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NOTICE

Please read:

'Myanmar:' for 'Burma'

'Yangon' for 'Rangoon'

'Ziwaka Road' for 'Zafar Shah Road'

Page 55	for	Page 1	Page 95	for	Page 41
" 63	for	" 9	" 99	for	" 45
" 50	for	" 15	" 102	for	" 48
" 75	for	" 21	" 105	for	" 51
" 81	for	" 27	" 109	for	" 55
" 87	for	" 33	" 111	for	" 57

Immune mechanisms in mouse malaria (I): Period of survival correlates with immune deficient status, but not with antibody titres and immune complex levels <i>Maung-Maung-Oo, Susumu Ikehara & Yoshihiro Hamashima</i>	1
Pulmonary host defence mechanism in newborn non-human primate lungs <i>Maung-Maung-Cho</i>	9
Isoenzyme variation in gametocytes of <u>Plasmodium falciparum</u> isolates from Myanmar <i>Myint-Oo & Sein-Min</i>	15
Comparison of two health education methods (Talk & Pamphlet Study) <i>Soe-Soe-Aye, Kyaw-Sein, Nyunt-Lwin, Maung-Maung-Gyi, Pe-Toe, Win-Lwin-Nyun, Ye-Hla, Khin-Than-Oo, Kyin-San-Yin & Thar-Din</i>	21
Experimental amoebiasis in mice immunosuppressed with cyclophosphamide injection and whole-body irradiation <i>Myint-Oo & W.E. Ormerod</i>	27
Description of currently unknown larval and pupal forms of <u>Aedes</u> (<u>Aedimorphus</u>) <u>nigrostriatus</u> (Barraud) 1972 <i>Myo-Paing & Thi-Thi-Naing</i>	33
Clinical trial of two Myanmar traditional medicine formulations TMF-03A and TMF-21 for diuretic efficacy <i>S.L. Tha, Aung-Naing & Kyi-Minn</i>	41
The effect of artemether (Qinghaosu derivative) on the development of <u>Plasmodium falciparum</u> trophozoites <i>Tin-Shwe, Khin-Sar-Mu, Pe-Than-Myint, Lin-Soe, Win-Myint & Ye-Htut</i>	45
Isolation of <u>Salmonellas</u> from stool, foods, flies and currency notes <i>Khin-Nwe-Oo, Myint-Myint-Lwin & Aung-Mon</i>	48

A preliminary study on knowledge and practices on ascariasis in rural Taikkyi <i>Myat-Lay-Kyin & Thein-Hlaing</i>	51
A prospective study of amodiaquine induced agranulocytosis and liver damage in malaria patients <i>Thet-Naing, Hla-Win, Aye-Aye-Mu & Tin-Shwe</i>	55
Malaria situation in Kowthaung Township, January 1989 <i>Tin-Shwe & Soe-Aung</i>	57

Immune mechanisms in mouse malaria (I) : Period of survival correlates with immune deficient status ,but not with antibody titres and immune complex levels

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Plasmodium berghei NK65 or *Plasmodium yoelii* (lethal strain) was inoculated into normal BALB/c mice, BALB/c nu/nu mice, neonatally thymectomized mice (NTx) and antithymocyte serum treated neonatally thymectomized mice (NTx + ATS). Periods of survival were compared to one another. The nu/nu, NTx and NTx + ATS mice survived significantly longer than normal BALB/c mice after *P. berghei* infection, whereas no such difference was noticed after *P. yoelii* infection. Antimalarial antibodies and circulating immune complex (CIC) levels were measured in order to examine their correlation with the survival period. A significant difference in the state of equilibrium between antibodies and CICs was revealed. Most of the antibodies induced by *P. yoelii* formed CICs whereas a portion of the antibodies induced by *P. berghei* remained unbound. However, no correlation between mortality pattern and serological changes was found. Therefore it seems unlikely that antimalarial antibodies and CICs are harmful factors responsible for the earlier death of the immunocompetent mice.

INTRODUCTION

Immunity to malaria is a complex phenomenon involving a spectrum of immune mechanisms ranging from a non-specific removal of parasite from the infected red cells in the spleen (1) to specific immune responses by humoral as well as cellular elements of the host immune system (2,3,4).

High parasitaemia level and severe anemia normally lead to death of the host but there were occasions where hosts died at an early stage of infection with a relatively low level of parasitaemia. In other words, the survival time of a host is probably not dependent solely on the degree of parasitaemia (5,6,7). Evidences have accumulated showing that hosts with T cell dysfunction survive longer after

malarial infection than hosts with normal T cell functions. BALB/c mice die earlier than BALB/c nu/nu mice after inoculation with *P. berghei* NK65 (8) or *P. berghei* ANKA (5). Neonatally thymectomized or antithymocyte serum treated golden hamsters survived longer after malarial challenge than do normal hamsters (9,10,11). Malnourished children with impaired T cell functions were found to suffer a less virulent form of falciparum malaria in West Africa than well-nourished children from the same region (12). All these findings suggest that intact T cell functions are deleterious to the host after infection with some species of malaria.

By contrast, non-lethal strain of *P. yoelii* 17X causes markedly increased parasitaemia and shortened survival in athymic mice in comparison with normal BALB/c mice (4). It is conceivable that different

plasmodia behave differently inside one host and the same parasite behave differently in different hosts (13,14).

In order to elucidate what immune mechanisms are involved in such discrimination, we first tried to find a model system including two plasmodia with or without an ability to discriminate between the mice with intact or defective T cell functions. Secondly, antimalarial antibodies and CICs were measured in the infected mice to find out whether they are responsible for such discrimination.

MATERIALS AND METHODS

Mice

Mice with different levels of immunological competence were used, including BALB/c mice, nu/nu mice with a BALB/c background, neonatally thymectomized BALB/c mice (NTx) and antithymocyte serum treated neonatally thymectomized BALB/c mice (NTx+ATS). All mice used were between two and four months old.

Antithymocyte serum treatment

Antithymocyte serum was raised in two albino rabbits. Primary immunization was done by subcutaneous injection of 2 ml of C57BL/6J mouse thymus homogenate in phosphate buffered saline (PBS) with complete Freund's adjuvant. One month later, a booster injection of 5×10^6 thymocytes from the same strain of mouse was given intravenously. Ten days after the booster injection, sera were collected and absorbed with one tenth volume of packed spleen cells from nude mice at 4°C for 4 hours. After absorption, their antibody titre and non-specific lytic activity were checked by a cytotoxicity test using lymphoid cells of BALB/c mice in the presence of rabbit complement. The sera lysed approximately 30% of spleen cells, more than 95% of thymocytes but less than 5% of bone marrow cells. Antithymocyte serum (0.5 ml of 1 in 10 dilution) was injected intraperitoneally to NTx mice 3 times a week for 2 weeks.

Inoculation for the study of survival pattern

Ten BALB/c mice, 4 nu/nu, 3 NTx and 3 NTx+ATS were each inoculated intraperitoneally with the lethal strain of *P. yoelii* 17X as 10^7 infected red cells. The other group inoculated in the same manner with *P. berghei* NK65 included 10 BALB/c mice, 5 nu/nu, 3 NTx and 3 NTx+ATS. Giemsa stained tail blood smears were prepared every day to determine the parasitaemia level. The survival periods were noted.

Inoculation and serum collection for serological tests

Ten BALB/c mice and 5 BALB/c nu/nu mice were inoculated with 10^7 *P. yoelii* (lethal strain) infected red cells per mouse intraperitoneally. Similar group of mice was inoculated with *P. berghei* NK65 in the same manner. It was planned to sacrifice 2 BALB/c and 1 nu/nu mice from each group on days 2,4,6,8,10 and 12 in order to obtain sera. No mouse remained alive after the sixth day in *P. yoelii*-infected group, and after the eighth day in the *P. berghei*-infected BALB/c group. The day 2 blood was omitted in the case of the nude mice. All the serum samples were immediately stored at -70°C for simultaneous assay at a later date.

Hyperimmune sera were obtained from 2 BALB/c mice which had been repeatedly infected with *P. berghei* NK65 and treated 4 days later with chloroquine injections (8 mg/kg/day for 2 days), for 3 cycles at 10 day-intervals. They were pooled and used as a positive control in all determinations. Heat inactivated serum pooled from 3 normal BALB/c mice was used as a negative control in all tests.

Antimalarial antibody titres (IgM and IgG) and CICs were measured in all of these samples. Correlations between these results and survival days were examined.

Antimalarial antibodies

Antimalarial antibody titres were measured in both IgM and IgG fractions by indirect fluorescence antibody technique (IFAT) using fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgM goat IgG (Cappel; lot.no. 18115) and FITC-conjugated antimouse IgG goat IgG (Cappel; lot.no.19644). Antigen spots were prepared on a glass slide by dropping malaria-infected mouse red cells, which had been washed 3 times and resuspended in PBS. Each of the test sera, the hyperimmune serum and normal mouse serum were assayed neat and at 6 fourfold serial dilutions.

Circulating immune complexes

Estimation of CIC levels was performed by Clq solid phase radioimmunoassay using radioiodinated protein-A (^{125} I-Prot.A). Clq was purified from human sera obtained from 5 healthy donors. The purification procedure was as previously described (15). Heat aggregated human crude globulin was used to make standard in serial dilutions. Hyperimmune mouse serum was used as a positive control, and heat-inactivated normal mouse serum was used as a negative control. The test procedure was as previously described (16). Mean count per minute values of the duplicate results were calculated to read the concentration from a standard curve as equivalences of aggregated globulin in µg/ml.

RESULTS

Survival periods in different mouse groups with varied immunological status

Survival periods are shown in Table 1 and the corresponding parasitaemia levels are shown in Figure 1. In comparison with BALB/c mice, nu/nu mice survived significantly longer ($p < 0.001$) to *P.berghei* infection, but not to *P.yoelii* infection.

Table 1. Periods of survival after malarial inoculation in different groups of mice.

Type of infection	Type of mice	Mean survival days standard deviation	Significance of difference from BALB/c group
<i>P. yoelii</i> infection	BALB/c	5.50 ± 1.0903	-
	NTx*	5.00 ± 1.7327	NS†
	NTx+ATS‡	4.00 ± 0.0000	$P < 0.05$
	nu/nu	5.50 ± 0.5774	NS
<i>P. berghei</i> NK65	BALB/c	6.25 ± 1.1650	-
	NTx	10.00 ± 2.0000	$P < 0.01$
	NTx+ATS	11.00 ± 0.0000	$P < 0.001$
	nu/nu	10.40 ± 0.8944	$P < 0.001$

*Neonately thymectomized mice.

‡Anti-thymocyte serum treated neonatally thymectomized mice.

†Not significant.

The survival days of nu/nu and BALB/c mice were the same in *P. yoelii* infection. The most obvious difference in survival was found between normal BALB/c and NTx + ATS group. The latter group survived longest with *P. berghei* infection ($p < 0.001$), but shortest with *P. yoelii* infection ($p < 0.05$). Mean survival days of the NTx group lay between those of normal BALB/c and NTx + ATS groups. The difference between the mean survivals of BALB/c and NTx + ATS groups after *P. yoelii* infection was not marked, though it was statistically significant at the 5% level. The NTx + ATS group showed the least variation in the number of survival days whereas all other groups including the nu/nu mice displayed some variation of survival days within the group.

Antibody titres and circulating immune complex levels

Seriological results are shown in Table 2. After *P. yoelii* infection, the rise in antibody titre was almost nil in the BALB/c group, and a mild rise was detected in the nu/nu group. By contrast, antibody titre rose markedly in *P. berghei* infected groups, both BALB/c and nu/nu. However, CICs appeared early during the *P. yoelii* infection in BALB/c mice in contrast to a slow rise in nu/nu mice infected with the same parasite. Therefore,

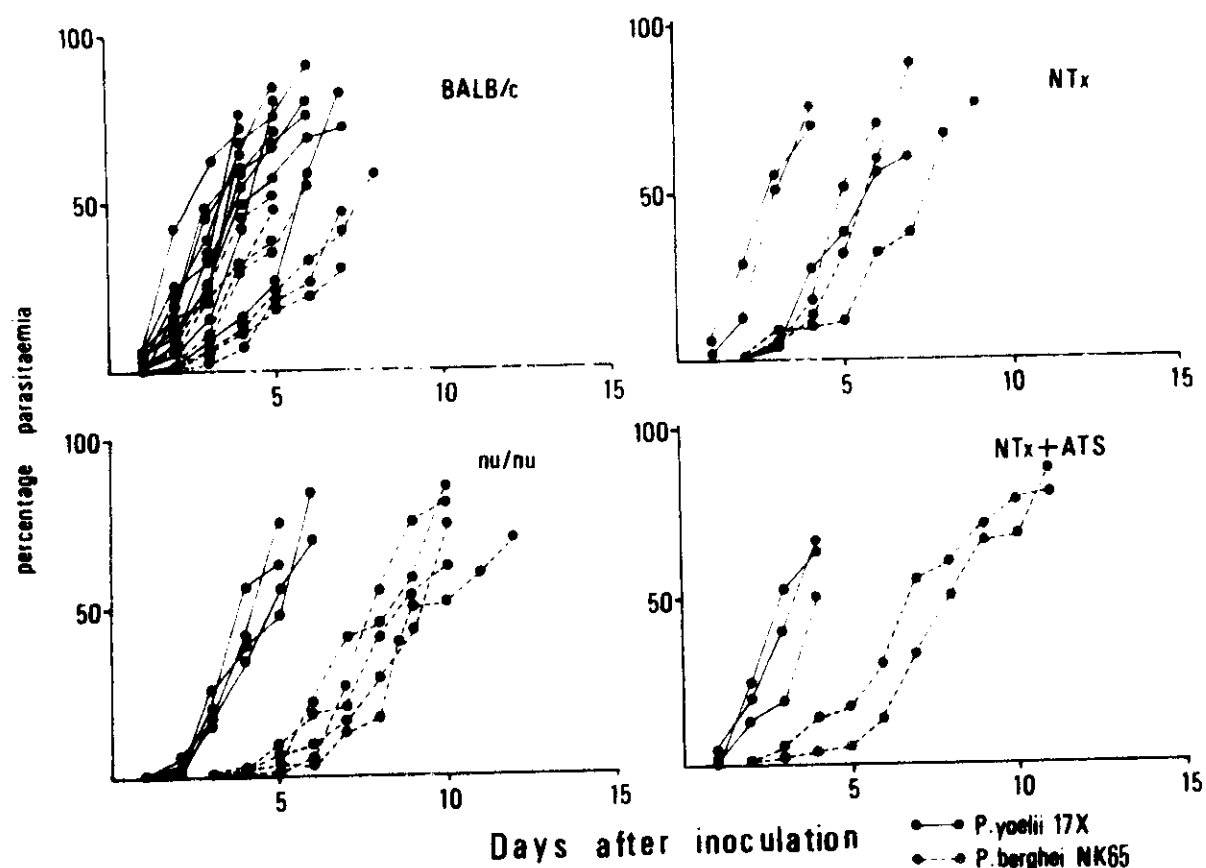


Figure 1. Parasitaemia levels from the different groups of mice as the percentage of infected red cells from the peripheral blood films. NTx, neonatally thymectomized mice; NTx + ATS, neonatally thymectomized and antithymocyte serum treated mice.

it could be deduced that the reason for a very low measurable antibody titre in BALB/c mice particularly at the early stage was that the majority of the formed antibodies during *P. yoelii* infection immediately bound to the circulating soluble antigens to form CICs.

The positive control showed IgM titre 1 in 4, IgG titre 1 in 1024 and 20.5 ug/ml of CIC; whereas the negative control showed

no antibody activity and less than 0.39 ug/ml of CIC. CIC levels rose with increasing days of infection. Mice with longer survival had higher levels of CIC and higher titres of antibody. Extremely high level was found in the hyperimmune serum. Therefore it is unlikely that antibody production and consequent immune complex formation are responsible for the untimely death in this system.

Table(2) Antimalarial antibody titres and circulating immune complex levels in different groups of mice.

Type of infection	Type of mice	Days after inoculation	IgM titre	IgG titre	CICs in ug/ml
<i>P. yoelii</i> 17X	BALB/c	2	0	0	10.50
	"	2	0	0	9.80
	"	4	0	0	29.00
	"	4	0	0	29.50
	"	6	0	1	37.50
	nu/nu	4	0	1	40.39
	"	6	1	4	17.50
	"	"	"	"	"
<i>P. berghei</i> NK65	BALB/c	2	0	0	0.39
	"	2	4	0	15.50
	"	4	4	1	26.00
	"	4	1	1	16.20
	"	6	4	1	32.00
	"	6	4	4	40.39
	"	8	16	64	62.50
	"	8	4	16	15.60
	nu/nu	4	1	0	12.70
	"	6	4	256	25.50
	"	8	16	4	10.20
	"	10	NS	NS	4.35
	"	12	16	256	12.00
Controls	Hyperimmune serum		4	1024	20.50
	Normal mouse serum		0	0	40.39

Abbreviations: NS=serum was not sufficient.

DISCUSSION

Our experiments revealed that the T cell deficient mice such as NTx, NTx + ATS and nu/nu mice survived longer than normal BALB/c mice when infected with *P. berghei*, but not when infected with *P. yoelii*.

Many explanations and speculations have been put forward regarding the prolonged survival of T cell deprived hosts with malarial infection. One postulation was that the delayed death in a T cell deficient host was due to a defect in the formation of T dependent serum agglutinins

which were injurious to endothelial cells by forming intravascular agglutinates and by blockage of small blood vessels leading to ischaemic injuries and early death (10,11). Our results showed no significant difference between the antibody titres of *P. berghei* infected nu/nu mice and BALB/c mice. Therefore it is difficult to claim the antibody as a discriminating factor.

It had been shown (17) that nu/nu mice possess functional T cells and that these T cells increase with age, showing some variations among nu/nu mice. NTx mice also seemed to possess some T cells which probably having already matured to the functional state at the time of thymectomy and thus remained circulating in the peripheral blood. They seemed to be totally destroyed after antithymocyte serum treatment resulting in the total deprivation of T cell functions. These assumptions could explain the different survival days of the mouse groups according to the degree of T cell deficiency. Sharply delineated figures of survival days in NTx + ATS group in contrast to a slight variation of survival days in nu/nu mice could also explain the completeness of T cell deficiency in NT + ATS mice and the presence of a few functional T cells in variable number in nu/nu mice which had a slightly shorter survival than NTx+ATS group.

Another T cell dependent pathway of tissue injury was probably through the formation of CICs in the blood. T dependent antiplasmodial antibodies bound to circulating soluble antigens to form CICs which were thought harmful to the host by complement activation after deposition on certain tissues or on red cells (18,19). Electron dense deposits were demonstrated in cerebral blood vessels of hamsters infected with *P. berghei* (7). Formation of secondary complexes such as idiotype-antiidiotype complexes is a possibility (5) because marked polyclonal activation of B cells and production of a series of autoantibodies during malarial infection has been documented (2,20). The presence of CICs during malarial infection and their role in pathogenesis has been extensively discussed (19,21,22). Cerebral malaria is considered due possibly to immune complex deposition in choroid plexus (18). In our results, CICs were found to be raised in all four groups regardless of

their difference in the survival period. However, the fatality pattern was not correlated with CIC levels. Strikingly high levels of CICs in hyperimmune serum had no lethal effect to those chronically infected mice.

Some interesting differences were revealed in the dynamics of antibody and CIC formation in different infection groups. Nu/nu mice showed less liability to form CICs even after a considerable degree of antibody production. This suggests that antibody produced by nu/nu mice may be of low affinity.

Antibodies induced by *P. yoelii* tended to form CICs to an extent that free antibody could not be detected in our assays during the early days of infection in BALB/c mice. Antimalarial antibody response has been found to be blunted in BALB/c mice during infection with the lethal strain of *P. yoelii* but no reason was found to explain that phenomenon (4). Here, we get a suggestive evidence that the failure to detect antimalarial antibody was not due to a lack of production but due to their immediate transformation into CICs.

The assumption that *P. berghei* is more susceptible to natural killer (NK) cell activity may help to explain the longer survival of nu/nu and NTx + ATS mice to *P. berghei* infection because these mice possess higher NK activity than normal BALB/c mice (23). A correlation has been demonstrated between the susceptibility to malarial infection and the levels of NK cell activity of the host (24). In a preliminary experiment, we have found that NK activity was enhanced several folds after malarial infection.

Macrophage activity may also be correlated with fatality patterns. Ample evidences suggest that macrophage products damage malarial parasites to a considerable extent (25) and their action is found to be partly T dependent (26). Liver macrophages in cooperation with circulating T cells are shown to be most active, particularly in the vaccinated hosts (25,27).

The changes in other aspects of the immune system in this model will be presented later.

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Pulmonary host defence mechanism in newborn non-human primate lungs

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Using the technique of Broncho-alveolar lavage (BAL), alveolar macrophages (AM) from the lower respiratory tract of five newborn rhesus monkeys (1 to 2 days of age) with five adult controls were isolated. Chemotactically responsiveness, phagocytic assays and killing capacity of the above macrophages were tested. It was found that the alveolar macrophages from the adult primate lung were chemotactically responsive while the alveolar macrophages from the newborn lung were chemotactically impaired. In phagocytic assays, the percentages of alveolar macrophages from the lung which had phagocytised 3 or more *candida albican* blastospores ranged from 56 to 89 while macrophages from newborn scored as 0 to 10. The killing capacity of alveolar macrophages from adult lung ranged from 70 to 92 percent while macrophages from newborn scored at 1 to 12 percent. Moreover, a substantial increase in neutrophil count was obtained in the second series of lavages and a significant migration of neutrophils into the adult lungs was confirmed. These results clearly indicate that the newborn non-human primate lungs are functionally deficient in defence mechanism compared to adults.

INTRODUCTION

To work as a protective functional barrier against invading microbes, several host defence mechanism must be operative including the mucociliary clearance mechanism of the upper respiratory tract as well as the alveolar macrophage phagocytic mechanism and the migrating neutrophil back-up mechanism of the lower respiratory tract.

Schwartz and Christman (1) have shown the healthy lung contains within the alveoli a resident population of alveolar macrophages. During episodes of bacterial insult, Green & Kass (2) had shown the active role of these alveolar macrophages to phagocytise and kill the invading microbes. While much research has been conducted on macrophage function, very little is known of their ontogeny, functional characteristics, competence or maturity in the newborn lung. such knowledge is particularly important in view of the vulnerability of the newborn

lung to infection, environmental insult and bacterial pneumonia.

Kazmierowsky, Gallen & Reynolds (3); Miller & Cheung (4), documented that neutrophils could migrate to the lung of the healthy adult in response to lower respiratory tract perturbation like bacterial infection or broncho-alveolar lavage. This brisk mobilization of neutrophils to sites of lower respiratory tract perturbation provides a phagocytic back-up mechanism for the alveolar macrophage host-defence mechanism. Again not much information is available on migrating neutrophils in newborns. In general, the functional capability of host defence in newborns is not known.

To solve this problem, the present study has investigated two major aspects of the pulmonary host defence mechanism; alveolar macrophage function as a first line of bacterial defence and induced neutrophils migration into the lung as a back-up defence.

MATERIALS AND METHODS

Five newborn rhesus monkeys with five adult controls were tested. Broncho-alveolar lavage was performed under general anesthesia induced by katamine hydrochloride at a dosage of 10.15 mg per kg body weight. Local anesthesia induced by 2 percent cetacaine spray was applied to the posterior pharynx. A double-lumen balloon-tipped bronchial catheter-4F for newborns & 10F for adult controls was used for each lavage. A laryngoscope equipped with a pediatric flagg blade was used to visualize the glottis and the sterile catheter was carefully passed into the trachea and wedged into the lower lobe bronchus. The balloon was then inflated with 1.5 ml of air and the lobe was lavaged with aliquotes of sterile Dulbecco's phosphate buffer saline. For a newborn monkey a total of 7.5 ml of buffered saline was used in 3-4 lavage cycles. For the adult control, 120ml buffered saline was used in 3-6 lavage cycles. The total amount of buffered saline was used in each lavage and the amount of fluid recovered was recorded. At the completion of the lavage, the balloon was deflated, the catheter removed carefully and the animal was returned to the hospital for observation until the anesthesia was resolved (usually 1-2 hours).

Two lavage, 24 hours apart, were carried out for each newborn and adult control in identical manners.

The recovered buffered saline from the lavage was centrifuged at 500 g for 15 minutes and the cell pellet was suspended in 2ml of McCoy's 5a modified culture medium enriched with L-glutamine and 10% heat-inactivated calf serum. The cells from the first lavage were mostly alveolar macrophages and the cell concentration was adjusted to 10^6 cells/ml for the chemotatic chamber assay and phagocytic/killing assay.

Before adjusting the macrophage concentration, three smears were made for differential cell measurement from 2 ml McCoy's suspension. The smears were air-dried and stained with methyl violet stain. Cells were classified and scored as macrophages and neutrophils per 100 cells counted. Cells viability was determined by the Trypan Blue Exclusion Test. Total cell concentration of the 2 ml McCoy's suspension was determined by the standard haemocytometer procedure and the original concentration of cells in the lavage fluid could then be calculated.

Each newborn monkey (as well as adult control) was lavaged twice - the first lavage to provide baseline score for differential macrophage and neutrophil counts and a second lavage - 24 hours later to give the count for induced neutrophil migration arising as a result of lower respiratory tract perturbation.

Chemotactic responsiveness was studied by a modification of the Boyden assay. Briefly, alveolar macrophages from the first lavage of each test were placed in the upper portion of the blind well chemotactic chamber over a 8 μ m pore-sized cellulose ester filter. The lower portion of the chamber was filled with 0.2 ml of endotoxin activated plasma in 0.4 ml of plasma from the same monkey as the chemoattractant. Triplicate assays were performed on the adult samples (control) while duplicate assays were performed on the newborn samples as there might not be enough cells for the triplicate assays. Chambers removed and stained with the modified Hematoxylin-Wright procedure. The number of alveolar macrophages per high power field (100 x oil immersion) which had migrated through the micropores to the lower surface of the filter was counted. Ten fields under high power were counted per assay of each test sample and the average number of macrophages per field was calculated.

Phagocytosis and killing capacities were evaluated in triplicates as modified by Schuit. Briefly, 3 precleaned glass cover slips were used for each test. Two drops of alveolar macrophages, from the first lavage fluid of each test, were allowed to adhere to the glass cover slip for 15 min at 37°C. At the end of the incubation, non-adherent macrophages were removed by gentle washing with buffered saline. Two drops of *Candida albicans* suspension, which was standardized to 2×10^6 *Candida* blastospores in 1.0 ml of the McCoy's 5a modified culture medium (with 5% rhesus monkey serum added), were put on each cover slip to be phagocytized by the macrophages. At the end of the 4-hour incubation, each cover slip was carefully rinsed to remove non-phagocytised *Candida* blastospores, stained with acridine orange and mounted on a glass slide with permount for scoring. Scoring was

conducted under fluorescence microscope by counting the number of alveolar macrophages which had ingested *Candida* blastospores. The killing capacity of the macrophages for ingested *Candida* was assessed on the basis of the fluorescing color of the *Candida*. The fluorescence of live intracellular *Candida* blastospores was palegreen while that of dead (killed) one was copper red. At least 100 macrophages were examined and scored for each cover slip and all the actual scores were tabulated.

Results for phagocytosis are expressed as the percentage of macrophages which had ingested 3 or more *Candida* blastospores.

The results of intracellular cytotoxicity (killing) are expressed as the percentage of ingested *Candida* blastospores which had been killed.

RESULTS

Table 1. Lavage fluid measurements and cell counts for adult monkeys

MMU	(Age)	Lavage	Lavage fluid in MLS. (used / recovered)	Fluid recovery (%)	Total cell count from lavage fluid	Differential cell counts in (%) (AM) (N)		
1	10 yr	(1)	120/110	92	7.4×10^6	82	10	
		(2)	120/ 90	75	7.2×10^6	45	56	
2	8 yr	(1)	120/110	83	9.6×10^6	84	9	
		(2)	120/ 95	79	10.4×10^6	52	44	
3	9 yr	(1)	120/100	83	20.4×10^6	86	9	
		(2)	120/105	88	18.6×10^6	48	52	
4	12 yr	(1)	120/110	92	10.6×10^6	80	12	
		(2)	120/100	83	10.4×10^6	45	48	
5	6 yr	(1)	120/ 90	75	8.6×10^6	84	12	
		(2)	120/100	83	9.2×10^6	51	10	
Average						1st day-	83%	10%
Recovery 83%						2nd day-	48%	49%

MMU : Rhesus Monkey (*Macaca Mulatta*)

AM : Alveolar Macrophages

N : Neutrophil

Both adult and newborn monkeys tolerated the lavage well and ill effects arising as a result of the lavages were not observed.

In adult monkeys 85 per cent of the lavage fluid used for the first series of lavages and 82 per cent in 2nd series, with an average recovery percentage of 83 for all lavage performed was recorded. Differential cell counts indicated that 83 per cent of the cells obtained from the first series of lavages were macrophages while 10 per cent were neutrophils. Second series of lavages generated a differential count of 48 per cent macrophages and 49 per cent neutrophils (Table 1). A substantial increase in neutrophil count was obtained in the

second series of lavages and a significant migration of neutrophils into the adult lungs was confirmed.

In newborn monkeys, 80 per cent and 82 per cent of the lavage fluid was recovered on 1st series and 2nd series respectively. Differential cell count indicated that 88 per cent of the cells obtained from the first series were macrophages and 6 per cent were neutrophils. The 2nd series of lavage generated a differential count of 89 per cent macrophages and 6 per cent neutrophils. The differential counts for the first and 2nd lavages were practically identical and there was no evidence of neutrophil migration into the newborn lungs (Table 2).

Table 2. Lavage fluid measurements and cell counts for new-born monkeys

MMU	(Age)	Lavage	Lavage fluid in MLS. (used / recovery)	Fluid recovery (%)	Total cell count from lavage fluid	Differential cell counts in (%)	
						(AM)	(N)
1	1 day	(1)	7.5/6.0	80	1.4×10^6	90	7
		(2)	7.5/6.5	87	1.3×10^6	91	6
2	1 day	(1)	7.5/5.5	73	1.1×10^6	88	7
		(2)	7.5/5.7	75	1.0×10^6	89	5
3	1 day	(1)	7.5/6.2	83	0.9×10^6	92	5
		(2)	7.5/5.8	77	0.8×10^6	87	6
4	1 day	(1)	7.5/6.4	85	1.2×10^6	94	3
		(2)	7.5/6.6	88	1.0×10^6	91	5
5	2 days	(1)	7.5/5.9	79	1.1×10^6	86	7
		(2)	7.5/6.3	84	1.2×10^6	88	8
Average						1st day 88%	6%
Recovery 81%						2nd day 89%	6%

MMU : Rhesus Monkey (Macaca mulatta)

AM : Alveolar Macrophage

N : Neutrophil

Table 3. Chemotactic chamber assay with alveolar macrophages from adult monkeys

MMU (Age)	Average number of cells per field in medium control (Non-chemottractant)			Average number of cells per field in endotoxin-activated plasma (Chemo-attractant)		
1 10 yr	0, (0)	1		12, 16, 10		
2 8 yr	0, 1, 1	1		(8) 16, 12		
3 9 yr	1, 1, 0			16, 12, 14		
4 12 yr	(2) 1, 0			18, 15, (19)		
5 6 yr	0, 1, 1			10, 14, 16		

MMU : Rhesus Monkey (Macaca Mulatta)

Moreover, it is found that the alveolar macrophages from the adult primate lung were chemotactically responsive (Table 3) while the alveolar macrophages from the newborn lung were chemotactically impaired (Table 4).

Table 4. Chemotactic chamber assay with alveolar macrophages from new-born monkeys

MMU (Age)	Average number of cells per field in medium control (Non-chemottractant)			Average number of cells per field in endotoxin-activated plasma (Chemo-attractant)		
1 1 day	0, (0)			(2)	1	
2 1 day	1, 0			0, 1		
3 1 day	1, 0			1, (0)		
4 1 day	1, 1			2, 1		
5 2 days	(3) 1			2, 2		

MMU : Rhesus Monkey (Macaca Mulatta)

In phagocytic assays, the percentage of alveolar macrophages from adult lung which had phagocytized 3 or more *Candida blastospore* ranged from 56 to 86 per cent while macrophages from newborn scored at 0 to 15 per cent. The killing capacity of alveolar macrophages from adult lung ranged from 67 to 89 per cent while macrophages from newborn scored at 2 to 16 per cent (Table 5).

These results overwhelmingly indicate that the alveolar macrophages from newborn lung are functionally deficient.

Table 5. Phagocytic and killing capacity of alveolar macrophages

MMU	(Age)	Phagocytic Score %			Killing Score %		
1	10 yr	(56)	60, 51	70, 75, 81			
2	8 yr	46, 58, 62	74, 85, 78				
3	9 yr	60, 71, 56	70, 78, 82				
4	12 yr	66, 75, 64	74, 85, (89)				
5	6 yr	84, 68, (86)	82, 78, (67)				
6	1 day	0, 4, 7	2, 6, 8				
7	1 day	0, 2, 8	4, 6, 10				
8	1 day	(15) 1, 4	(16) (2) 8				
9	1 day	14, (0) 2	14, 5, 4				
10	2 days	0, 6, 8	2, 8, 12				

MMU : Rhesus Monkey (Macaca Mulatta)

COMMENT

With the utilization of bronchoalveolar lavage technique, it is evident that the alveolar macrophage is the predominant cell type obtained from the first lavages and that on 24 hr subsequent lavages, there is always a marked increase in neutrophils in adult lungs but not in newborn. Previous work by Miller and Cheung (4) has established and characterized deficiencies in neutrophil function in human and non-human primate neonates. Therefore, deficiency in neutrophil migration in newborn monkeys documented in this study is consistent with previous findings on chemotactic non-responsiveness in newborn.

With fully functional alveolar macrophages and the reinforcement of migrating neutrophils, adult lungs are able to resist invading microbes. Deficiencies in the functional capability of alveolar macrophages as well as the absence of neutrophil migration to provide phagocytic back-up for host defence in the newborn leave the newborn lung defenceless and has important clinical implications.

These findings may serve to explain, to a great extent, the relative susceptibility of newborn to lower respiratory tract infection and pulmonary complications.

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Isoenzyme variation in gametocytes of *Plasmodium falciparum* isolates from Myanmar

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Seven isolates of blood containing *Plasmodium falciparum* gametocytes were collected from hospital in-patients in Yangon and the gametocytes were harvested for starch gel electrophoresis of the following parasite isoenzymes-adenosine deaminase (ADA) (EC. 3.5.4.4), glucose phosphate isomerase (GPI) (EC. 5.3.1.9), NADP-dependent glutamate dehydrogenase (GDH) (EC. 1.4.1.4), lactate dehydrogenase (LDH) (EC. 1.1.1.27), 6-phosphogluconate dehydrogenase (6PGD) (EC. 1.1.1.43) and peptidase F (PEPF) (EC. 3.4.11 or 13). Variations were found in GPI, LDH and ADA. The other three isoenzymes appeared to be invariant in all the isolates. The isoenzyme patterns of gametocytes showed no marked difference from those of cultured schizonts.

INTRODUCTION

Isoenzyme characterization, using starch gel electrophoresis, has proved to be a valuable technique for identifying genetic differences in populations of rodent malarial parasites Carter (1, 2, 3), human malarial parasites, *Plasmodium falciparum* Carter and Voller (4); Carter & McGregor (5); Hempelmann, Ling & Wilson (6); Sanderson et al. (7); Thaithong et al. (8); Myint-Oo et al. (9) and *P. vivax* Myint-Oo (10). In most of the previous works by myself and others, the characterization was done only on the cultured and harvested schizonts of the malarial parasites.

In this paper we tried to harvest and concentrate the gametocytes of *P. falciparum* and compare their isoenzyme patterns on starch gel electrophoresis in the hope of identifying new forms of isoenzymes additional to those of other stages of malarial parasites.

MATERIALS AND METHODS

Ten to 15 ml of venous blood samples with suitable amount of gametocytes (5 gametes/ 10^3 erythrocytes) of *P. falciparum*

were collected in acid-citrate-dextrose (ACD) and transported in ice to the Parasitology Research Division, Department of Medical Research, within one to three hours of collection.

Gametocytes were separated from non-infected erythrocytes, leucocytes and platelets by the method of Gutteridge & Coombs (11) by low speed centrifugation (225g for 5 min) on a linear sucrose gradient (0.25 to 0.75M). Recovery of gametocytes varied from 10-25% and contamination of ring trophozoites and white cells was less than 0.1%. After saponin lysis of the erythrocytes (Sanderson et al.), gametocytes were again lysed by adding 200 μ l of 1% Triton-X100 dissolved in EDTA-Tris-KCl buffer pH 7.4. The lysate obtained was centrifuged at high speed (3,500g) and the clear supernatant was then absorbed on square pieces (6 x 6 mm) of Whatman filter paper No. 43 before insertion into the starch gel.

Starch gel electrophoresis and staining of enzyme bands for adenosine deaminase (ADA) (EC. 3.5.4.4), glucose phosphate isomerase (GPI) (EC. 5.3.1.9), NADP-dependent glutamate dehydrogenase (GDH) (EC. 1.4.1.4), lactate dehydrogenase

(LDH) (EC. 1.1.1.27), 6-phosphogluconate dehydrogenase (6PGD) (EC. 1.1.1.43) and peptidase E (PEPE) (EC. 3.4.11 or 13) was carried out basically as described by Carter (2) and Sanderson et al. (7). Previously characterized Burmese isolates of *P. falciparum* Myint-Oo et al. (9) were also examined to compare the enzyme patterns of schizont stages with those seen in gametocytes.

Recording of starch gel zymograms

Stained zymograms were recorded either by photography or by permanent mounting on Whatman filter paper by the method previously described by Myint-Oo et al. (9).

RESULTS

The harvested gametocytes after saponin lysis is shown in Fig. 1. Both macro- and micro-gametes maintained their original shapes and structures.

Isoenzyme patterns of gametocytes of *P. falciparum* were characterized by pattern analysis as shown in Fig. 2. and Fig. 3.

The results for six enzymes from seven parasite isolates are summarised in Table 1.

Substantial polymorphism was found with ADA, GPI and LDH; the other three isoenzymes appeared to be invariant by the tests used. The patterns of all isoenzymes of gametocytes appeared to be the same as those of schizont stage.



Figure 1. Harvested gametocytes of *Plasmodium falciparum*

Table 1. Distribution of enzyme types in gametocytes of *Plasmodium falciparum* from Myanmar

Isolate No.	GDH		GPI				PGD		LDH		ADA		PEPE	
	1	2	1	2	1+2	3	1	2	1	2	1	2	1	2
FG-1	+	-	+	-	-	-	+	-	+	-	-	+	+	-
FG-2	+	-	+	-	-	-	+	-	+	-	+	-	+	-
FG-3	+	-	+	-	-	-	+	-	+	-	+	-	+	-
FG-4	+	-	+	-	-	-	+	-	-	+	+	-	+	-
FG-5	+	-	-	-	-	+	+	-	+	-	+	-	+	-
FG-6	+	-	-	+	-	-	+	-	+	-	+	-	+	-
FG-7	+	-	+	-	-	-	+	-	+	-	+	-	+	-
	7	0	5	1	0	1	7	0	6	1	6	1	7	0

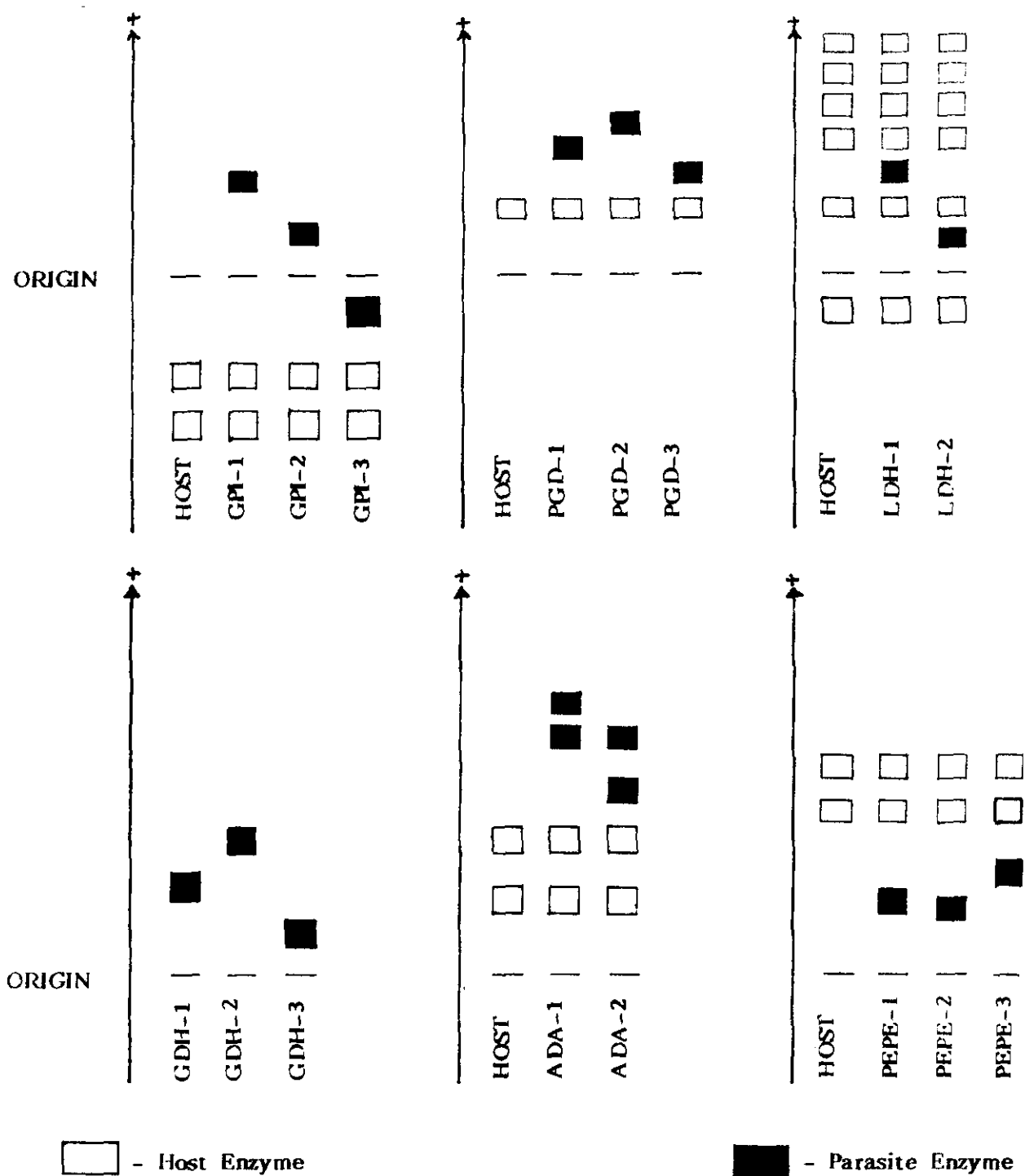


Figure 2. ELECTROPHORETIC FORMS OF GPI, PGD, LDH, GDH, ADA, AND PEPE GAMETOCYTES OF *P. FALCIPARUM*

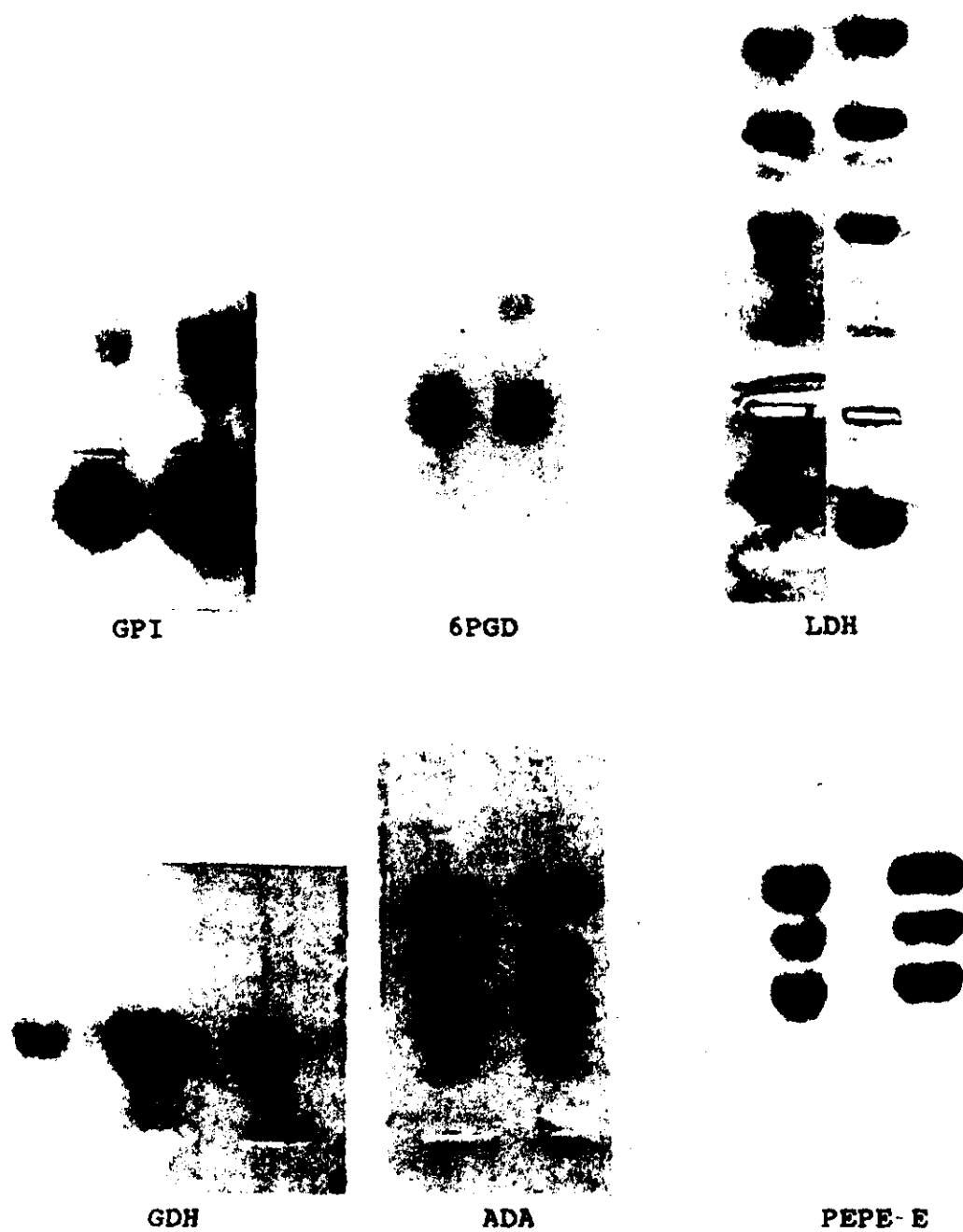


FIGURE 3. ELECTROPHEROGRAMS OF GPI, 6PGD, LDH, GDH, ADA AND PEPE-E IN GAMETOCYTES OF PLASMODIUM FALCIPARUM

DISCUSSION

Although there have been reports on isoenzyme variation in schizont stages of human malarial parasites Carter & McGregor (5); Carter & Voller (4); Hempelmann et al. (6); Sanderson et al. (7); Thaithong et al. (8); Myint-Oo et al. (9); Myint-Oo (10), isoenzyme variation in gametocytic stage has never been documented. This paper is the first report of isoenzyme typing of harvested gametocytes of P. falciparum.

It is well known that in P. falciparum infections, especially in people living in endemic areas, gametocytes generally appear in the peripheral blood. It is therefore possible to harvest and concentrate the gametocytes from blood samples and carry out the starch gel electrophoresis without involving long-term cultivation for gametogenesis. The use of the concentration method described here gave a 10 to 25 times increment of gametocytaemia from the original samples of less than 10%.

It is notable that all of the electrophoretic forms of the enzymes examined in gametocytes of P. falciparum possessed similar patterns to those of cultured schizonts of P. falciparum; the only exception was that each isolate of gametocytes possessed a single isoenzyme form where some isolates of cultured schizonts contained more than one forms of certain isoenzymes (e.g. LDH-1 & -2 or GPI-1 & -2) Myint-Oo et al. (9).

No other additional forms were recorded by using various dilutions of electrode buffers (data not shown).

The method of isolation and harvesting of gametocytes direct from patient's blood could be useful for further studies such as susceptibility of infection by Anopheline mosquitoes and using as antigens to test transmission blocking antibodies by Indirect Fluorescent Antibody Technique.

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Comparison of two health education methods (Talk & Pamphlet study)

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Two health education methods, "talk" and "pamphlet" study, were studied in order to plan health educational activities regarding rheumatic fever in school children in Mandalay Division. The two health education methods, Talk and Pamphlet study, were studied in two groups of school children. Eighty students from V standard and 80 students from IX standard, randomly selected from 4 randomly selected schools : one school from each of the 4 townships of Mandalay City.

Each group of 80 students from the V and IX standards were subdivided into two groups using random numbers one group being assigned to the health education talk and the other to the pamphlet study. Pre-test and post-test scores were obtained from the 160 students involved in the study.

Analysis of the data using appropriate statistical tests was done. The mean health education level achieved (post-test scores) after health education talk was found to be significantly better than that after the pamphlet study.

INTRODUCTION

A situational analysis report on rheumatic fever in Myanmar (1), data collected through the cardiovascular disease (CVD) project in Myanmar and local publications (2,3,4,5,6,7,8) substantiate the existence of a high risk of rheumatic fever and rheumatic heart disease population, i.e. the school going age group children in Myanmar.

Apart from initiating secondary prophylaxis with long acting penicillins, one of the ways to prevent rheumatic fever and rheumatic heart disease would be to disseminate health knowledge regarding rheumatic fever to the school children.

There are several educational methods for disseminating the health message. From which, two educational methods "talk" and "pamphlet study" are chosen for this study.

This study is undertaken to compare the two educational methods (talk and pamphlet study) with a view to help plan health educational activities regarding rheumatic fever in school children.

MATERIALS AND METHODS

a. Subjects

A total of 160 students (80 each from V

standard and IX standard) was studied. Twenty students each from V standard and IX standard were randomly selected from one school, randomly selected in each of the four townships, North-West, North-East, South-West and South-East townships of Mandalay. Ten students out of the twenty students selected in each standard in one school in each of the four townships of Mandalay were assigned by random numbers to listen to the health education talk whilst the other ten students were assigned to pamphlet study (Table 1)

Table 1. Allocation of the students for the two methods of health education.

Township	Number of Students			
	V Standard		IX Standard	
	Talk	Pamphlet	Talk	Pamphlet
MDY NW	10	10	10	10
MDY NE	10	10	10	10
MDY SW	10	10	10	10
MDY SE	10	10	10	10
	40	40	40	40
	80		80	

MDY NW = Mandalay North West

MDY NE = Mandalay North-East

MDY SW = Mandalay South-West

MDY SE = Mandalay South-East

b. Materials

The contents of the health education talk and the pamphlet were actually similar. Also two sets of pamphlets were prepared according to the school education levels (V and IX standards). Although the contents of these were the same in essence the language and illustrations used were simpler for the V standard level.

c. Design of the Study

Ten students each from V and IX standards and another ten students each from V and IX standards were assigned to either talk or pamphlet study by random numbers in one school of each township of Mandalay Division on four consecutive days.

A pre-test was given to all the students in the study.

The health education talks were given by one of the authors (Soe Soe Aye) to the eight "talk-groups" on eight occasions using transparency slides with an overhead projector. The V and IX standard students were given talks separately in each of the four schools in this study.

The pamphlets were distributed to the V standard and IX standard students in separate groups and the students were asked to read and study the contents of the pamphlets.

A post-test was given to each of the student groups after the health education either by talk or pamphlet study. The pre and post-test questions consisted of 20 simple multiple choice questions prepared separately for V standard and IX standard students.

The results of the pre-test and post-test for the different groups were analysed.

RESULTS

Table 2 illustrates the mean value for each group of students being tested. Mean values for the two methods of health education i. e the health education talk and the pamphlet study methods, are shown for both the V standard students and the IX standard students from each of the four Mandalay Townships. It will be noted that every group of students showed an increase in the mean score after the health education (irrespective of the method) as seen by comparing the corresponding post-test and the pre-test mean values.

Table 2. The mean score (out of 20) for the two health education methods for two education levels in four Mandalay townships

Township	Health Education talk				Pamphlet			
	V Standard		IX Standard		V Standard		IX Standard	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
MDY NW	7.5	15.5	8.7	15.5	7.5	11.8	10.6	15.3
MDY NE	8.0	12.9	8.3	15.7	5.9	12.0	9.6	15.3
MDY SW	6.4	14.4	9.2	16.4	5.4	11.4	9.4	15.2
MDY SE	7.1	13.1	7.9	15.4	6.0	12.4	9.3	13.2
All townships	7.205	14.333	8.525	15.75	6.200	11.900	9.974	15.128

The pairs of mean scores (the pre-test value and the corresponding post-test value) given in Table 2 are compared using the "t"-test and the t values are shown in Table 3.

Table 3. Results of t-test comparing the post-test and pre-test mean scores on corresponding groups.

Township	Health Education talk		Pamphlet	
	V	IX	V	IX
MDY NW	*** 6.708	*** 6.893	** 3.204	*** 5.403
MDY NE	*** 4.892	*** 9.033	*** 5.263	** 4.122
MDY SW	*** 7.667	*** 6.095	*** 5.379	*** 5.031
MDY SE	*** 5.601	*** 6.708	*** 5.488	** 4.111
All Township	*** 7.819	*** 14.278	*** 7.422	*** 8.951

** P less than 0.01
*** P less than 0.001

The mean post-test score was found to be significantly (P less than 0.001 in many groups) greater than the mean pre-test score in every group of students being tested. The probability level of less than 0.01 was found in only two groups of students in the pamphlet study series. Considering as a whole i. e. (students from all the townships) the difference between pre- and post-test mean for each methods of health education in the V and IX standard groups of students were found to be also significant at the 0.001 level.

The IX standard pre-test mean scores were greater than the V standard pre-test mean scores. The IX standard post-test mean scores were found to be significantly greater than the corresponding score for the V standard (Table 4). From this it may be seen that the education level for the IX standard students was higher than that for the V standard students, (a) before any health education had been given (pre-test results) and (b) after some forms of health education (post-test results).

Table 4. Result of the t- test comparing the mean scores for the V and IX standard students

	Pre-test	Post-test
	V vs IX	V vs IX
H E TALK	1.327	2.404*
PAMPHLET	6.279***	4.288***
COMBINED	4.703***	4.723***

* P less than 0.05

*** P less than 0.001

The increase in score represents the effect of health education. From Table 2 the V standard students gained an average of 14.333-7.205 or 7.128 points after listening to the health education talk and 11.900-6.200 or 5.700 points after studying the pamphlet. Comparing these two values using the t-test gave a "t" value of 1.535; therefore the increase in mean score for the health education talk showed a upward trend but not to a significant level.

Table 5. Result of t- test comparing the increment in score between the two health education methods

H E TALK	vs	PAMPHLET
V Standard	1.535	
IX Standard	2.780**	
Both Groups Combined	2.988**	

** P less than 0.01

The IX standard students, however, gained an average of 15.75 - 8.525 or 7.225 points by the health education talk method which is higher than the mean gain obtained by studying the pamphlet i. e. 15.128 - 9.974 or 5.154 points. The t-test for these figures gave a value

of 2.780 ($P < 0.01$). This illustrates the fact that in the IX standard students in this study, the gain (increment in mean score obtained) was significantly greater after the health education talk than after pamphlet study.

Final health education level attained after health education (as indicated by the post-test mean score) was found to be higher in the group of students given health education talk than in the group of students who had to study pamphlets only.

The V Standard students attained a significantly greater level after listening to the talk than by studying the pamphlet ($P < 0.01$) Table 6.

Table 6. Results of t-test comparing the post-test mean score for the two methods of H.E

H E TALK	vs	PAMPHLET
V Standard	2.914**	
IX Standard	1.335	
Both Groups Combined	3.022**	

** P less than 0.01

DISCUSSION

The contents of the health education given for both the test groups viz the health education talk and the pamphlet study are the same. However, that given to the V Standard group, is simpler in presentation and language.

Though the contents are similar in essential points, the health education talk involved two-way communication and some clarification on certain points raised by the students at the end of the talk.

When the increment in knowledge after the health education i. e. the difference between the mean post-test score and the mean pre-test score, was analysed (Table 5), the combined group gave a significant gain in score after the health education talk than after the pamphlet study. When the students are subgrouped

according to the school educational standards, the IX standard group's gain for the health education talk was significantly ($P < 0.01$) greater than that for the pamphlet study, whereas the V standard group gave a higher but not significant gain. This finding is as expected since students with a better and higher educational background level can achieve a better gain in knowledge.

When pre-test scores which reflect the health education level already possessed by the students before the health education, were analysed the health education level already possessed by the IX standard students was found to be higher than the V standard students. This is probably due to the fact that the IX standard students have a wider general knowledge (including health knowledge) and a greater likelihood of experiencing the actual diseases (rheumatic fever and rheumatic heart disease) themselves or of being acquainted with friends having such a disorder.

The health education talk method of disseminating health knowledge was found to be significantly better than the pamphlet study method when all the students were taken as a whole (Table 5).

But when the students are subgrouped according to the school educational standard it was found that the V standard group attained a significantly higher level of health education after the health education talk method than after studying the pamphlet. The IX standard group also had a higher post-test mean score (indicating the level of attainment of health education after the health education) but the level attained by the talk and by the pamphlet study was significantly different although that obtained by the talk was greater. This may probably be explained by the fact that the V standard students needed more explanation and clarification during the talk sessions in order to understand and remember the facts presented for dissemination of health knowledge, which was lacking in the pamphlet study method.

From this study, it is noted that health education talk method is significantly better than pamphlet study method. This is as expected because the most effective way of communication with people is to talk with them. In health education talks, we have many opportunities to talk with people.

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Experimental amoebiasis in mice immunosuppressed with cyclophosphamide injection and whole-body irradiation

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Mice were infected intracaecally with two strains of *Entamoeba histolytica* after 48 hr of cyclophosphamide injection and whole-body sub-lethal X-radiation. An overdose of ether was used to sacrifice mice at 10 days after inoculation of amoebae and their caecum and livers were examined for degree of infection. Only few caecal ulcerations and no hepatic lesion were observed in control mice but ulcerations in caecum and marked lesions in livers were found in mice receiving cyclophosphamide injection and whole body sub-lethal irradiation prior to inoculation with amoebae. Cyclophosphamide injection of 200 mg/kg/ 2 days was found to be a better immunosuppressive agent for having experimental amoebiasis than whole-body sub-lethal X-radiation of 700R.

INTRODUCTION

Several techniques have been employed for the experimental hepatic amoebiasis in laboratory animals, particularly that resulting from inoculation with *Entamoeba histolytica* obtained from human sources. Early attempt to infect rats, rabbits, and guinea pigs were seldom successful but in the investigations of Goodwin, Hoare and Sharp (1) and Jones (2), the rats have been found susceptible to short-term infection. Carrera and Faust (3), Sohler and Peleux (4) and Taylor, Greenberg, Highman and Coatney (5) also succeeded to produce amoebic dysentery in guinea pigs by intracaecal inoculation with trophozoites. There have also been many methods developed for the production of amoebic liver abscess in hamsters (6, 7, 8, 9, 10) but only one has partially succeeded to produce amoebic liver abscesses in mice (11). The present paper revealed a high production of amoebic liver abscesses in mice by using immunosuppressive agents like cyclophosphamide injection and X-radiation.

MATERIALS AND METHODS

Experimental animals

4-6 week-old c3H/mg strain of inbred mice weighing between 15-20 gram were used throughout the experiment. Each group consisted of 10 mice for every experiment.

Method of immunodepression

One group of mice was given sterile saline injection 0.1 ml/day for two days; second group received cyclophosphamide injection (ENDOXANA : Ward Beckinsop Pharmaceutical Ltd., Brecknell Berks, United Kingdom) at a dose of 200 mg/kg body weight, intraperitoneally in two divided doses just 48 hr before the inoculation with *E.histolytica* trophozoites. Another group of mice received whole-body X-radiation (700R) at Middlesex Hospital, London. The whole body irradiation was done 48hr prior to amoebal inoculation.

Culture of amoeba for inoculation

The Biswas and Wheatle isolates of *E. histolytica* grown in Robinson's (12) medium with associated bacteria obtained from Dr. D. C. Warhurst of London School of Hygiene and Tropical Medicine, London, England, was used throughout the experiment. The amoebae were grown in Robinson's (12) medium until a healthy growth was obtained. The isolates were then transferred into 100 ml Erlenmeyer flasks containing 25 ml of equal volumes of completed medium BRS and 0.05 M phosphate diluent pH 6.5. Approximately 150 mg of starch with bacto-peptone and erythromycin approximately 0.5 ml each was added. A healthy growth was obtained after 48 hr of incubation at 37°C.

Infecting inoculum

Deposits from culture flasks were concentrated by centrifugation at 900 rpm x G for 5 minutes. Upper portion of supernatant was discarded to increase the population of amoebae in culture. The number of amoebae per ml was counted by using Neubaur Brightline counting chamber after adding equal amount of Lugol's iodine. On some occasions a direct counting was made in addition to the counting chamber method. A 0.02 ml of the suspension was taken on glass slide, covered by 22 x 22 mm coverslip, and sealed with parafin wax. This was then counted under microscope (100 X magnification) and multiplying the number counted by 50 represented the approximate number of amoeba per ml.

Site of inoculation

The mice were fasted for 24 hr before inoculation with amoebae. After laparotomy under light ether anaesthesia, 0.4 ml of culture containing approximately 40,000 unwashed active trophozoites of *E. histolytica* was inoculated intracaecally (5). The needle was inserted at the base of the caecum and inoculum was injected towards the caecal apex. Mice were injected randomly one from each group.

The abdominal wound was closed with Michel clips, and a small amount of vaseline was applied on the wound. The application of vaseline was continued until it is healed.

Examination at autopsy

Table 1. Caecal scores given by the method of Neal (13)

WALL:	Normal	0
	Slight thickening	1
	Marked local thickening and contraction	2
	Extensive thickening and contraction	3
	Caecum shapeless, extensive ulceration with abscess formation	4
CONTENTS:	Normal	0
	Slightly less solid than normal	1
	Slightly mucoid	2
	Mucoid, some solid matter present	3
	No solid matter, white or yellow mucus only	4

Mice were killed with an overdose of ether at 10 days after inoculation with amoebae. The caecum was removed after clamping the proximal end of the ascending colon and the distal end of the ileum. The caecum was opened longitudinally and the appearance of the caecal wall and contents were examined and the findings were given one of the five scores according to the method of Neal (13) (Table 1). A saline smear of the caecal contents was made with a drop of calf serum and fixed for staining with haematoxylin. The content of the caecum was also cultivated in Robinson's medium for further examination. The liver of every infected mouse was examined for hepatic lesions. The sternum of the animal was opened carefully and the liver was taken out from the base, blotted on a filter paper and examined for lesions and abscesses; if present, portions were fixed in 10% normal saline for histological study. Sections were cut at a thickness of 5 µ and stained with haematoxylin-eosin for further examinations.

RESULTS

Table 2. The effect of immunodepression in experimental amoebiasis in mice

GROUP	Type of Immunodepression	Strains of <i>E. histolytica</i>		Average Caecal Scores			
		BISWAS	WHEATLE	BISWAS		WHEATLE	
				Wall	Contents	Wall	Contents
A	CYCLOPHOSPHAMIDE 200 mg/kg/2 days	10/10 ^a	8/10	3.4 ^b	3.2	2.8	2.4
B	IRRADIATION 700K/mouse	9/10	7/10	2.7	2.3	2.0	1.7
C	NOT IMMUNODEPRESSED	6/10	4/10	1.5	1.3	0.7	0.6

^a Number of mice infected/Number of mice inoculated

^b Mean values

The effects of cyclophosphamide injection and irradiation in the experimental amoebiasis is shown in Table 2. It was found that the degrees of infection vary with the methods of immunodepression and the strains of amoebae used for infection. In all the groups inoculated with Biswas strain, the average caecal scores of the walls were 3.4 in group A receiving cyclophosphamide injection, 2.7 in group B having irradiation and 1.5 in group C controls. Again, the average caecal scores of the contents followed the same pattern with 3.2 in group A, 2.3 in group B, and 1.3 in group C. It was found that cyclophosphamide injection of 200 mg/kg for two days gave a higher degree of susceptibility to amoebic infection than whole-body irradiation at 700 R. The same effect of immunodepression was also observed in another three groups of mice inoculated with Wheatle strain. The average caecal score of the walls and contents were 2.8 and 2.4 in group receiving cyclophosphamide injections, 2.0 and 1.7 in irradiated group, 0.7 and 0.6 in non-immunodepressed controls respectively. According to these results Biswas strain seems to be more virulent than the Wheatle strain. The statistical difference between the two

strain's infectivity was highly significant at $P < 0.01$. In the caecal sections examined, superficial ulcerations of the mucosa were seen. No lesions were detected which penetrated the muscle layers. An increase in number of mucus secreting goblet cells was noted in the infected caeca. Amoeba were detected in the mucoid debris overlying the ulcerations. In both the immunodepressed groups, a prominent feature was the scaly cellularity of the lamina propria.

The occurrence of hepatic lesions in immunodepressed and control mice inoculated with two strains of *E. histolytica* is shown in Fig 1. The percentages of hepatic lesions by two methods of immunodepression and two strains of amoebae inoculated were very variable. In all non-immunodepressed control mice, livers appeared normal. However, there were marked lesions occurred in the livers of the immunodepressed mice. By looking at the groups receiving cyclophosphamide injections, hepatic lesions were observed in 8 out of 10 mice infected with Biswas strain, and 2 out of 8 mice infected with Wheatle strain. Again in the whole-body irradiation, hepatic lesions were observed in 6 out of 9 infected with Biswas strain,

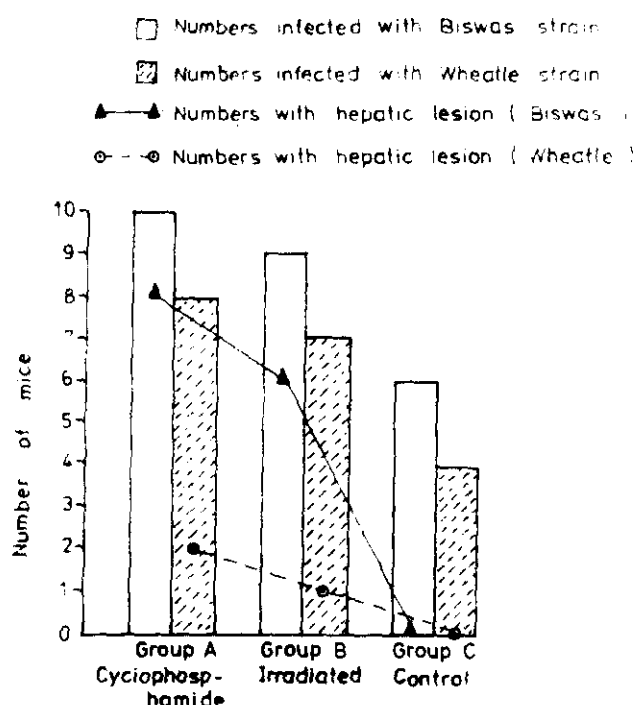


Fig 1 - The occurrence of hepatic lesion in immunodepressed and control mice infected with two strains of *E. histolytica*

and 1 out of 7 infected with Wheatle strain. The lower percentages of hepatic lesions in mice infected with Wheatle strain could be presumed that this strain possesses a lesser virulency. In 5 out of 8 mice infected with Biswas strain, lesions were focal, well demarcated, creamy white areas scattered irregularly on the liver lobes, varying from 0.2 to 0.5 cm in size. In all the rest of the lesions were much more extensive, occupying about $\frac{1}{4}$ of the total liver space. The lesions were mostly sub-capsular and cut-surfaces were firm. The sub-capsular nature of the lesions was evident although focal lesions were also seen scattered in the liver substances. The whole liver showed gross thickening with proliferation of mesothelial cells on fibroblasts. Most of the amoebae were seen at the edges of the lesions surrounded by a clear zone.

DISCUSSION

Teodorovic, Ingallis and Greenberg (14) have found that administration of corticosteroids result in marked exacerbation of pathology in animals with amoebiasis. Wijesundra (11) has also reported that the injection of cyclophosphamide at 200 mg/kg/2 days depress the immune mechanism of experimental mice and make susceptible to *E. histolytica* infection. She has also observed a high degree of caecal scores and hepatic ulcerations in mice receiving cyclophosphamide injections. The effect of wholebody irradiation in experimental amoebiasis has not been reported so far but Hopkins and Zajac (15) found that sub-lethal irradiation of 550R delay the rejection of *Hymenolepis diminuta* by immune mice. Gadeberg, Rhodes and Larson (16) have also demonstrated that whole-body irradiation of 500R and cyclophosphamide injection of 100mg/kg decrease the phagocytosis capacity of mouse macrophages *in vitro* and reduce the ability of PE cells to degrade ^{125} I-labelled HSA-antibody complexes.

In the present study it was found that the condition of immunodepression caused by cyclophosphamide injection was more severe than the effect of whole-body irradiation at 700R in mice. The significant differences ($P < 0.01$) in susceptibilities of two strains of *E. histolytica* proved that cyclophosphamide injection of 200 mg/kg for 2 days was more potent than whole-body irradiation of 700R.

The most promising finding in the present experiment was the liver lesions seen in the immunodepressed mice. Ten out of 18 infected mice injected with cyclophosphamide and 7 out of 16 infected mice treated with irradiation showed hepatic lesions on the 10th day after amoebal inoculation. This finding also supported the study of Wijesundra (11) who, surprisingly for the first time,

found the liver lesions in five immunodepressed mice infected with Biswas strain. There was no such experiment except the study of Wijesundra (11), reported any records on hepatic lesions in mice infected intracaecally with trophozoites of *E. histolytica*. Even with intrahepatic or intraportal inoculation with strains of *E. histolytica*, induction of hepatic lesions in normal rats and guinea pigs has not been successful (17,18,19,20). Therefore it was reasonable to accept that there is an inherent resistance in many animals preventing extraintestinal invasiveness of *E. histolytica*, which in this study, had apparently reduced by the injections of cyclophosphamide and whole-body irradiation. Since the mode of changes for these situations was not clear yet, the results so far suggest several lines of future investigations into immunosuppression-infection-interaction.

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Description of currently unknown larval and pupal forms of

Aedes (Aedimorphus) nigrostriatus

(Barraud), 1972

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During routine mosquito collections in a rice cultivation area near Yangon, one blood fed *Aedes (Aedimorphus) nigrostriatus* female was collected. It was allowed to oviposit and a colony was raised. Adult specimens, male terminalia slides, pupal and larval skin slides were prepared and preserved in the Department of Medical Research (DMR) collections. The morphological characters of the pupa and larva, currently unknown, are described in detail.

INTRODUCTION

Survey teams from the Medical Entomology Research Division, Department of Medical Research (DMR), conducted routine mosquito collections in different ecological areas to monitor anopheline fauna and also to collect mosquito specimens for taxonomical study and for preserving in DMR collections. Routine mosquito collection was carried out during November at Kyauk-ain village, Hle-gu Township, Yangon Division. This village is in a paddy-cultivating plain area about 25 miles north of Yangon and is situated about one mile west of the Yangon-Mandalay highway. During the time of survey, the paddy fields were no more flooded but small muddy pools still existed in and near the paddy fields. Small seepage streams were also found near the mosquito collection site. Mosquito collection was done by cattle-baited big bed-nets (11' x 11' x 6'), the net being operated near swampy paddy field from dusk till 0430 hours the next morning. The mosquitoes collected were transferred to paper cups and transported back to Yangon in ice chests.

PROCESSING THE SPECIMENS

On separating the mosquitoes collected, one golden-scaled female mosquito, fully fed with cattle blood, was found. This female was held separately in a beaker and allowed to oviposit. After the first oviposition, another blood meal was given and second oviposition was obtained. The larvae were reared in plastic trays each containing one litre of pond water. First and 2nd instars were given powdered mouse chow while 3rd and 4th instar larvae were fed on DMR larval food consisting of Casein, corn flour, Ox liver and vitamin B-complex. From the colony raised, adult specimens, male terminalia specimens, larval and pupal skin slides were prepared and preserved according to the method of Ramalingam (1).

IDENTIFICATION AND DESCRIPTION

On detail examination of the specimens and slides, it was identified as *Aedes (Aedimorphus) nigrostriatus* (Barraud), 1927 mainly from its distinctly characteristic male terminalia morphology as

described by Reinhart(2). Adult specimens, male terminalia slides and aquatic stage skin slides were sent to Dr. Ralph Harbach and Mrs. Rampa Rattanathrikul of Armed Forces Research Institute for Medical Science, Bangkok for examination and our identification was confirmed. The adult male and female morphology have been described in detail, with illustrations, by Reinhart(2) and also by Barraud(3). However, Reinhart stated that the larval and pupal forms are unknown. This fact was also confirmed by Ralph Harbach (personal communication).

The morphology of larval and pupal stages are described in this paper after detailed examination of 13 larval skin slides and 12 pupal skin slides. The specimens were all preserved in DMR collections.

PUPA

Chaetotaxy as figured and recorded in Figure 1.

Cephalothorax

1-C single, long, slender; 2-C 2-3, usually 2 branches, long, slender; 3-C 1-3 branches; 4, 5-C 2-3 branches; 6-C single, short; 7-C 2-3 long branches; 8-C normally 2-3 branches; 9-C 1-2 long branches.

Trumpet

Index 5.5, width almost uniform throughout its length, lightly pigmented, integument of trumpet having appearance of scales.

Metanotum

10-C 1-2 very long branches; 11-C usually single, long; 12-C 2-4 branches, normally 2.

Abdomen

Segment I :

Float hair 1-I 13-19 main branches each dividing further into 3-5 branches; 2-I single, stout at base and tapering to a fine point; 3-I single, long; 4,5-I 3-5 branches; 6-I single, long; 7-I 2-3 branches, long; 10-I single, fine (observed only in one slide).

Segment II :

0-II single, very fine; 1-II 4-6 branches; 2-II single, small; 3-II single, very long; 4,5-II 1-3 branches, usually 2; 6-II single, long; 7-II 1-3 branches, usually 3; 8,9-II single; 14-II single, very fine.

Segment III :

0-III single, very fine; 1-III 2-3 branches, usually 3; 2-III single, short; 3-III single, very long; 4-III 1-3 branches; 5-III 2-3 branches, usually 2; 6-III single, long; 7,8-III 1-2 branches; 9-III single, small; 10-III 1-2 branches, usually 2; 11-III single; 14-III single, very fine.

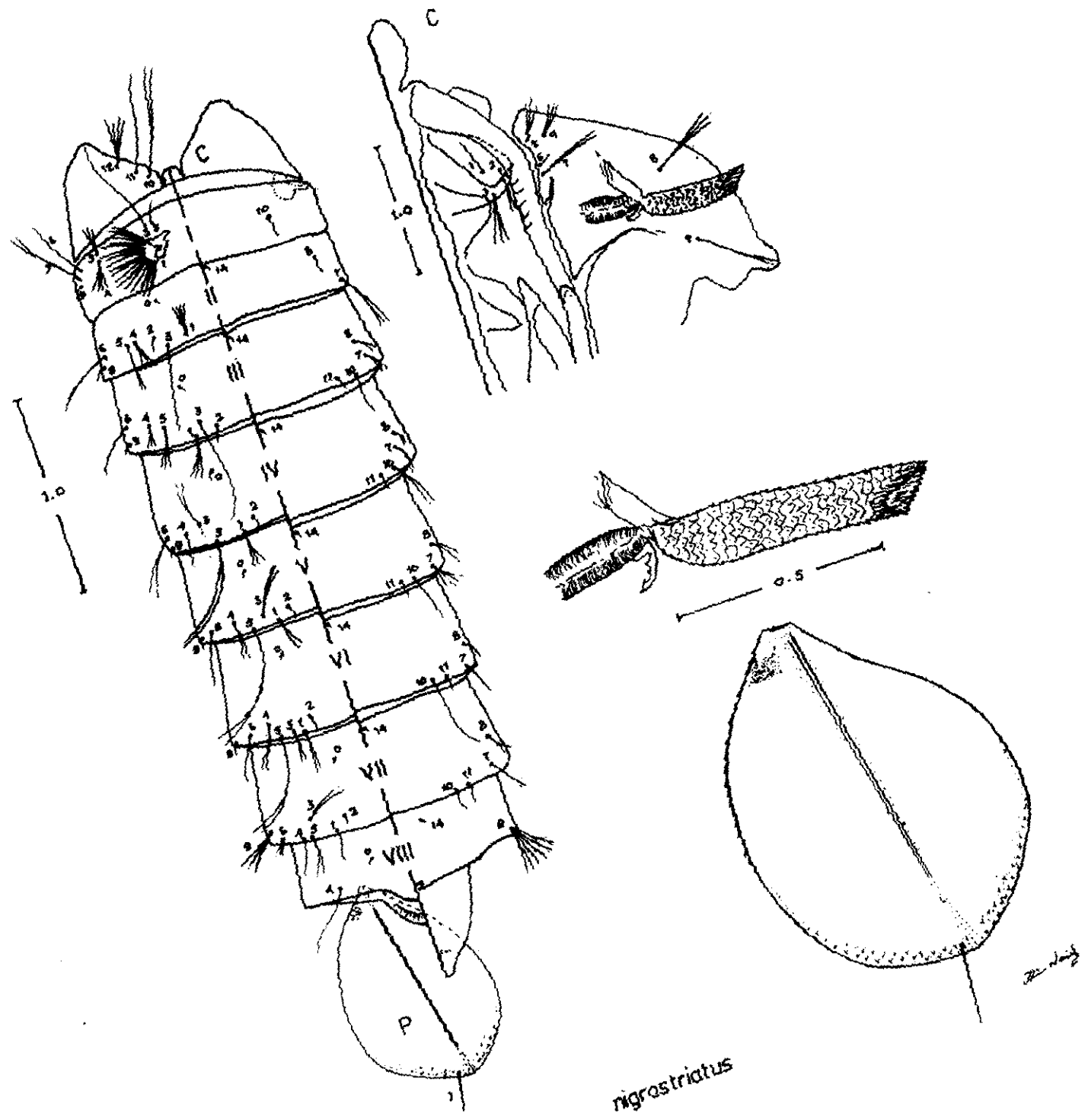
Segment IV :

0-IV single, very fine; 1-IV 2-3 branches; 2-IV single, fine; 3-IV 2-4 branches; 4-IV 1-2 branches, fine; 5-IV usually 2, very long branches; 6-IV 1-2 branches, long; 7-IV 1-2 branches; 8-IV 1-3 branches; 9-IV single, small; 10-IV 1-3 branches, long; 11-IV single, fine; 14-IV single, very fine.

Segment V :

0-V single, very small; 1-V usually 2 branches; 2-V single; 3-V 2-3 branches, usually 2; 4-V 1-2 branches; 5-V 2 very long branches; 6-V single, long; 7-V 1-4 branches; usually 3; 8-V usually 2 branches; 9-V single, small; 10-V single, long; 11-V single, short; 14-V single, very fine.

Fig. 1



nigrostriatus

Segment VI :

0-VI single, very small; 1-VI 2-3 branches; 2-VI single; 3-VI 2-3 branches; 4-VI 1-4 branches; 5-VI usually 2 very long branches; 6,7,8-VI 1-2 branches; 9-VI single, small; 10-VI single very long (much longer than 10-V); 11-VI single; 14-VI single, very small.

Segment VII :

0-VII single, very fine; 1-VII 1-2 branches; 2-VII single; 3-VII 2-3 branches; 4-VII 1-2 branches; 5-VII 2 branches; 6-VII 1-3, usually 3 branches; 7-VII single, long; 8-VII 2 branches; 9-VII 2-3, usually 3 branches, slightly pigmented and strong; 10,11-VII single; 14-VII single, very small.

Segment VIII :

0-VIII single, very small; 4-VIII 1-2 branches, long; 9-VIII 5-7 strong, pigmented and barbed branches, usually 7; 14-VIII single, very small.

Paddle

Oval shaped. Very lightly pigmented near the base, rest of the paddle clear. Distinct mid-rib present but does not reach tip of paddle. Paddle Index 1.35. Paddle fringe without fringe teeth or filaments. 1-P single, pigmented and stout. Conspicuous spicules present in irregular rows on the apical part of the paddle.

Larva :

Chaetotaxy as figured and illustrated in Figure 2.

Head:

Width of head about 1.1 of its length. Mental plate without prominent median tooth and with about 10-11 teeth on each side. Hair 1-C single, stout, pigmented and slightly curved inwards; 3-C single,

very small; 4-C 2 main branches dividing into 12-14 fine branches; 5-C 8-9 long branches with numerous fine barbs; 6-C 6-8 long branches with numerous fine barbs; 7-C 11-13 long branches with numerous fine barbs; 8,9,10-C 2 branches; 11-C 5-8 small branches, usually 5; 12-C 7-8 branches; 13-C 3-5 long branches, usually 5; 14-C single, stout and spinous; 15-C 2-4 branches, usually 4.

Antenna:

Length about 0.49 mm, curved inwards, strong spicules present on shaft. Hair 1-A arising about 0.4 from base, 5-7 usually 7 long, finely-barbed branches reaching to tip of antenna. Other antenna hairs as illustrated.

Thorax:

Prothorax

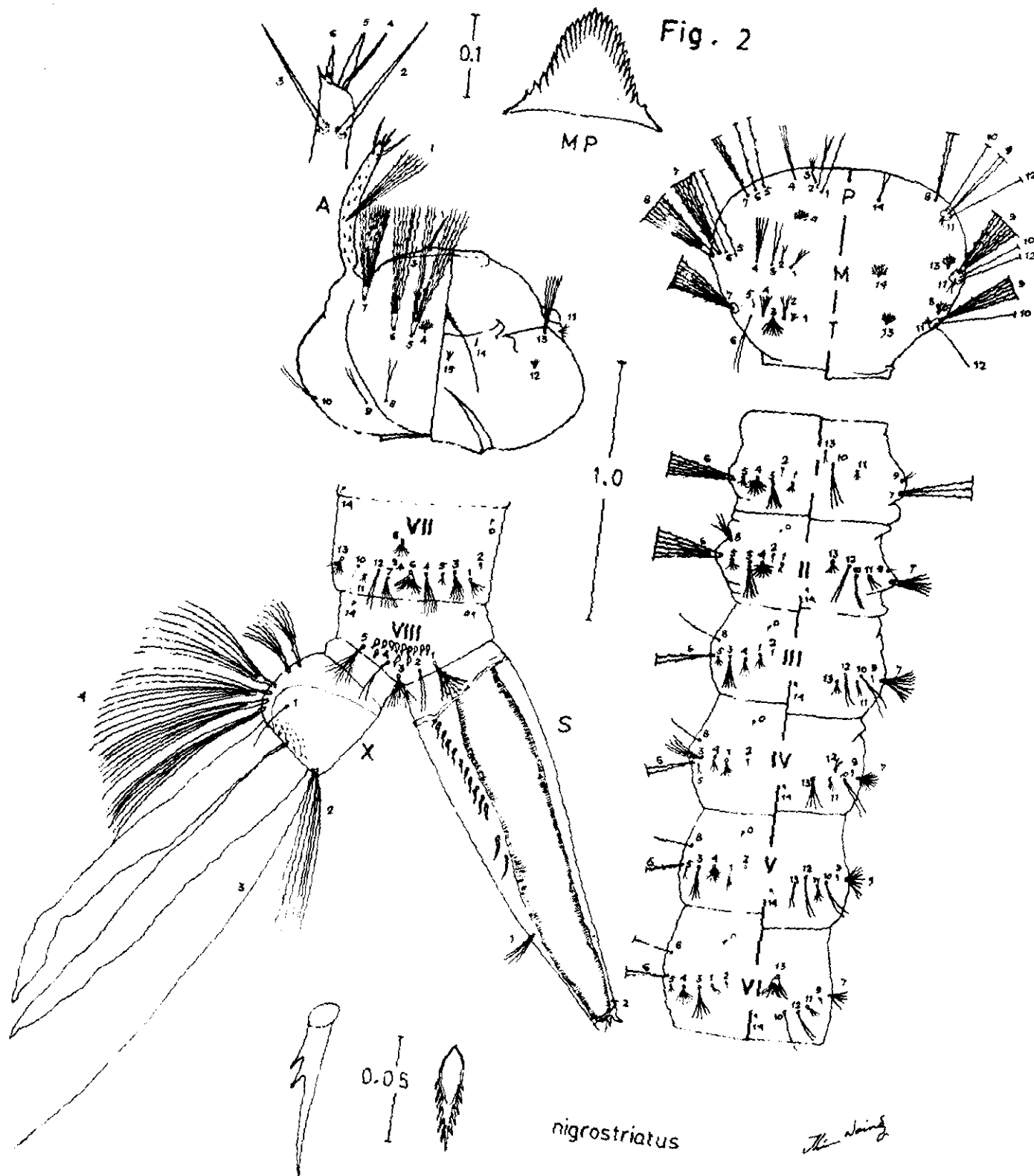
Hair 0-P 13-14 branches; 1-P single, long; 2-P 2 branches; 3-P 3 branches; 4-P 3 long branches; 5,6-P single, very long and finely-barbed; 7-P 3 branches, very long and finely-barbed; 8-P 2 long, finely-barbed branches; 9-P 2 long branches; 10,12-P single, long; 11-P 3-4 small branches; 14-P 2 branches.

Mesothorax

Hair 1-M 3 branches; 2-M 2 small branches; 3-M 1-2, usually 2 long branches; 4-M 4-5 branches, usually 4; 5-M single, long and finely-barbed; 6-M single, long; 7-M 5-7 branches, long and finely-barbed; 8-M 6-9 branches, long & finely-barbed; 9-M 6-8 branches, long and finely-barbed; 10,12-M single, long and with very fine, sparsely distributed barbs; 11-M 2 small branches; 13,14-M dendritic, 3-4 main branches subdividing into 20-24 branches.

Metathorax

Hair 1-T 3 small branches; 2-T 4 branches; 3-T 10 branches; 4-T 5 branches; 5-T single, very small, spinous; 6-T 2 branches, long; 7-T 10 branches, very long and with dense, fine barbs; 8-T



3 main branches subdividing into about 20 branches; 9-T 6 branches, very long, finely-barbed; 10-T single, very long & stout; 12-T single, very long, filamentous; 11-T 3 very fine branches; 13-T dendritic, with 2 main branches.

Abdomen:

Segment I :

1-I 4 small branches; 2-I single, small; 3-I 4-5 branches; 4-I 8-10 branches; 5-I 5 branches, rather small; 6-I 6 very long branches with dense fine barbs; 7-I 2-3 long branches with fine barbs; 9-I 2 small branches; 10-I 2,3 long branches, usually 3; 11-I 3-4 small branches, usually 4; 13-I 2 branches.

Segment II :

0-II single, very fine; 1-II 2-3 branches; 2-II single, short, spinous; 3-II 5 branches; 6-II 5-6 long branches with barbs; 7-II 4-9 branches, usually 6, with very fine sparsely distributed barbs; 8-II 2-3 branches; 9-II usually single, small; 10-II 1-2 long branches; 11-II 3 branches; 12-II 1-2, usually 2 long branches; 13-II 4-5 branches; 14-II single, very fine.

Segment III :

0-III single, very fine; 1-III usually 5 branches; 2-III single, small and spinous; 3-III 4-7 branches; 4-III 4 branches; 5-III 3-4 small branches; 6-III 2-3 very long branches with fine barbs; 7-III 10-12 branches; 8-III single, long; 9-III single, small and spinous, slightly curved; 10-III 2-3 long filamentous branches; 11-III 2-4 short branches; 12-III 2-3 long branches; 13-III 2-4 short branches; 14-III single, very fine.

Segment IV :

0-IV single, very fine; 1-IV 5-6 branches; 2-IV single, small & spinous; 3-IV 5 branches, long; 4-IV 3 branches; 5-IV 2-3 short branches; 6-IV 2 very

long branches with fine barbs; 7-IV 12-14 branches; 8-IV single, long; 9-IV single, spinous; 10-IV 2 long branches; 11-IV 2-4 short branches; 12-IV 2 short branches; 13-IV 4 long branches; 14-IV single, very fine.

Segment V :

0-V single, very fine; 1-V 2-3 branches; 2-V single, small and spinous; 3-V 2-3 branches; 4-V 5-8 branches; 5-V 2-4 short branches; 6-V single, long and with fine barbs; 7-V 10-11 branches; 8-V single; 9-V single, small; 10-V 2-4 branches, long; 11-V 3 small branches; 12-V 2 branches, long; 13-V 2-4 branches, long; 14-V single, very fine.

Segment VI :

0-VI single, very fine, 1-VI 4-6 small branches, usually 4; 3-VI 3-5 branches, long; 4-VI 4-6 branches; 5-VI 3-4 small, short branches; 6-VI 1-2 very long branches, not barbed; 7-VI 8-10 branches; 8-VI single, long; 9-VI single, small & spinous; 10-VI single, long; 11-VI 3-4 short branches; 12-VI 2 branches, long; 13-VI 5 main branches subdividing into over 20 fine branches; 14-VI single, very fine.

Segment VII :

0-VII single, very small; 1-VII 5 branches; 2-VII single, small & stout; 3-VII 5 branches; 4-VII 6 long branches; 5-VII 3 small branches; 6-VII 12-14 branches; 7-VII 3-5 long branches; 8-VII 9 branches; 9-VII 2 very small branches; 10-VII single; 11-VII 3 small branches; 12-VII 2 branches, long; 13-VII 8 branches; 14-VII single, very small.

Segment VIII :

0-VIII single, very fine; 1-VIII 7 branches with fine barbs; 2-VIII 2 long branches; 3-VIII 14-16 branches; 4-VIII 2 branches, long; 5-VIII 6-7 long branches.

Comb Scales

16-28 scales, average 24; scales arranged in 3 nearly regular rows roughly forming a triangle; individual scales pointed apically and with filamentous spines on the lateral margins.

Segment X :

1-X single, long, filamentous; 2-X 7-8 branches, very long; 3-X single, very long (longer than anal gills); 4-X 6 groups of ventral brushes on 6 grids; Saddle - pigmented area covers 0.70 - 0.75 of segment, 3 regular rows of strong, pointed spicules present along posterior margin of segment.

Siphon : - Smooth, moderately long and lightly pigmented; siphon index 4.0; 1-S 4-5 branches, arising from about 1/3 from apex; 2-S single, small and very fine.

Pecten teeth : - 12-17, usually about 13; apical 2 or 3 teeth longer and smooth; separated from the basal teeth which have one or two basal denticles.

Terminology and numbering of hairs used in this description was according to Knight and Laffoon (4) and Knight (5).

Taxonomic Discussion

The adult Aedes (Aedimorphus) nigrostriatus is easily distinguishable by its characteristic markings on scutum, wings and by its golden scales on the abdomen. From the male terminilia morphology, it can also be identified easily by distinctly forked and expanded gonostylus. In the subgenus Aedimorphus, other species like culicinus and mediolineatus have expanded gonostylus which are not forked. On the other hand in species like caecus, orbitae and pallidostratus, gonostylus are forked but not expanded, Reinhart (2).

Larval morphology as observed by us was distinctly different from other species of the subgenus Aedimorphus as described by Reinhart (2). Aedes (Aedimorphus)

pallidostratus was morphologically close to nigrostriatus. However, the shape and width of siphon, number and shape of pecten teeth and the presence of 3 rows of spicules in saddle in nigrostriatus are differentiating points. Also, in pallidostratus the saddle is almost totally covered by chitinous plate.

In the pupal morphology, nigrostriatus can be differentiated from other species in the same subgenus by having only 2 long branches in Hair 10-C, whereas in pallidostratus, orbitae and mediolineatus, Hair 10-C have more than 16 branches. In mediolineatus, pallidostratus and orbitae, Hair 1 of Abdominal segments II and III were found to have more than 18 branches, with the branches in mediolineatus being barbed. In nigrostriatus, Hair 1 of segment II and III was never more than 3-4 branches.

The paddle hair 1-P of nigrostriatus was found to be longer and stouter than other species of subgenus Aedimorphus except orbitae but can be easily distinguished from it by trumpet morphology. The trumpet in orbitae is funnel-shaped whereas in nigrostriatus the trumpet is of uniform width throughout.

ACKNOWLEDGEMENT

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Clinical trial of two Myanmar traditional medicine formulations TMF-03A and TMF-21 for diuretic efficacy

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INTRODUCTION

Some traditional medicine formulations (TMF) as diuretics have been in use to treat anasarca and oedema. These are distributed at the grass-root level without any report of their toxic effects. The aim of this trial is to scientifically evaluate the diuretic potentials of drug formulations - namely, TMF-03A and TMF-21 - using frusemide as positive control.

MATERIALS AND METHODS

The trial was conducted on a total of 40 healthy male volunteers. The traditional medicine formulations were supplied by authorities of Traditional Medicine Council of the Department of Health Services and their ingredients were shown in Table 1. The clinical trial was conducted at the Traditional Medicine Clinical Research Unit, Department of Medical Research, Yangon, from September 1987 to February 1988.

Selection of subjects

All volunteers who wished to participate in the trial were examined medically for relevant diseases, especially renal insufficiencies. Routine examinations of urine were done to exclude renal diseases. Out of them, 40 volunteers were chosen as subjects for this trial, after obtaining the informed consent and setting of the following criteria.

(1) Inclusion criteria:

- (a) sex - male;
- (b) age - 16 to 35 years;
- (c) free from renal diseases.

(2) Exclusion criteria:

- (a) sex - female;
- (b) age - less than 16 and more than 35 years;
- (c) any relevant major organ diseases.

(3) Withdrawal criteria;

- (a) absence for two weeks during the trial;
- (b) any intolerable or allergic condition, e.g., angioneurotic oedema, hypotension, cramps;
- (c) subjects who committed non-compliance anytime during the trial.

Main Trial

Healthy male volunteers, aged 24 ± 5 years, were put under the controlled setting of overnight fasting. Fluid intake was restricted to 250 ml and allowed one standardized meal of 2 slices of bread and butter immediately preceding the drug administration time. Their urine output under these conditions within 4 hours was collected as for the control readings. On the next day the subjects were again put under the similar condition, and the test drug (2 g) or frusemide (40 mg) orally administered according to the pre-determined single-blind (observer blind) schedule. The urine outputs were collected hourly for 4 hours. Urine samples for later electrolytes determination were stored at minus 20 degree Celsius. Urine volumes were measured to the nearest millilitre and the urinary electrolytes (i.e., sodium and potassium) were assayed, using the SP-9/600 Model atomic absorption spectrophotometer (Pye Unicam, England).

At weekly rest interval, the same procedure was repeated, except the subjects administered according to the

the pre-determined cross-over design, until each individual subject had undergone the whole series of the trial. Only a few subjects complained to a minor degree of side-effects, such as nausea, vomiting, cramps, giddiness or allergic manifestations. Such cases were stopped to participate in the trial and prompt and adequate treatment also instituted, where none required major medical intervention. Thirty four subjects completed the trial with the exclusion of 6 drop-outs.

Net increase of total 4-hour urine volume was calculated to estimate the extent of diuretic response, and the diuretic efficacy was expressed in terms of percentage of that of frusemide. Total ionic (sodium or potassium) contents of the 4-hour samples were calculated to evaluate the respective saluretic potential. The findings were analysed statistically using the Student's *t* test between any two groups and the results presented graphically.

RESULTS

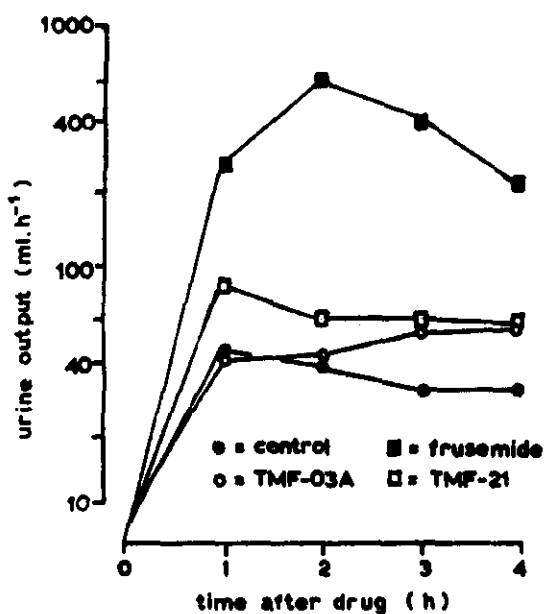


Figure 1. Time course diuretic effects of Burmese traditional medicine formulations TMF-03A and TMF-21 and frusemide on healthy volunteers.

Time course diuretic effects of TMF-03A, TMF-21 and frusemide are illustrated in Figure 1. Peak urine volume were reached within one hour, and the plateau effects maintained for the next three hours. Only the test drug TMF-21 produced noteworthy ($p < 0.002$) diuresis, having a 19% diuretic efficacy of the frusemide (see Figure 2a).

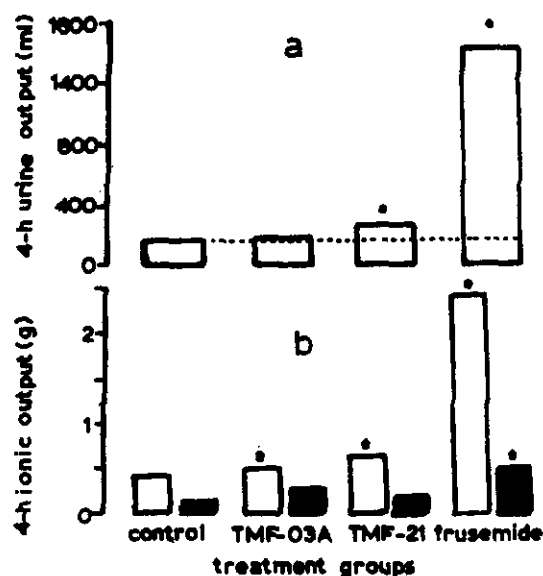


Figure 2. Diuretic and saluretic potentials of TMF-03A, TMF-21 and frusemide.

The upper panel (a) shows diuretic potential of respective treatment if evidence above the dotted line.

The lower panel (b) illustrates ionic excretion of sodium (open column) and potassium (closed column) respectively. Asterisk (*) indicates the difference to that of the control at the $P < 0.002$ of statistical significance level, employing Student's *t* test.

Saluretic potentials of the three drugs employed are depicted in Figure 2b. Either TMF-03A or TMF-21 excreted sodium ions well in excess ($p < 0.002$) of that of the control, but not as potent as frusemide. Urinary excretion of potassium ions was significantly ($p < 0.002$) increased only with the frusemide group, whereas the test drugs produced no changes.

Table 1. Ingredients of Burmese traditional medicine formulations

TMF-03A and TMF-21		
Scientific / common name (part used)	Myanmar name, transliterated	Amount, per cent
TMF-03A		
	<u>Hsar ngarbar say</u>	
1. Ammonium chloride	Zawetthar	20.0
2. Black salt	Hsabok	20.0
3. Magnesium sulphate	Myanmar sakhar	20.0
4. Natural sodium chloride	Theindaw	20.0
5. Synthetic sodium chloride	Hsar yoyo	20.0
TMF-21		
	<u>Hsizay byu</u>	
1. Calcium carbonate	Myebyu	41.2
2. <i>Kaempferia</i> sp (rhizome)	Pan u	20.6
3. <i>Arundo donax</i> (rhizome)	Alokyu	2.9
4. Algae filaments	Yehnyi	2.9
5. <i>Pterocarpus santalinus</i> (wood)	Nathani	2.9
6. <i>Santalum album</i> (wood)	Nathabyu	2.9
7. <i>Cypraea eglantina</i>	Kywaybok	2.9
8. <i>Goniopora stuehbunyi</i>	Kyaukset	2.9
9. <i>Hystrix bengalensis</i> (ash)	Hpyuzu pya	2.9
10. <i>Sepia esculenta</i>	Pinleiyayhmyok	2.9
11. <i>Trigonostegia</i>	Leikkyauksu	2.9
12. Ammonium chloride	Zawetthar	2.9
13. Borax	Letchar	
14. Potassium aluminium sulphate	Kyaukchin	2.9
15. Potassium nitrate	Yanzein	2.9
16. Talc	Kankusan	2.9

The trial recorded four subjects complaining of giddiness due to hypotension, and two subjects of muscular cramp. All are from the positive control frusemide group, where such side-effects are well documented (2). These adverse effects disappeared by the oral rehydration therapy. No other major adverse effects were observed in subjects administered with the traditional medicine formulations.

DISCUSSION

The urine volume was significantly increased by TMF-21 only, whereas TMF-03A produced no changes and thereby disfavoured as a diuretics. TMF-21 was approximately one-fifth as efficacious as frusemide in producing diuresis. Even though the TMF-21 had a lesser diuretic effect than frusemide, it showed to possess the potassium-sparing action, a factor which contributes to

cautious therapeutic application of frusemide. The mechanisms could not be attributable on the basis of potassium ions replacement, because potassium content in the drug formulation was negligible. The mechanism is questionable to the presence of any mineralocorticoids in this multiple-drug formulation—an information not yet available. An added advantage is that the drug did not produce any adverse effects, inherently accompanying those potent diuretics, e.g., frusemide. The study confirmed that, rationally, the TMF-21 could be safely used as an effective diuretic at the traditional medicine hospitals and dispensaries.

ACKNOWLEDGEMENT

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The effect of artemether (Qinghaosu derivative) on the development of plasmodium trophozoites

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INTRODUCTION

Most of the present antimalarial drugs act on schizont stage of parasites. Hence, are known as schizontocidal but their actions may differ from each other. The difference in the action of mefloquine, and qinghaosu on tiny, small and large ring forms of blood trophozoites was reported by Jing-Bo Jiang et al. (1) in 1982 but the site of action of other drugs especially for compound as artemether is not yet known. Hence, this study is done.

PATIENTS AND METHODS ;

The patients who were admitted to Tharyarwadi Civil Hospital during 1986 and 1987 malaria seasons were chosen for study. They were febrile and had positive blood slides for falciparum asexual parasite with counts above 1,000 per cu.mm.

Urine was tested for chloroquine, amodiaquine, sulfonamide and quinine by Wilson & Edeson test, Lelijveld & Kortmann test, Lignin test and Mayer-Tarner reagent test respectively.

Patients with positive reaction to any single antimalarial drug was treated with another drug at standard dose. Only patients who were negative for antimalarial drugs in the urine were treated with artemether (200mg initially followed by 100mg 12 hourly up to total dose 600mg given intramuscularly).

Patients with complications such as jaundice, severe anaemia (PCV less than 30%) and those with cerebral signs and symptoms were not included in the study.

The patients were kept in hospital for 7 days and were followed up on days 14, 21 and 28.

Thick and thin blood films were taken every 6 hours till the parasites were negative and then 12 hourly for 7 days and once at weekly follow-up visits.

The sensitivity of the drugs on the patients were assessed according to the criteria laid down for chloroquine resistance. Of these, patients who are sensitive to respective drugs were finally chosen for study in detail.

From the thin blood smear, stages of development of 200 asexual parasites were assessed as tiny, small and large ring forms as suggested by Jing-Bo Jaing et al. (2) in 1982.

The method suggested is as follows:

- (a) tiny ring form, in which the width of the cytoplasm band is less than half of the diameter of the nucleus; this generally appears within the first 6 hours of the asexual cycle.
- (b) small ring form, in which the width of the cytoplasm band is greater than half of the diameter but less than the diameter of the nucleus; this generally appears within 6 to 16 hours of the asexual cycle.
- (c) large ring form, in which the greatest width of the cytoplasm band is equal to or greater than the diameter of nucleus; this generally appears within 16 to 26 hours of asexual cycle.

The results were expressed as percentage of the tiny, small and large forms of parasites seen.

RESULTS

Six patients who were sensitive to Chloroquine, nine to Amodiaquine, six to Sulphadoxine pyrimethamine, ten to Quinine and ten who were sensitive to artemether were studied.

Among the 6 patients who were sensitive to chloroquine, the percentage of tiny forms of parasites gradually decreased according to the time of treatment until its disappearance from circulation and there was a proportionate increase in small and large ring forms in relation to time of treatment. See table 1 for details.

Table 1. Percentage distribution of tiny, small and large ring forms in chloroquine sensitive cases

Hours of treatment	Tiny Ring form (%)	Small Ring form (%)	Large Ring form (%)
0	74	16	10
6	68	25	7
12	53	27	20
18	35	36	29
24	21	31	48
30	10	28	62
36	12	28	60

This typical pattern of action of the drug on the parasite was also seen in patients treated and sensitive to amodiaquine, sulfadoxine-pyrimethamine, quinine and mefloquine-sulfadoxine-pyrimethamine. The action of sulfadoxine-pyrimethamine was comparatively slower than other drugs.

Among the patients treated with artemether, there was reduction in large ring forms in relation to time of treatment. See table 2 for details.

DISCUSSION

In this study, all the patients who were sensitive to chloroquine, amodiaquine, sulfadoxine-pyrimethamine, quinine and mefloquine-sulfadoxine-pyrimethamine showed a gradual reduction in percentage of tiny ring forms of parasites in circulation in relation to time of treatment. So there could be a blockage

Table 2. Percentage distribution of tiny, small and large ring forms in artemether sensitive cases

Hours of treatment	Tiny Ring form (%)	Small Ring form (%)	Large Ring form (%)
0	72.7	18.6	8.7
6	63.6	28.4	8.0
12	54.6	38.1	7.30
18	53.55	41.00	5.45
24	45.50	50.40	4.10
30	32.0	60.60	7.40
36	37.00	55.67	7.33
42	19.67	77.33	3.00

in release of tiny forms into the circulation. Hence, the action of the drugs was tissue schizontocidal.

Among the patients treated with artemether, there was a gradual reduction in percentage of large ring forms. The blockage is in the interval between the small and large ring forms. Hence, the action of artemether was not only schizontocidal. It might also act on large ring forms of parasite.

Jing-Bo Jiang et al. in 1982 also reported the similar difference on the action of mefloquine and qinghaosu on the different stages of parasite.

Guo-Qiao Li et al. in 1984 reported the effect of mefloquine, qinghaosu, pyrimethamine-sulfadoxine on morphology of malaria trophozoites by this same method. They also claimed that artemether acts early in asexual development cycle when compared to mefloquine or sulfadoxine-pyrimethamine. Mu Mu Sein Myint et al. (3) in 1986 studied the effect of antimalarials on the development of *Plasmodium falciparum* trophozoites. Their study shed some light on the action of artemether, quinine, sulfadoxine-pyrimethamine, chloroquine and amodiaquine in three different ring forms of parasites. But

definite conclusion could not be drawn because some cases which were resistant to antimalarial drugs were included in the study. They suggested that the study should be extended.

Our group (4) in 1987 has also reported similar action of chloroquine and amodiaquine on the patients who are sensitive to the drug. We also reported that there is a difference in the percentage distribution of tiny, small and large forms during various period of treatment between sensitive and resistant cases.

Thus, it may be claimed that the action of artemether is different from other antimalarials. It acts on late trophozoites more precisely on small and large ring forms.

In our study on individual patients, in 5 out of 10 patients treated with artemether, there was an increase in tiny ring forms in circulation between 12 to 24 hours of treatment. This may be due to lack of action of this drug on the schizont.

ACKNOWLEDGEMENT

The authors wish to thank the gift of artemether from Chinese sources.

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Isolation of salmonellas from stool, foods, flies and currency notes

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INTRODUCTION

In Myanmar, *Salmonella* spp. is responsible for 0.9% of acute diarrhoea in children (1). Children's foods may be contaminated by flies and fingers and this is probably one of the important ways by which diarrhoeal pathogens including *Salmonella* are transmitted. Since currency notes are handled by many people daily they are liable to get contaminated. These contaminated money may in turn contaminate fingers of other people who handle them and act as a potential vehicle for disseminating *Salmonella* and other pathogens to susceptible persons. This paper attempts to show that the *Salmonellas*, which were found in children's stool and foods may also be isolated from flies and currency notes.

MATERIALS AND METHODS

Children's stool samples were collected from children under 5 years old admitted in Yangon Children's Hospital during July 1985 - September 1985. They were inoculated on *Salmonella Shigella* (SS) media and enriched in Selenite-F (SF) media. Then *Salmonellas* were isolated

by standard procedures.

Flies were captured alive in cages from animal pens, bazaar, kitchens, latrines, refuse dumps and food stalls during September 1984 to August 1985 in a community in Yangon. They were collected in sterile wide mouth tubes from the cages and anaesthetized by exposure to 0°C. An approximate 10% suspension of a pool of 10 flies were made using 0.9% normal saline (N/S). Then *Salmonella* spp. was isolated from all the samples.

Children's food samples were taken in the mornings from their morning meals. They were collected by field workers in sterile containers after the child's food had been prepared and was just about to be fed to the child. The study was conducted in Yangon, during July to September 1986. 10 gm of these samples was mixed with 90 ml of peptone water and homogenized employing the stomacher lab blender. They were incubated at 37°C overnight as pre-enrichment. Then Selenite-F and *Salmonella shigella* media were used to isolate *Salmonella* spp.

Specimens of one-kyat notes were randomly collected from vendors selling chicken, pork, beef, fish, prawn, fish paste, Myanmar snacks, vegetables and eggs during December 1986 to July 1987 in a local market in Yangon. They were collected in sterile containers in the mornings and transported to the laboratory for processing on the same day. They were put in 1% peptone water, preincubated at 37°C for 4 hrs and then *Salmonella* spp. was isolated by standard procedures.

Serological identification was done to know the *Salmonella* serotypes on all isolates. It was done in the Enterobacteriaceae Section, National Health Laboratory.

RESULTS

Table 1. *Salmonella* isolated from different sources, Yangon, 1984-87

Sources	No. of Samples	No. of <i>Salmonella</i> positive samples	% of <i>Salmonella</i> positive samples
Children's stool	170	11	6.5
Cooked children's foods	779	6	0.8
Flies	186	25	13.4
Currency notes	60	9	15

Salmonella isolations from children's stool, cooked foods, flies and currency notes are shown in Table 1. *Salmonella* were found in 6.5% of children's stool specimens, 0.8% of cooked children's foods, 13.4% of flies and 15% of currency notes samples.

The *Salmonella* serotypes which were isolated from these different sources are shown in Table 2. *Salmonella* spp. such

as *S. derby* was not only found in children's stool and cooked foods but also isolated from flies and currency notes samples. It was predominant in environmental sources but relatively uncommon in children's stool. *S. anatum* was found in cooked children's food as well as in environmental samples. Likewise, *S. enteritidis* which was isolated from children's stool and cooked foods was also found in flies samples. Some of the *Salmonellas* such as *S. krefeld*, *S. paratyphi* B and *S. typhimurium* were only isolated from children's stool.

DISCUSSION

Salmonellas were found in 6.5% of children's stool specimens, 0.8% of cooked children's foods, 13.4% of flies and 15% of currency notes samples. Isolation of *Salmonellas* from children's stool was done for 2 months only and therefore the exact bacteriological picture could not be established. We collected children's food specimens after they had been prepared. So *Salmonella* spp. was isolated from only 0.8% of them. Percentage of *Salmonella* isolation in cooked food is lesser than that in raw food (2).

Table 2. Serotypes of *Salmonellas* isolated from different sources, Yangon, 1984-87

Serotypes	Children's stool (1985)	Cooked children's foods (1986)	Flies (1984-85)	Currency notes (1986-87)
<i>S. derby</i>	1	2	11	8
<i>S. anatum</i>	0	2	5	1
<i>S. agona</i>	0	0	5	0
<i>S. enteritidis</i>	1	2	2	0
<i>S. stanley</i>	3	0	0	0
<i>S. senftenberg</i>	2	0	1	0
<i>S. krefeld</i>	2	0	0	0
<i>S. paratyphi</i> B	1	0	0	0
<i>S. typhimurium</i>	1	0	0	0
<i>S. weltevreden</i>	0	0	1	0

From our study we come to know that flies can transmit many different *Salmonella* serotypes. Ostralenk & Welch in 1942, found that flies may remain infected with *S. enteritidis* for as long as 4 weeks. Thus, flies are one of the sources of enteric pathogens.

Cooked children's foods can be contaminated from utensils and mother's hands which are used in feeding. In our country most of the mothers feed their children by hand, some wash their hands properly with soap and water but the majority do not wash properly. A small inoculum of *S. anatum* is still recoverable from fingers after 3 hrs of contamination (3). So the cooked foods can be easily contaminated through handling during feeding.

The paper money which are handled daily by many people can also carry *Salmonellas*. After a simple soap and water hand wash, organisms are no longer recoverable from the convalescent carriers (4). Thus we have to wash hands after handling them, particularly before food handling or eating.

This study shows that some *Salmonella* serotypes which were found in Children's stool and cooked foods were also isolated from flies and paper money notes. Thus we have to take care not to get contamination of food by flies and to wash hands after handling paper money notes, especially before food handling or eating.

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A preliminary study on knowledge and practices on ascariasis in rural Taikkyi

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INTRODUCTION

Ascariasis has been identified as one of the priority health problems in the third country health programming cycle in 1985 (1). This may be due to occurrence of high prevalence of ascariasis in the community particularly in children and of relatively large numbers of ascariasis cases and its abdominal complications treated in hospitals (2). In developing countries with poor socio-economic status, regular periodic mass chemotherapy is the most effective immediate measure for reducing intensity of *Ascaris* infection and ascariasis morbidity (3). For effective implementation of community based mass chemotherapy against ascariasis in Myanmar in the future, we undertook a preliminary study to determine the extent of knowledge on ascariasis the mothers had, and their practices towards ascariasis control by chemotherapy.

Study area and population

The study was conducted in August 1986 in three villages, In-ga-la-gyi, Gyo-gya-kwin and Chauk-taing-kwe in Taikkyi township, Yangon Division. The study villages were among the 13 control villages (no chemotherapy was given before the study) of the 2-year field research project on the study of the relationship between the control of *Ascaris* infection and child nutritional status, ending in August 1986. The villages were highly endemic for ascariasis, the prevalences being between

79 to 85%. The study population comprised 106 mothers out of the 112, who had children less than 12 years of age from the villages.

Study method

An interview data collection method was used by employing a standardized questionnaire consisting of the following multi-choice questions among others, on knowledge and practices relevant to control of ascariasis by chemotherapy. (a) Do you believe that eating fresh meat and fish and green vegetables can cause ascariasis? (b) Do you believe that summer/rainy season is the best time for deworming children? (c) Do you believe that a life-guardian worm should exist in human body? (d) How frequently do you deworm your child? The interviewers are two medical officers and five graduate technicians from the Epidemiology Research Division, appointed for the USAID-funded ascariasis-nutrition relationship research project. The data were analysed according to educational status of the mothers.

RESULTS

76.5% of illiterate and 65% of literate mothers believed that ascariasis was due to eating meat and fish, and the difference by literacy status was statistically significant ($\chi^2 = 4.588$; $p < 0.05$). On the other hand, only 5.5% of literate and 15.7% of illiterate mothers said that ascariasis was caused by eating green vegetables (see Table), and the difference was also statistically significant ($\chi^2 = 4.303$; $p < 0.05$). The majority of mothers (over 75%) favoured that children should be dewormed in the rainy season, and a few percentage (less than 10%) of mothers, however accepted deworming during the summer. In addition, 85 to 90% of rural mothers believed that there should be a life-guardian worm in the human body.

Percentage distribution of 106 mothers pertaining to knowledge
on ascariasis and practice on chemotherapy

Knowledge/ practice	Measurement scale	Illiterate		Literate		Total	
		No.	%	No.	%	No.	%
Causation of ascariasis							
Eating meat and fish	Yes	39	76.5	36	65.5	75	70.8
	No	2	3.9	11	20.0	13	12.2
	Don't know	10	19.6	8	14.5	18	17.0
Eating green vegetables	Yes	8	15.7	3	5.5	11	10.4
	No	29	56.9	40	72.7	69	65.1
	Don't know	14	27.4	12	21.8	26	24.5
Time for deworming							
Summer	Yes	2	3.9	5	9.1	7	6.6
	No	36	70.6	46	83.6	82	77.4
	Don't know	13	25.5	4	7.3	17	16.0
Rainy season	Yes	39	76.4	46	83.7	85	80.2
	No	6	11.8	7	12.7	13	12.3
	Don't know	6	11.8	2	3.6	8	7.5
Traditional belief on worm							
Need a life-guardian worm in body	Yes	46	90.2	47	85.5	93	87.7
	No	2	3.9	1	1.8	4	3.8
	Don't know	3	5.9	6	10.9	9	8.5
Practice on chemotherapy							
Frequency of deworming	Twice a year	2	3.9	7	12.8	9	8.5
	Once a year	27	52.9	30	54.5	57	53.8
	More than a year	1	2.0	1	1.8	2	1.9
	Only when necessary	12	23.5	9	16.4	21	19.7
	Never	9	17.7	8	14.5	17	16.1

In general, don't -know responses to questions occurred more in illiterate than in literate mothers.

With regard to deworming practice, 50% of both literate and illiterate mothers dewormed their children once a year and seldom dewormed more than once a year.

DISCUSSION

Since this study was undertaken at the end of the main ascariasis-nutrition-relationship study, the team members were so intimate with the village mothers that they were treated as one of them and reliable information were obtained when the mothers were interviewed.

In this study, we were able to quantify the belief that ascariasis was caused by eating meat and fish. This is corresponding to the old Myanmar belief that meaty and fishy nature or smell of food might cause ascariasis. The local population must be explained that not only eating meat and fish cannot cause ascariasis, but also they are required for child nutrition and growth. The knowledge that children should be dewormed in the rainy season and not in the summer may not be epidemiologically sound, although ascariasis cases are mostly admitted to hospitals in the months of May, June and July every year corresponding to the rainy season in Myanmar(4). Since the prepatent period takes about 2 months from the time of ingestion of infective Ascaris egg to the development of mature adult worm, deworming in the rainy season will definitely delay the prevention of ascariasis morbidity. Therefore it is strongly recommended that health education should be given to deworm children before the start of the rainy season if once a year chemotherapeutic regimen is adopted. The peculiar belief that there should be at least one life-guardian worm in human body to guard the existence of life of its host should be corrected by pointing out that

even a single worm could endanger life when it blocked a vital duct. Moreover, health education should include explanation of the life cycle of Ascaris lumbricoides and how man gets the infection.

In fact, children should be dewormed at least twice a year in hyperendemic areas because the mean worm burden of the children reach their precontrol level 12 months after chemotherapy (5), especially to children predisposed to high worm burden(5); from large family size (5,6) or from low socio-economic status (7). Hence a health education programme to correct wrong beliefs and to adopt desire health practices of mothers and other people should be incorporated whenever and wherever a suitable community oriented mass chemotherapeutic program (8) is implemented.

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A prospective study of amodiaquine induced agranulocytosis and liver damage in malaria patients

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INTRODUCTION

Amodiaquine is structurally related to chloroquine. It is effective against some strains of *P. falciparum* which are resistant to chloroquine, but amodiaquine induce agranulocytosis and liver damage has been reported (1,2). Prophylactic use of amodiaquine is also associated with unacceptable risk of neutropenia of one in two thousand (3). 7 cases of hepatitis associated with amodiaquine has also been reported. Resistance to chloroquine is widespread in Myanmar especially in Shan State. The first-line antimalarial drug used in Sao San Tun Hospital, Taunggyi is amodiaquine. In our study of falciparum malaria in pregnancy, we found no significant difference in the response to quinine and amodiaquine in both pregnant and control patients. In another study, we have reported the percentage of chloroquine failure as compared to amodiaquine failure as 48.7% and 20% respectively.

Since amodiaquine is cheap and readily available, this will be the main stay antimalarial in Sao San Tun Hospital and in Shan State. Hence the urgency and importance of the study to establish or dispute the fact that in therapeutic and prophylactic dose, agranulocytosis and liver damage could be induced by amodiaquine, is called for.

MATERIALS AND METHODS

Adult patients age of 12 years and above admitted to medical ward of Sao San Tun Hospital, Taunggyi, with positive smears for asexual forms of *P. falciparum* was studied. Patients with the following clinical condition were excluded from the study.

1. pregnant women.
2. patients with systemic diseases.
3. patients with suspected or confirmed liver diseases or bone marrow depression or history of taking medicines which could produce agranulocytosis.
4. chronic alcoholic with enlarged liver.
5. patients who fit into the category of severe falciparum malaria i.e. parasite count more than 5% of RBCs, cerebral malaria, black water fever, etc.

All the chosen patients were admitted to hospital for minimum of 7 days and were followed weekly for 6 weeks. Amodiaquine was given orally according to WHO recommendation of 25mg/kg total dose divided into 3 days. During follow-up period they were given prophylactic amodiaquine 300 mg weekly to all the patients.

Apart from routine examination of blood film for malaria parasite and blood PCV, the following tests were also done at days 0, 7, 14, 21 and 28.

- blood for complete picture
- blood for platelets count
- liver function test; serum bilirubin, SGPT, thymol turbidity, total protein and albumin/globulin ratio.

RESULTS

Out of 141 patients treated with therapeutic and prophylactic amodiaquine, the following completed the 6-week study.

Group 1: 34 patients who came to follow-up clinic weekly regularly for 6 weeks. Of these, 14 patients relapsed with *P. falciparum* malaria. Raised serum bilirubin was recorded in 6 patients during admission to hospital. Raised level of SGPT was,

recorded in 4 patients but during the day of discharge from hospital and at follow-up visits, the LFT tests and blood picture were all normal.

Group 2: 7 patients who came to follow-up clinic irregularly, but came on last week ie. 6th week of the study. all of them showed no changes in LFT tests and blood pictures during follow-up period.

Group 3: 6 patients who attended the follow-up clinic irregularly but were taking prophylactic amodiaquine. They came to hospital for malaria while they were on amodiaquine prophylaxis.

Group 4: 11 patients who were city dwellers, apart from initial amodiaquine therapy, they had not taken amodiaquine prophylaxis. They showed no abnormal changes in blood pictures and LFT tests.

The platelet count of the patients was reduced in 60% of the patients. But they increased to normal level from third day of hospital admission. They are not associated with bleeding tendencies.

DISCUSSION

In this study, out of 34 patients who had taken prophylactic amodiaquine weekly and regularly, 14 relapsed with malaria. There were also 6 patients who suffered from malaria while they are provided with prophylactic amodiaquine. Hatton et al. (3) has also reported that the prophylactic use of amodiaquine was associated with an estimated risks of severe neutropenia in one in two thousand. Hence, we wish to claim that amodiaquine is not recommended as a prophylactic drug especially at the area with established resistance to chloroquine.

In this study there were a few patients with changes in LFT during admission to

hospital. Such changes in LFT had also been reported by Pe-Than-Myint et al. (4). But all the patients in this study had no changes in blood picture and LFT tests from the day of discharge from the hospital till 6 weeks follow-up period. The fall in the level of platelets count on admission to hospital is a common feature reported in literatures (4). But the level of platelets returned to normal level from the third day of hospital admission. Hence, amodiaquine in therapeutic dose and weekly prophylaxis for 6 weeks may have no harmful effects to the patients.

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Malaria situation in Kowthaung Township, January, 1989

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In January 1989 we had the chance to study the situation of malaria at Kowthaung Township. Kowthaung Township consists of a group of rocky hilly islands, situated at southernmost part of Myanmar with population over 38,000 (50% urban and 50% rural). It has heavy rainfall throughout the year. The main occupation of the local people is fishing in deep sea water and trade with Thailand. There are also people going into forest for extraction of kayin oil and canes. There are three government economic projects going on at the township. They are as follows:

1. Extraction of Timber (Kanyin Phyu Project). Timber is extracted from the multiple small islands by imported personnel from the timber extraction department. Their base is at Ma Thae village. The logs are extracted from the multiple small islands which have very few local inhabitants and loaded to the ships at the same area by skilled workers from Port Corporation Yangon (Total workers population about 600).
2. Timber extraction at Nam Thorn (Lone Phaw Gyi Village). The site is close to Thailand. There were 150 workers from Myanmar and 1000 workers from Thailand. It is separated from Thailand by a river. There were also administrative personnel who came from various parts of Myanmar. They were treated with Mefloquine-Sulfadoxine-Pyrimethamine 3 tablets and 30

mg primaquine if they suffered from malaria by malaria staff from Thailand.

3. Palm Oil Project. This project was started in early 1981. The yebets (convicts from various jails of Myanmar) had to clear the tropical jungle for cultivation of palm oil plants.

There were ten camps distributed in different small islands which are far from each other. They have planted 6423 acres of land and 5872 acres were successful with plants. The plants are now yielding fruits. The yebets, the jail staff and special police force (Lone Htains), total population about 2000-3000, were all imported from Myanmar proper.

Kowthaung has a 50-bedded hospital, one maternal and child-health centre and 4 rural health centres to look after the health of the population. There is also a clinic for the yebets of palm oil project.

According to Kowthaung hospital statistics during the last two years (1987-1988) there were 5023 patients admitted to hospital. Of these 1825 (27%) were due to malaria. There were 140 deaths due to malaria. The mortality rate is 7.9%. Malaria is present throughout the year.

During our stay at Kowthaung Township there were 18 malaria patients admitted to hospital. Of these two were proved to be quinine resistant (one patient was treated with quinine 600 mg tds by infusion for 4 days with no clinical improvement and parasites count was 71,000/cumm on 4 th day. Another patient was also treated with quinine infusion 600mg tds for three days. There was no improvement and parasite count was 86,000/cumm at end of day 3. The serum quinine level of the two patients before the change of drug to mefloquine was 11.2 mg% and 16.4 mg% respectively. We were unable to confirm quinine resistance by in vitro technique because of lack of electricity. But since their serum quinine levels were above MIC level we can assume both the patients were quinine resistant.

Most of the malaria patients of the hospital were from timber extraction department jail, police, yebets and from army and navy. During the week while we were at the hospital there were 4 deaths with signs and symptoms of cerebral malaria. All the patients died within 2-12 hours of admission to hospital.

Sixty six samples of blood collected at night from contacts of hospital patients showed 3 with *Plasmodium falciparum* one with *vivax* and one with *microfilaria*. Three important species of *Anopheles* such as *dirus*, *annularis* and *maculatus* were also collected from the township.

Two hundred and forty-eight samples of blood collected from Shwe Hin Tha ward of the township (population 2400) showed *P. falciparum* 14, *P. vivax* 2, prevalence rate is 5.6%. Quinine sensitivity tests were done on 20 patients by field method (WHO). There were no patients with R_2 and R_3 resistance to quinine.

In summary:

1. Malaria is Hypo- mesoendemic in Kowthaung Township.
2. Most of the patients were imported personnel from Myanmar (so far there were 817 deaths from yebets and 32

from service personnel during the last 8 years at palm oil projects. The main cause of death is probably malaria).

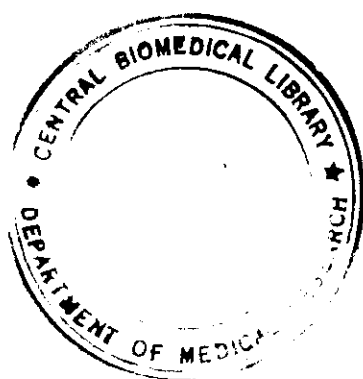
3. There were 2 cases with quinine R_2 and R_3 resistance during our visit to hospital. To reduce the mortality, intramuscular quinine is needed to be given to all patients with complicated malaria before referring to hospital. More injection quinine is needed to be supplied to the staff of Rural Health Centres.

Primaquine, a gametocidal drug has not been used. It is encouraged to use 45 mg primaquine before the patients are discharged from hospital.

For the control of mosquitoes, and selection of camp sites for non-immune migrant project workers technical advice from the medical personnel is needed to be given.

The use of prophylactic drugs is needed to encourage all imported persons.

Parent new drugs such as mefloquine, and Artemether are needed to be supplied for those patients with slow response (resistance) to quinine.



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(a) One author: Khin-Maung-U. In vitro determination of intestinal aminoacid (14-C-L-Glycine) absorption during cholera. American Journal of Gastroenterology 1986;81(7):536-538.

(b) More than 6 authors: Pe-Than-Myint, Mg-Mg-Lay, Aung-Shwe-Saw et al. A comparative study of Artemether (Qinghaosu derivative) and standard anti-malarial drugs in the treatment of falciparum malaria in Burma. Burma Medical Journal 1985; 31(1): 36-43.

(c) Book: Thein-Hlaing. Ascaris lumbricoides infections in Burma. In: Ascariasis and its Public Health Importance. Crompton, DWT, et al (Eds) Taylor and Francis, London, 83-112.

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