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Electrocardiographic effects of quinine and quinidine in the treatment of falciparum malaria

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Quinidine has been suggested as an acceptable alternative to quinine in the treatment of falciparum malaria. To compare the electrocardiographic effects of quinine and quinidine in falciparum malaria, sixty patients with more than 2% of parasitized red cells were chosen and paired as closely as possible. The study was double-blind. 15 mg/kg quinine or quinidine was given as a loading dose infused over 4 hours followed by 2 doses of 7.5 mg/kg base at 8 hourly intervals. Oral quinine or quinidine as 7.5 mg/kg base 3 times/day was then continued till day 7. Satisfactory 12 lead electrocardiograms were obtained from 58 patients on day 0, after 24 hours, on day 3 and on day 7. Overall there was no significant change in PR interval or QRS duration. The pretreatment QTc was 443 ± 30 msec in the quinine group (n=30) and 438 ± 25 msec in the quinidine group (n=28) with no significant difference. Maximum QTc prolongation occurred on the third day in both groups being 481 ± 28 msec in the quinine group and this was significantly less than 532 ± 55 msec seen in the quinidine group. QTc ≥ 550 msec was found in 46.6% in the quinidine group but in only one in the quinine group. T wave flattening occurred in 62% in the quinidine group but in only 25% in the quinine group. No dysrhythmias were recorded in this study but because of the excessive prolongation of the QTc caused by quinidine, we conclude that we should be cautious about the use of quinidine for falciparum malaria.

INTRODUCTION

Quinidine, an optical isomer of quinine was first described in 1848 by van Heyningen and prepared and given its present name by Pasteur in 1853. Quinidine shares all the pharmacological actions of quinine, including its antimalarial, antipyretic and oxytocic effects (1).

Many years ago, both quinine and quinidine were used for treatment of malaria, and it was noted that patients with malaria who also had certain cardiac arrhythmias would occasionally

be cured of arrhythmia by quinidine (1).

The action of quinidine on cardiac muscle was found out to be more intense than those of quinine. Quinidine came to be employed as an antiarrhythmic drug and quinine came to be used mainly for its antimalarial effect. In recent years, the use of quinidine in the treatment of falciparum malaria has been resurrected (2) and claims have been made that it is as effective and as safe as quinine (3).

It is the purpose of this investigation to compare the electrocardiographic effects of quinine and quinidine in the

treatment of falciparum malaria with a view towards detecting possible toxic effects of quinidine on the cardiac muscle compared to quinine.

PATIENTS AND METHODS

Sixty patients with more than 2% parasitized red cells were chosen and paired as closely as possible (Table 1). The study was double blind. 15 mg/kg quinine or quinidine was given as a loading dose infused over four hours followed by two further infusions of 7.5 mg/kg at eight hourly intervals. This is followed by oral quinine or quinidine 7.5 mg/kg three times a day till day 7. Satisfactory 12 lead electrocardiograms were obtained from 58 patients on day 0, after 24 hours, on day 3 and on day 7.

E.C.G.s were recorded at a paper speed of 25 mm/s and were read under magnification. The QT interval was measured from the lead showing greatest T-U dissociation. The mean of 5 measurements was taken and corrected QT interval or QTc was calculated according to the formula

$$QTc = \frac{QT}{\sqrt{R-R \text{ interval}}}$$

E.C.G.s were read without the knowledge of whether quinine or quinidine had been used on each particular case. The code was broken when all E.C.G.s had been read and interpreted.

Students' t test was used to analyse electrocardiographic time intervals.

RESULTS

There was no significant change in the P wave morphology, or PR interval in any of the patients in both groups treated with quinine or quinidine.

Overall, there was no significant prolongation of the QRS duration in either quinine or quinidine treated cases although in 2 of the quinidine treated cases QRS duration was prolonged by 50%.

The pretreatment QTc was 443 ± 30 msec in the quinine group and 438 ± 25 msec in the quinidine group and the difference is not significant. Normal QTc is not to exceed 420 msec in

Table 1. Patient characteristics in quinine and quinidine groups

	Quinine	Quinidine
Initial parasite count	152,000 $\pm$ 253,000/cmm	153,000 $\pm$ 361,000/cmm
Number of patients	30	28
Sex ratio (male:females)	26:4	25:3
Mean age	28.8 $\pm$ 9.6 yr	34.3 $\pm$ 13.8 yr
Mean body weight	47.3 $\pm$ 5.1 kg	48.8 $\pm$ 15.7 kg
Hyperparasitaemia (>5%)	10%	11%
P.C.V. <30%	10 (2 transfused)	11 (4 transfused)
Clinical jaundice	4	10
Raised blood urea	1	2
Initial hypoglycaemia	1	2
Hyperpyrexia > 105°F	1	1
Hypoglycemia	0	0
More than one complication:	9	12

men and 430 msec in women (4). Maximum QTc in quinine treated patients occurred at the end of the third day, 481 ± 28 msec i.e. 8.58% increase over the base-line value and this was significantly less than the maximum QTc prolongation produced by quinidine, 532 ± 55 msec, i.e. 21.55% increase over base-line value. The longest QTc in the quinine group was 550 msec seen at the end of 3rd day in one patient only whereas QTc ≥ 550 msec was found in 46.4% of the quinidine group. QTc of 620 msec was seen in one patient in the quinidine group. However, no significant dysrhythmias were detected in any of the patients in both the groups. T wave flattening occurred in 62% of the quinidine group but in only 25% of the quinine group. (Table 2.3 & Graph 1).

DISCUSSION

Quinidine syncope, commonly due to TORSADE DE POINTES (a form of ventricular fibrillation) appears to occur as a result of both individual suscepti-

bility and excessive plasma concentration. Fatalities from this complication have been reported to occur in 0.5% - 4% (5). Excessive prolongation of the QT interval may herald susceptibility to this complication. However, the actual values that constitute "excessive prolongation" are uncertain and it is regarded that a QTc of more than 550 msec is undesirable and a value of 600 msec or more is an indication to stop therapy. (5).

In this study, longest QTc caused by quinine was 481 ± 28 msec and the longest QTc recorded was 550 msec seen in one patient in this group.

QTc ≥ 550 msec was seen in 46.4% of quinidine treated patients and QTc of 620 msec was seen in one patient.

Although no dysrhythmias were seen in the quinidine group which produced greater prolongation of the QTc, it would be prudent to say that larger numbers of patients would need to be observed to establish that quinidine is as safe as quinine in the management of malaria. The normal range of QT prolongation with therapeutic quinidine levels would also need to be established. Perhaps the effects of QTc prolongation

Table 2. ECG changes in quinine group (n = 30)

	QRS msec	% increase over base line	P value	QTc msec	% increase over base line	P value	T wave flatten- ing
Base line	83 ± 10			443 ± 30			
End of 24 hr	85 ± 10	2.41	N.S	468 ± 39	5.64	< 0.02	
End of 3rd day	86.6 ± 10	3.16	N.S	481 ± 28	8.58	< 0.001	25% of cases (8)
End of 7th day	88 ± 10	5.68	N.S	460 ± 28	3.84	< 0.02	

N.B. QTc longest 550 msec in one patient (Day 3)

Table 3. ECG changes in quinaldine group (n = 28)

	QRS msec	% increase over base line	p value	QTc msec	% increase over base line	p value	T wave flattening
Baseline	84 ± 9			438 ± 25			
End of 24 hr	90 ± 12	7.14	N.S	504 ± 40	15.07	< 0.001	
End of 3rd day	94 ± 13	11.9	N.S	532 ± 55	21.55	< 0.001	62% of cases(18)
End of 7th day	93 ± 11	10.71	N.S	501 ± 36	14.38	< 0.001	

N.B. QTc ≥ 550 msec in 46.4% (13/28). Longest QTc 620 in on patient.
QRS 50% prolongation seen in 2 patients.

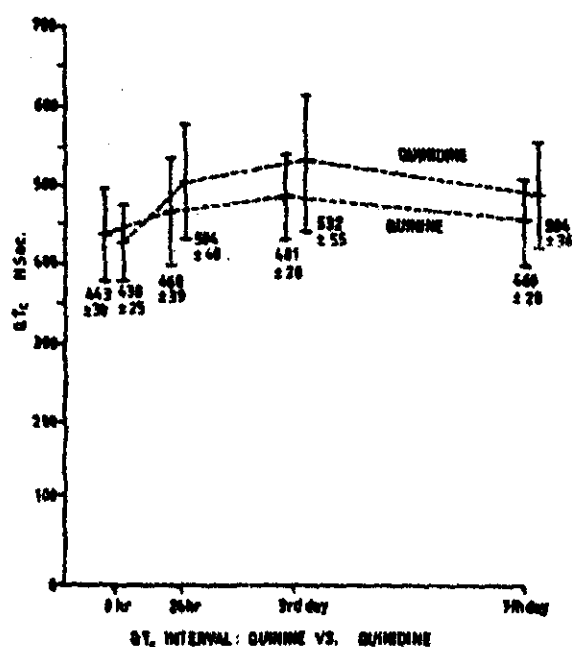


Figure 1. QTc interval in patients treated with quinine or quinidine

on a previously normal heart and a diseased heart may not be the same. It is said that widening of the QRS complex is also an indication of a cardiac toxicity. However, small changes in E.C.G. intervals could well be missed by visual inspection of traces and QRS prolongation of 50% was seen in only 2 patients in the quinine treated group.

Thus we wish to conclude that Unless quinine can be demonstrated to be superior that quinine in the treatment of falciparum malaria and as safe as quinine in large scale studies, we would recommend that quinidine be used cautiously in the treatment of malaria only when quinine is unavailable because of its pronounced effects on the E.C.G.

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**Inhibition of Plasmodium falciparum merozoites
invasion by immune sera from malaria
infected subjects**

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This study was conducted on sera samples of 92 subjects from malaria endemic areas. All 92 subjects have experienced either previous or present infection with different parasitaemia levels of Plasmodium falciparum. The relationship between frequency of malarial infection, parasite densities and immunofluorescence malarial antibody titres of P. falciparum were studied. There was no significant correlation between IFA titres and parasitaemia levels or frequency of malarial infection. The serum inhibition to invasion of P. falciparum merozoites in vitro was found to vary according to the sera obtained from subjects with various malarial immune status. Parasite inhibitory antibody was not evident in sera from acutely infected subjects but was significantly observed in sera from convalescent subjects with an inhibition of P. falciparum merozoite invasion up to 85 percent.

INTRODUCTION

In man, repeated attacks of malaria are necessary for the development of protective immunity. Effective immunity to malaria is associated with high levels of circulating specific antibodies which are believed to protect the host by preventing merozoite invasion of erythrocytes (1) and by incuding mononuclear phagocytes to ingest schizonts (2,3). Acute P. falciparum infection in children also stimulates the production of specific antibodies but little of the immunoglobulin produced is believed to be protective (4). Such acute phase sera do not prevent P. falciparum multiplication in vitro (5) and the sera of individuals recovering from a primary attack of malaria induce phagocytosis of schizonts (2). Nevertheless, most children do recover from malaria despite

their apparent lack of protective antibody suggesting that some other form of parasites destruction may be involved in recovery from acute infection.

The present paper was conducted to study the in vitro inhibition of P. falciparum merozoite invasion by the sera collected from subjects with various immune status.

MATERIALS AND METHODS

Collection of sample

Blood samples were collected from subjects with various categories of P. falciparum malaria infections. There were altogether 92 subjects from Defence Services General Hospital, Mingaladon, Yangon Division; Irrigation Project,

Daik-Oo Township, Bago Division and Ban-Bwe-Gwin Middle School, Taikkayi Township, Yangon Division. Blood films and venous blood samples were collected on the basis of past and present history of malaria infection.

Separation and sera samples

Sera were separated from the clotted blood by centrifugation at 3000 r.p.m. x g for 15 minutes. 0.5 ml sera samples were aliquoted in sterile containers and stored at -20°C in a deep freezer.

Counting technique for parasites

Counting was done to assess the parasitaemia level before culture. The parasites were counted under the oil immersion lens (100x) of an Olympus 3 H.B. microscope. The parasite counting was done against 200 leucocyte or 10^4 erythrocytes. This was converted to the total parasites per cubic millimetre of blood as 8,000 leucocytes. So the number of parasites obtained in (200) leucocytes was multiplied by a factor of (40) to gain the number of parasite per mm^3 of blood. (Parasite per $\text{mm}^3 = (X) \times 40$, where (X) is the number of parasites counted against 20 leucocytes). In the case of parasite counts per 10^4 erythrocytes the number of parasite obtained (Y) was multiplied by a factor of 700 to gain the number of parasites per mm^3 of blood.

In vitro cultivation of malaria parasite was done according to the method described by Trager and Jensen (6).

Antigen preparation

Antigens were prepared from Schizonts stages of *P. falciparum* continuous cultures. The antigens were prepared in bulk to set sufficient number of slides for the whole study. The antigen slides were prepared by using the 12 drops applicator (7) where the applicator was

allowed to touch on the prepared blood suspension and again on the slide. The slides were allowed to dry at room temperature for 1-2 hours and then wrapped, and stored at -20°C, until use.

The indirect fluorescent antibody test (IFAT) was carried out according to the standard methods (8,9).

Invasion assays

To determine the inhibitory effects of malaria antibody, in vitro schizonts invasion assays were carried out as shown in figure (1) using harvested schizonts from continuous in vitro cultures (10).

Test procedure

Flat-bottom 96 well microtitre plates (Falcon 3072) were used to screen both immune and non-immune serum samples for in vitro parasite inhibitory activity. The plate was charged vertically from A to H. Each serum sample was diluted with the medium (RPMI 1640), Hepes buffer, Na_2CO_3 and gentamicin sulphate in A to G wells of the appropriate column respectively. Except one well (well H) was reserved as control well (i.e. non immune normal serum). Three replicates were used for one subjects; for example A_1, A_2, A_3 and B_1, B_2, B_3 , etc. Each well was added 100 μl of the schizonts suspension with the Eppendorf pipette from culture. The plate was cultured in a candle jar at 37°C for overnight. Each plate would be used for (28) subjects. Separate plates were used for 24 hours and 48 hours cultures. After overnight incubation, the rate of parasite invasion i.e. ring trophozoite susceptibility was examined by making a thin blood film. The film was counted for the number of ring trophozoites per 10^4 erythrocytes. The results were determined by the ability of the parasites to invade when challenged by various sera samples

Erythrocytes (AB Blood Group)

- Wash with RPMI1640 at 1800 r.p.m. x G for 10 mins
- Remove WBCs by using PLASMAGEL or PERCOLL.
- Wash 3 times with RPMI1640 at 1800 r.p.m. x G for 10 mins.

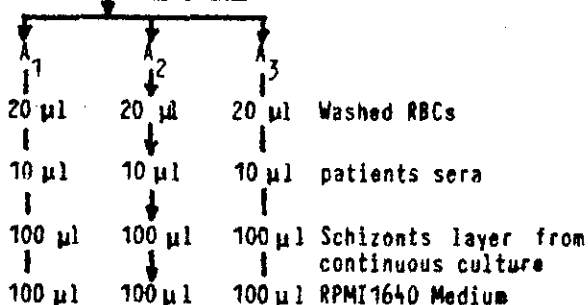
To each 0.5 ml. of packed RBCs, and 1 ml of RPMI1640.

Incubate at 37°C for 20 mins.

Wash 5 times with equal volume of RPMI1640 at 1800 r.p.m. x G for 10 mins.

Suspend the washed RBCs in double volume of complete medium.

In vitro microtiter plate



Incubate overnight.

- Take triplicate thin smears from each well.

- All films fix with methanol and stain one set with Giemsa.

- Count ring trophozoites per 100,000 RBCs under 100 x (Total parasitaemia).

Figure 1. Schematic flow diagram of in vitro invasion assays.

collected from patients with various immune status. The results were expressed in terms of the percentage of parasite invasion in normal serum.

RESULTS

In vitro micro invasion assays were carried out using P. falciparum schizonts harvested from in vitro continuous cultures. The specificity of parasite inhibitory antibody was assessed by testing the ability of acute, convalescent (recovery) and post treatment sera samples obtained from 24 subjects (paired samples) to inhibit invasion of their infecting parasite strains (local P. falciparum isolates from culture).

Fig. 2 shows the in vitro trophozoites susceptibility of P. falciparum inhibited by the sera collected from subjects with various immune status. During convalescence, sera from all subjects were significantly ($P < 0.01$) inhibitory to the infecting parasites strains. Sera from subjects with acute and post-treatment did not inhibit the growth of parasite strains.

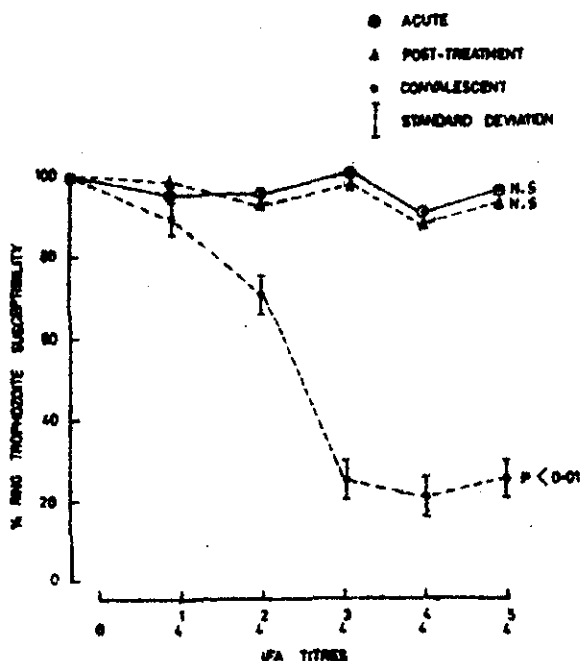


Figure 2. In vitro trophozoite susceptibility of Plasmodium falciparum in relation to sera with different IFA titres.

Fig. 3 represents the *in vitro* inhibitory effects of sera collected from three groups of patients. In contrast, parasite inhibitory was evident in sera from convalescent subjects (65-85 percent) but was not found in sera from acutely infected subjects and post-treated subjects (less than 5 per cent).

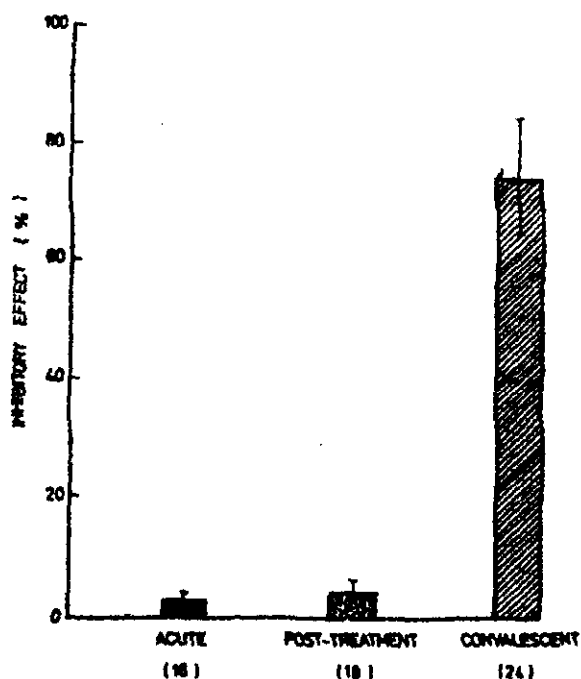


Figure 3. *In vitro* inhibitory effects of sera collected from three groups of patients.

Fig. 4 represents the relationship between frequency of malaria infection and IFA titres. It was observed that there is no correlation between frequency of malaria infection and IFA titres.

Fig. 5 shows the relationship between duration of post-infection of malaria and IFA titres. It is also noted that there is no correlation between IFA titres and days after post-infection.

Although the measurement of malaria antibody titres by immunofluorescent antibody technique (IFAT) is accepted as sensitive method, it was observed

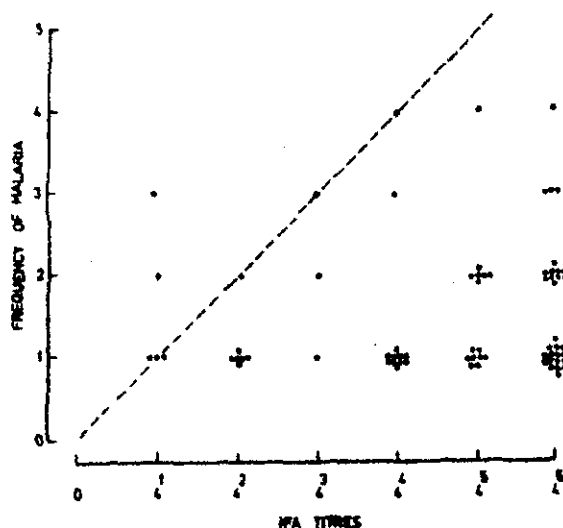


Figure 4. Relationship between frequency of malarial infections and IFA titres.

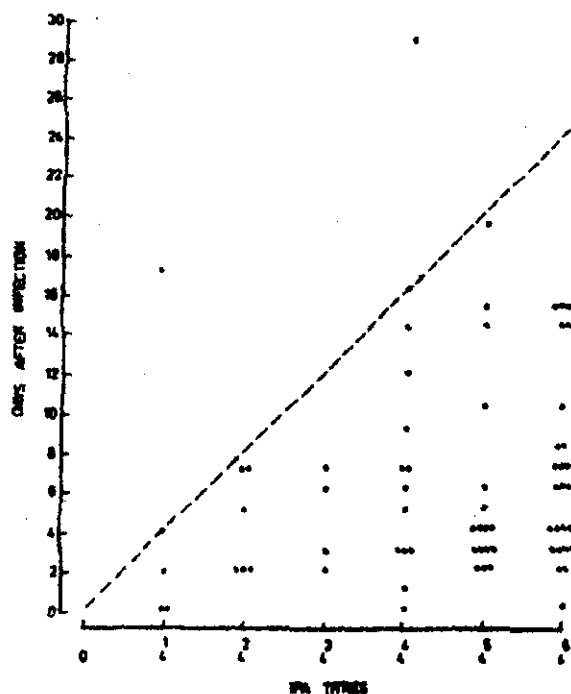


Figure 5. Relationship between duration of post-infection of malaria and IFA titres.

that there is no significant correlation between IFA titres and parasitaemia levels or frequency of malaria infection in this study.

DISCUSSION

In highly endemic areas, *P. falciparum* malaria infection of indigenous children during the first five years of life can cause severe and potentially fatal illness. In early months of life, the manifestations are usually mild with low grade parasitaemia, probably because of passive immunity acquired from immune mothers. The parasite rate increases with age from 0 to 10 per cent during the first three months of life to 50 to 90 per cent by one year of age and the rate persists at a high level during early childhood (11). The mortality rate in hyperendemic areas is highest during the first two years of life (11). By school age, a considerable degree of immunity has been developed and a symptomatic parasitaemia can be as high as 75 per cent in primary school children (9). In areas of low endemicity, however, where the immunity in the indigenous population is low, severe infection occurs in all age-groups including adults.

Current evidence suggests that specific antibodies may play an important role in mediating protective immunity, and it is probable that these antibodies require the cooperation of sensitized effector cells to achieve parasite destruction *in vitro*. *In vitro* merozoite inhibition techniques have been used to assay sera for protective antibodies, but the application of such techniques to malaria epidemiology is yet to be proven. All the methods used involve the quantification of the inhibition by sera of the invasion of red cells by merozoites in cultures of *P. falciparum*. This effect has been measured by directly counting parasites in conventionally stained smears (12), by the

uptake of radioactive isoleucine (12) by the use of fluorescent labels (13), by flow cytometry (13), and by fluorescence - activated cell sorters (14). In general, the methods have been based on microtest culture systems similar to those developed for drug sensitivity testing.

These methods have usually given results that accord well with the protective immune status of the host in defined situations where it has been possible to match parasites and sera in a homologous system. However, when applied under field conditions where matching of parasites and antisera is hardly possible inhibition tests have often yielded results that do not accurately reflect protective status.

Methods for measuring protective immunity to sporozoites and to gametocytes could also theoretically be devised on the basis of techniques that have been developed for the cultivation of these stages. These may have some relevance for measuring the impact of vaccines currently under development but would have little relevance to malaria epidemiology since it appears that antibodies to those stages are not induced to functional levels under condition of natural transmission and infection.

In vitro invasion assay described in this study measures the effective entry of merozoites and formation of rings in a population of cells. The time course under the present condition is ideal, taking up to 16 hours to reach a plateau. It appears to be quite practicable, however, as judged from the test runs with serum containing antimalarial antibody. A pure mature schizont input population should be achievable with improved methods for schizont-purification. In the present experiments the schizont - enriched input population contained a number of younger parasite forms, so that the

time course of infection correlates with the required for these forms they reach maturity prior to reinfection.

Using this assay, it was shown that the patient's serum inhibited infection of erythrocytes. Thus it is possible to employ the assay for quantitative assessment of some facts of humoral immunity to *P. falciparum* by measuring the effects of particular antibodies on merozoite invasion of specified erythrocytes. Similarly, it can be used to compare infection in a given erythrocyte population by different parasite strains or to study parasite invasion of erythrocyte variants.

Cohen, McGregor & Carrington (15) demonstrated that transfer of antibody from adult west African to children infected with *P. falciparum* rapidly reduced asexual parasitaemia. The antibody may have either affected the parasitized red cells or blocked merozoite invasion of red cells. Passive transfer of antibody has also been effective in animal malaria (16). In two primate system, *P. falciparum* in Aotus monkeys (17) and *P. knowlesi* in rhesus monkeys (18), immunity was associated with the ability of antibody to block invasion *in vitro*. Identification of the antigens to which these protective antibodies were directed is difficult because the immune sera contained a mixture of protective and nonprotective antibodies. Therefore, they used monoclonal antibodies produced by hybridomas to identify surface antigens on *P. knowlesi* merozoites against which protective monoclonal antibodies are directed.

A battery of sera from adult Gambians immune to *P. falciparum* blocked invasion of some strains *in vitro* but not others (12). Sera from adult Gambians injected into chimpanzees rapidly reduced parasitaemia from a west African strain of *P. falciparum* but had no effect on Asian strain (19). Variant antigens would probably be of little

value in vaccine development.

In addition to identifying merozoite determinants common to all strains, the antibodies should be highly effective in blocking invasion. The antibodies in the present study required high concentrations for an effect. It is possible, however, that a higher-affinity antibody may be more effective. It is also possible that antibodies to different components may act synergistically to block invasion, whereas each alone has a minimal effect. Synergism between 2 antigamete monoclonal antibodies were required to markedly reduce mosquito infectivity from *P. gallinaceum* (20). It may be possible, however, to block invasion more effectively by a monoclonal antibody to a different determinant. Freeman et al (21) observed that a single monoclonal antibody directed against merozoites protected mice infected with a virulent strain of *P. yoelli* (21). The question remains whether a single monoclonal antibody directed against an antigenic determination on merozoite will be highly effective against *P. falciparum* or *P. knowlesi* and react with a determinant common to all strains. If such an antibody existed, then it would identify an important antigen for vaccine development.

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In vitro and in vivo antimalarial activities of artesunate

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Artesunate, a derivative of Quinghaosu (Artemisinin) was tested for antimalarial activities against four isolates of Plasmodium falciparum in vitro and Plasmodium berghei- mouse model in vivo. The minimum inhibitory concentration of Artesunate in vitro was 6.0 μ Mol per well whereas in vivo ED₉₀ was 1.4 mg/kg/day.

INTRODUCTION

The therapeutic effectiveness of Quinghaosu and its derivatives (sesquiterpene lactone peroxides) and their possible development in accordance with international standards have continued to be the subject of intense study. These naturally occurring peroxide compounds have been extensively studied in China since 1970s. Research on the hemisuccinyl derivative, artesunate, and the methyl ether derivative, artemether (focusing on production methods), the standardization of formulations and the development of methods for pharmacokinetic and metabolic studies is being carried out (1).

Potentiation was also observed with artemisinin and primaquine against the P. berghei N strain and the primaquine resistant P strain. The effect of combinations of artemisinin and chloroquine were additive against N strain, whereas antagonism was observed between artemisinin and antifolate drugs, such as pyrimethamine, cycloguanil and sulfa drugs.

As the antimalarial activity of the Quinghaosu series of compounds appears to depend upon the 1,2,4- trioxane ring structure of the basic molecule (Fig.1) ,

a number of simpler compounds containing this ring have been synthesized (2,3,4,5,6,7), and are now being evaluated for efficacy against P. falciparum. They do appear to have blood schizontocidal activity.

Our study was conducted to test the efficacy of artesunate on four isolates of P. falciparum in vitro in parallel with chloroquine, quinine and mefloquine at the same time. We will have a better understanding of in vitro response of artesunate to our Myanmar isolates of P. falciparum. To backup the in vitro results, an in vivo rodent malaria (P. berghei) system was included in our study.

MATERIALS AND METHODS

P. falciparum isolates

Four isolates of P. falciparum were obtained from out-patients of Vector-Borne-Disease Control Unit, Department of Health, Yangon. The isolates were cultured and synchronised by the methods described by Trager and Jensen (8) and Lambros and Vanderberg (9).

In vitro testing

For In vitro drug testings of chloro-

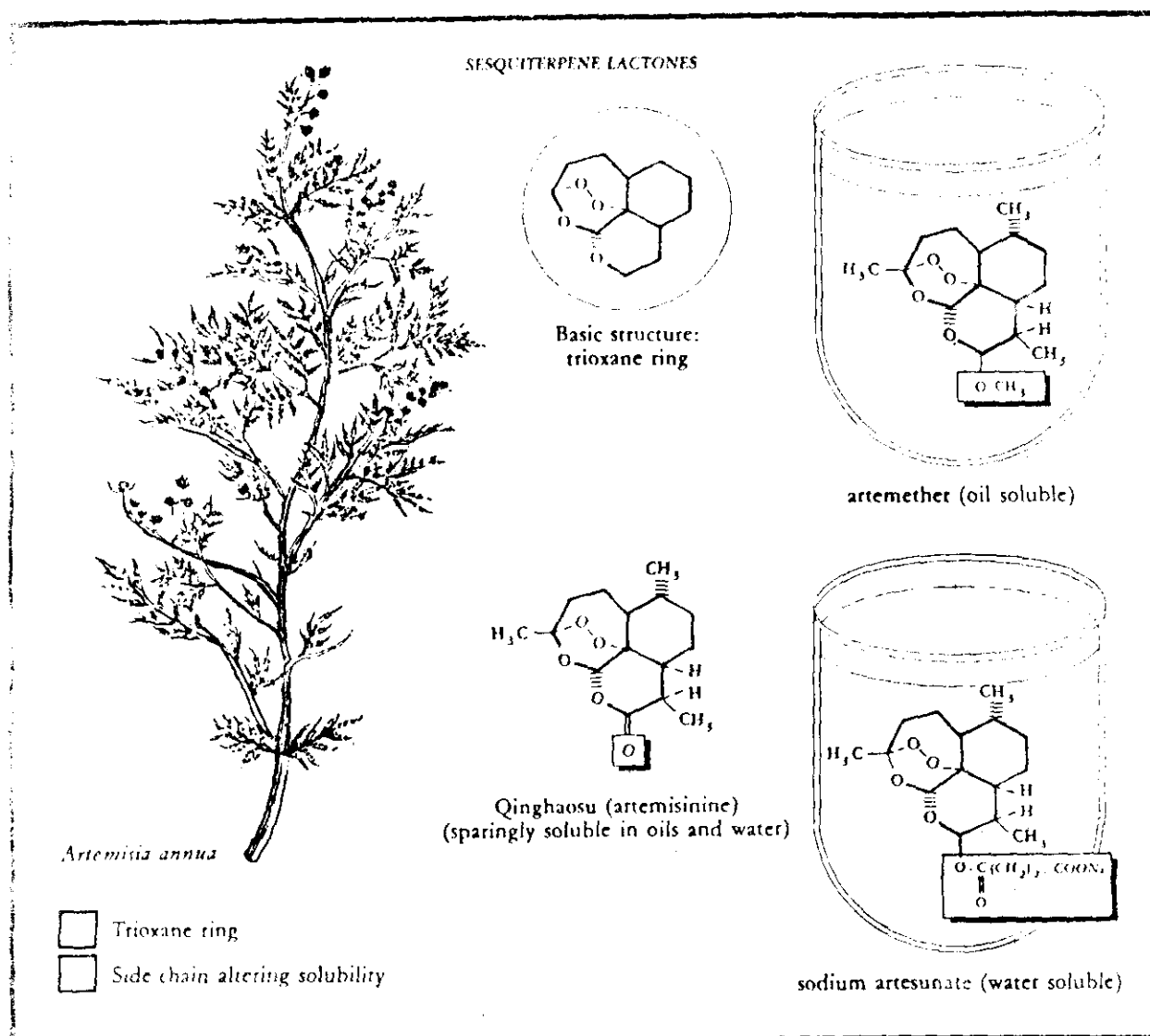


Figure 1. The plant morphology of *Artemisia annua* and the structure of the sesquiterpene lactones and its compound

quine, quinine and mefloquine, standard micro-titer charged plates supplied by WHO/SEARO were used. Artesunate was produced in dual pack form by the Guilin No. 2 Pharmaceutical Factory, Guangxi, China. For artesunate, flat bottomed 96 wells micro titre plates were charged in Parasitology Research Division, Department of Medical Research, Yangon.

In vivo Model

Plasmodium berghei - mouse model system as described by Myint-Lwin (10) was used to screen the effect of the drugs.

RESULTS

The effect of artesunate subcutaneous injection on mice infected with *Plasmodium*

berghei is summarised in table (1).

Table 1. The effect of subcutaneous artesunate injection on the mice infected with Plasmodium berghei (At day 6 after infection)

	Groups		
	A	B	C
Number of mice	18	18	18
Mean parasitaemia			
(at day 6)	29.0%	1.19%	0.54%
Standard Error	± 2.11	± 0.13	± 0.06
Survival rate			
at day 6 (n_1/N)	6/18	18/18	15/15
Mortality rate			
at day 6			
($n_2/N \times 100$)	33.3%	0	0

A - Control (Infected but untreated)

B - Artesunate 1.4 mg/kg/day subcutaneous infection for 4 days

C - Chloroquine 20 mg/kg/day orally for 4 days

n_1 - number of mice survived at day 6

n_2 - number of mice died at day 6

N - Total number of mice inoculated with Plasmodium berghei

The highest parasitaemia rates (29.0 $\pm$ 2.11 percent) were recorded in control mice (infected but untreated) with a mortality rate of 33.3 percent on 6th day after infection. The subcutaneous injections of artesunate (1.4 mg/kg/day for four days) was found to be effective for rodent malaria. There was no mortality in the groups of mice receiving oral chloroquine (20 mg/kg/day for four days).

The *in vitro* sensitivity of four isolates of P. falciparum to quinine, mefloquine, chloroquine and artesunate are shown in Figure 2. With quinine, only two isolates (I-4 and I-5) were found to be sensitive (above 90% inhibition) at the concentration of 232 p Mol, whereas the

isolates I-52 and I-53 were resistant with percent inhibition of 82 and 78. With chloroquine, all four isolates were found to be resistant up to 24 p Mol per well.

All the four isolates were found to be sensitive to mefloquine at a minimum concentration of 8 p Mol/well.

With artesunate, two isolates (I-5 and I-52) were 100% sensitive at MIC of 4 p Mol/well but another two (I-4 and I-53) were sensitive only at 6 p Mol/well.

DISCUSSION

Sodium artesunate displayed potent blood schizontocidal activity against the asexual forms of P. falciparum at erythrocytic stage in cultures as well as against P. berghei in mice and P. knowlesi in monkeys. The use of subcutaneous or intravenous route of administration will not alter the effect of artesunate on the parasites. The parasite clearance time was shorter than all other antimalarials currently in use. Comparative treatment with sodium artesunate and quinine dihydrochloride, both given intravenously to P. knowlesi infected Rhesus monkeys, indicated that the former was significantly better even at a dosage equivalent to one-tenth of the latter. Thus, time required for 100% parasite clearance by sodium artesunate at 3.16 mg/kg was only 13.2 hours, whilst by quinine dihydrochloride at 31.6 mg/kg was 50.5 hours. In our study it was observed that the potency of the drug as evaluated against P. falciparum in cultures was also higher than that of chloroquine and quinine.

Pharmacokinetic studies has been conducted on animals and men in China. After an intravenous injection, drug concentrations in blood were determined by RIA. The elimination half-life was 12-23 minutes in rabbits and 20-33 minutes in dogs. The distribution of the drug in animal body was relatively wide, concentrations in intestines, liver and kidneys were higher than that in the

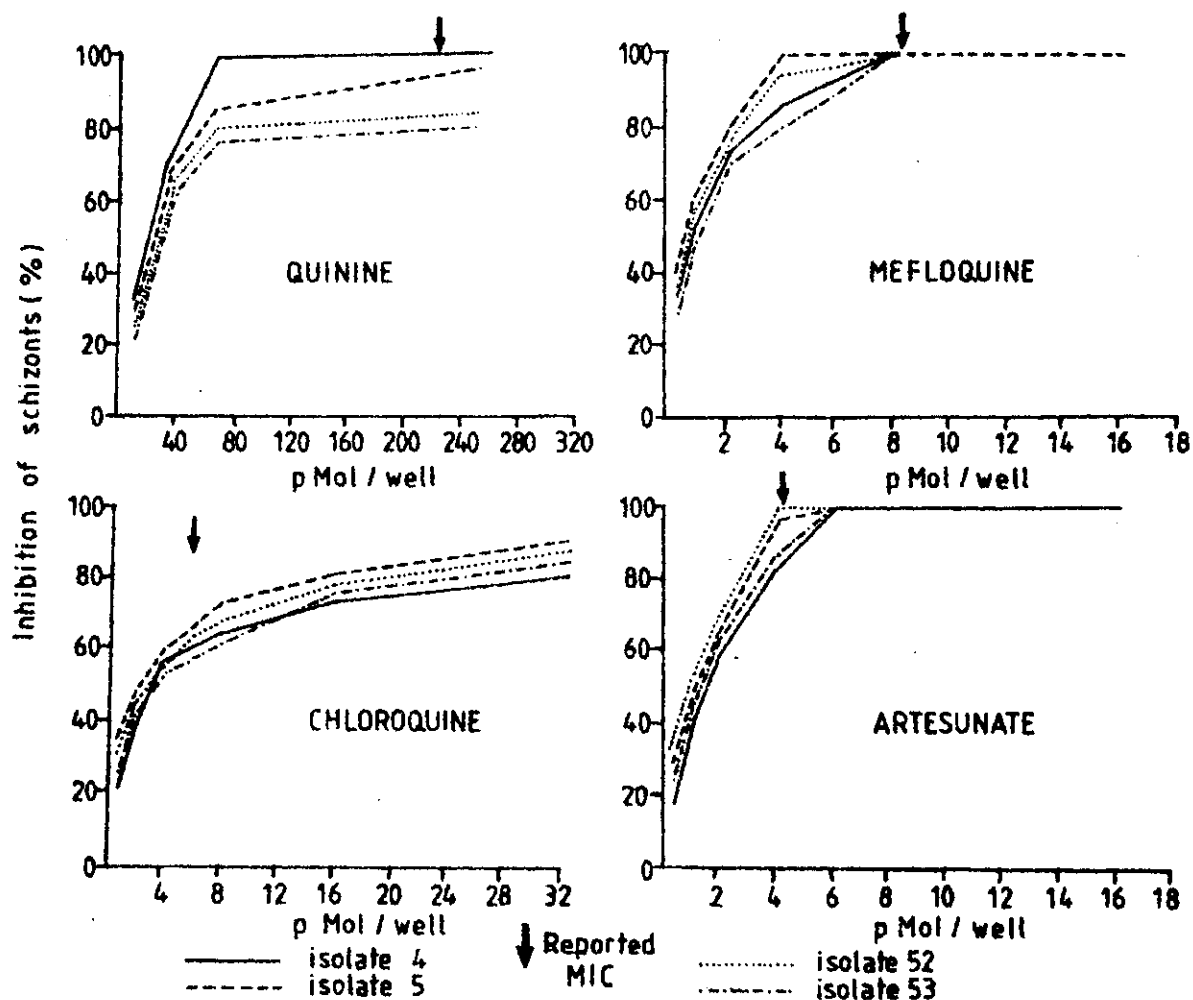


Figure 2. In vitro sensitivity of four isolates of *Plasmodium falciparum* to quinine, mefloquine, chloroquine and artesunate

other organs. Only a small amount of the unchanged drug was found in urine and faeces (1).

Results obtained from pharmacokinetic studies on healthy adult volunteers and malaria patients both indicated that the elimination half-life of the drug in blood was about 30 minutes after an intravenous injection. Only a small amount of the drug was found in excretion of urine and faeces, elimination half-life in which was about 70 minutes.

It has been reported that no general or

local adverse reactions were observed in 346 cases after treatment for 3 days with the drug at a total dosage of 240 mg given intravenously. No abnormal changes could be related to the drug in the examinations of heart rate, blood pressure, ECG, haematology, blood biochemistry, urine and faeces.

ACKNOWLEDGEMENT

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obtaining artesunate injections. This work was done by utilizing facilities donated by Japanese International Co-operation Agency, Japan.

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**Enterically Transmitted Non-A, Non-B, Hepatitis Immunity
in Rhesus and Cynomolgus Monkeys (Macaca mulatta
& Macaca fascicularis)**

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An experimental model of enterically transmitted non-A, non-B, hepatitis was established in rhesus and cynomolgus monkeys (Macaca mulatta and Macaca fascicularis). Two monkeys previously infected with 10% pooled stool suspensions of Myanmar patients with well-defined enterically transmitted non-A, non-B, hepatitis and three virgin control monkeys were infected simultaneously with pools of stool suspensions from the first-passage. None of the two previously infected monkeys developed signs of hepatitis. All the three control virgin monkeys exhibited hepatitis as evident by sharp rise in serum aminotransferases and pathological changes of acute hepatitis in liver biopsies. These findings document the establishment of immunity after infection with enterically transmitted non-A, non-B hepatitis virus in rhesus and cynomolgus monkeys.

INTRODUCTION

In Asia including Myanmar several outbreaks of non-A, non-B hepatitis have been reported (1-3) with a waterborne or fecal-oral mode of transmission hence called enterically transmitted non-A, non-B hepatitis (ET-NANBH). Many workers have discovered 27-nm-diameter virus-like particles (VLPs) in stools of patients and experimental animals (4-7). Our group has also reported the recovery of VLPs in stool as well as inside hepatocytes (8). This investigation studies the development of immunity in rhesus and cynomolgus monkeys after the rechallenge.

MATERIALS AND METHODS

Monkeys

Rhesus and cynomolgus monkeys (M mulatta & M fascicularis) born in the Forest Department, Hlawgar Garden, Myanmar, were quarantined for 3 months. The animals were housed in individual cages. The possibility of infection between any two animals was rigidly controlled. Two monkeys (Cy2 & MM6) who have developed ET-NANBH by first experiment were observed for 18 months and included in the rechallenge experiment when their liver enzymes and histology had returned to

normal. Each was negative for serum markers of both hepatitis A (anti-hepatitis A virus IgM determined by HAVAB-M Abbott Laboratories, USA) and hepatitis B (anti-hepatitis B core IgM determined by Corzyme-M Abbott Laboratories, USA). Venipuncture and liver biopsy were done after immobilization by ketamine hydrochloride a drug with no known hepatotoxic action.

Inocula

Two inocula were prepared with acute-phase stool specimens. Inoculum 1, was prepared as a 10% (w/v) suspension in phosphate buffered saline (PBS) from ten acutely ill Myanmar patients with epidemiologically and serologically defined ET-NANBH. Inoculum 2, was similarly prepared from a cynomolgus monkey (Cy 2) who has had ET-NANBH after i.v injection with inoculum 1. The stools of the monkey (Cy 2) were collected between 19 to 28 days after inoculation.

Liver biopsy

Percutaneous aspiration needle biopsy was taken at weekly interval for approximately 9 wks during quarantine and after inoculation for 12 wks or till sacrifice. The liver tissues were fixed in 10% buffered formalin, embedded in paraffin, cut at 2/ μ m thickness and stained with Hematoxylin and Eosin for histological examination. Animals with abnormal liver biopsies were omitted from the study.

Liver Function Tests

Blood specimens were obtained weekly simultaneously with liver biopsy before and after inoculation. Activities of AST and ALT were determined by GCT, GPT kit (GCT, GPT kit-K Nihonshoji Co. Ltd) and expressed in karmen U/ml. During the initial period of quarantine, weekly AST and ALT values were determined for each animal over a period of at least 9 wks and base-line

value for each animal was determined. After each inoculation, primates were bled weekly until the hepatitis resolved or until the animal was sacrificed. Serum AST or ALT levels of more than twice an individual animal's base-line value, accompanied by necroinflammation reactions in the liver biopsy were considered to represent an episode of hepatitis.

RESULTS

The study consisted of two parts. The first part of the study was to transmit and passage ET-NANBH from patients to primates and then primate to primate. Cy2 monkey was injected intravenously with inoculum 1 derived from patients stool. Serum AST & ALT started to rise 3 wks after inoculation and reached more than twice the base-line value 4 wks after inoculation (Fig 1).

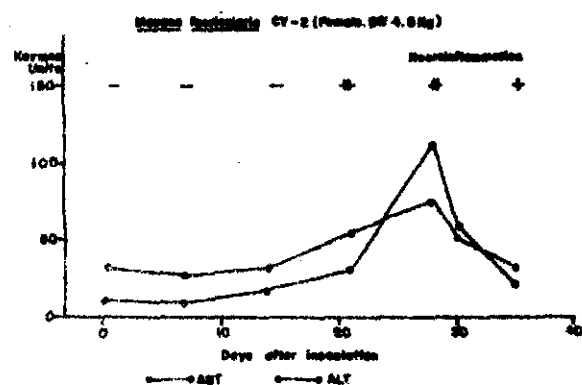


Figure 1. The clinical course of Cy2 following first challenge. Elevation of serum aminotransferases and hepatic necroinflammation were noted 3 wks after inoculation.

ALT, alanine aminotransferase. AST, aspartate aminotransferase. Necroinflammation; - absent, + minimal, + mild, ++ moderate.

Liver biopsies at the time of enzyme elevation revealed typical histologic

pictures of acute hepatitis which consisted of liver cell necrosis and cellular infiltration (necroinflammation). There was striking focal necrosis of single hepatocytes with total disappearance or formation of shrunken acidophilic bodies surrounded by inflammatory cells. The infiltrates were composed mainly of lymphocytes. Less numerous were histiocytes, polymorphonuclear leukocytes and plasma cells. Hydropic and ballooning degeneration of hepatocytes around central veins were also noted. Diffuse increase of lymphocytes within lobules and proliferation of Kupffer cells as well as periportal lymphocytic infiltration were also observed (Fig 2).

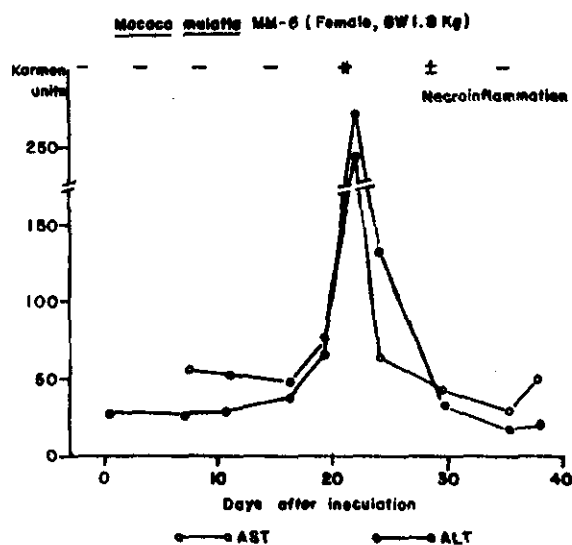


Figure 3. The clinical course of MM6 following first challenge. Elevation of serum aminotransferases and hepatic necroinflammation were noted 3 wks after inoculation. ALT, alanine aminotransferase. AST, aspartate aminotransferase. Necroinflammation; - absent, ± minimal, + mild, ++ moderate.



Figure 2. Liver biopsy from Cy2 monkey 3 wks after first challenge. focal necroses with predominant mononuclear cells infiltration and Kupffer cells mobilization were observed. (H&E stain X 400).

These histologic changes were the same as those of acute hepatitis in man (9) and chimpanzees (10).

Monkey (MM6) was inoculated intravenously with stool suspension of CY2 (inoculum 2). It developed sharp rise in AST & ALT more than 6 times the basal level 3 wks after inoculation. Liver biopsy at that time revealed full picture of characteristic histology for acute hepatitis (Fig 3). Thus the passage of hepatitis to the monkey was successfully accomplished.

The second portion of the study was aimed to test whether there is development of immunity or not after

ET-NANBH by rechallenge experiment. For test animals CY2 and MM6 were reused and as for control, three virgin monkeys were used. All 5 animals were inoculated with inoculum 2. Before second inoculation, both CY2 and MM6 had no residual evidence of previous hepatitis. Their enzymes were in their base-line level and their liver histology had returned to normal. Weekly monitoring of enzymes and liver biopsies were done for 3 months for MM6. The remaining 4 monkeys were sacrificed on 18 to 35 postinoculation days. The brief clinical courses of all 5 animals were shown in Fig 4-7. Both the test animals (CY2 & MM6) did not exhibit rise in their enzyme values nor there is appearance of necroinflammatory reaction in the liver as a sign of hepatitis. Thus these 2 primates did not contract hepatitis after second inoculation. On the contrary, all the three virgin control monkeys revealed increase in transferases and pathological changes of acute hepatitis in their liver biopsies 2 wks after inoculation. Apart

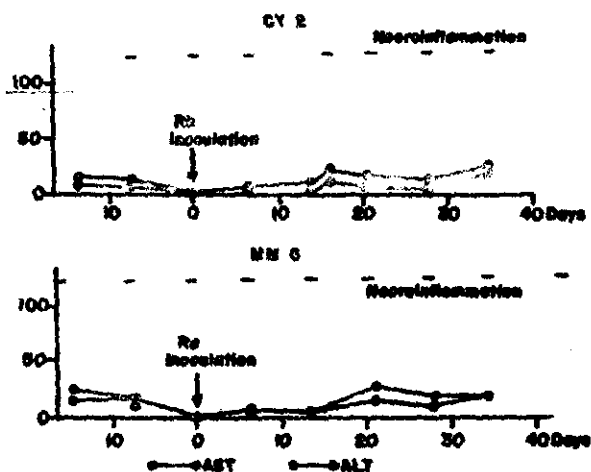


Figure 4. The clinical courses of Cy2 & MM6 after second inoculation. There were neither elevation in liver enzymes nor appearance of necroinflammatory reactions in the livers.

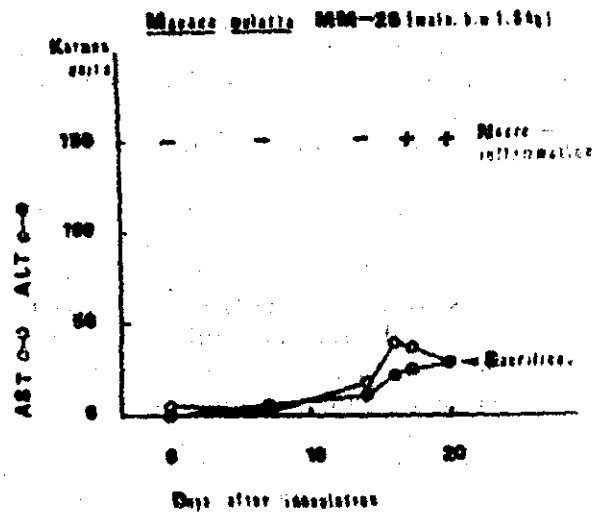


Figure 5. The clinical course of MM26 after inoculation. Hepatic necroinflammation was noted 2 wks after inoculation.

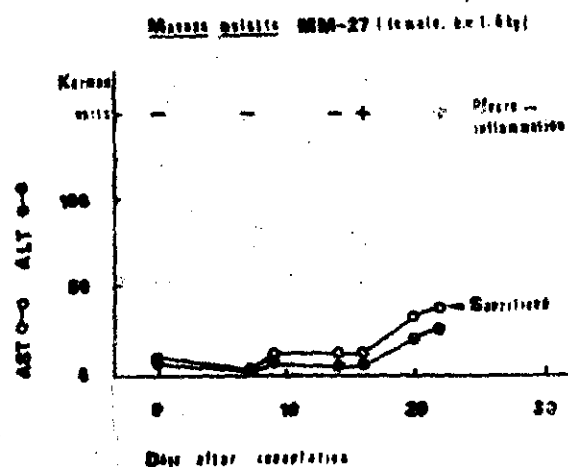


Figure 6. The clinical course of MM27 after inoculation. Hepatic necroinflammation was noted 2 wks after inoculation.

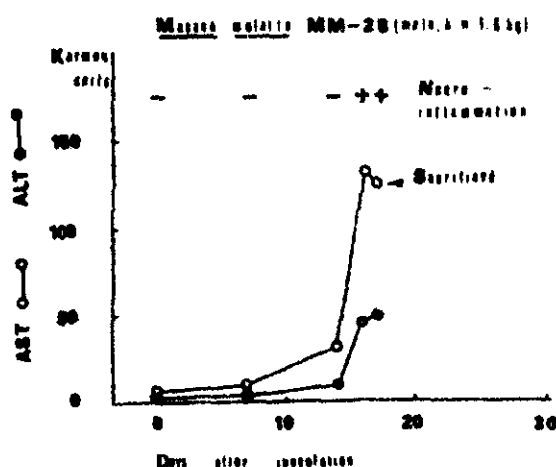


Figure 7. The clinical course of MM28 after inoculation. Hepatic necroinflammation was noted 2 wks after inoculation.

from one animal (MM28) the rise in liver enzymes was less than twice their base-line value. This may be due to earlier sacrifice of the animals before their maximum enzyme elevation.

DISCUSSION

From the results, it was clear that both rhesus and cynomolgus monkeys were susceptible to ET-NANBH which is prevalent in Myanmar. Balayan et al had shown transmission of ET-NANBH to cynomolgus monkeys (5). Kane et al (1) and Arankalle et al (7) had reported transmission of ET-NANBH to marmosets and chimpanzees respectively. Since we could transmit the disease from patients to primate and then from primate to primate our transmission study as well as passage experiment was successful. The findings of the second part of the study showed the occurrence of immunity in test animals. Because all the

control animals suffered from hepatitis when inoculated simultaneously with the same inoculum. Also the test animals suffered from the disease when they were inoculated with the infectious agents in the first part of the experiment. Thus development of ET-NANBH 18 months ago conferred immunity to ET-NANBH when challenged again. To our knowledge this is the first report to describe development of immunity in primates in ET-NANBH.

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Circadian rhythm of urinary oestrogen excretion during late pregnancy

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Six hourly urine samples for two consecutive days from 15 Myanmar women during late pregnancy (36 weeks onwards) were taken to study the presence of circadian rhythm of urinary oestrogen excretion in pregnant Myanmar women. A circadian rhythm of urinary oestrogen excretion was observed. The urinary oestrogen level fell in second fraction (13:00 hour to 19:00 hour), rose in third fraction (19:00 hour to 01:00 hour) and declined again in the fourth fraction (01:00 hour to 07:00 hour). Analysis of variance indicated significant variation in urinary oestrogen levels ($p < 0.01$). Moreover, high correlation between urinary oestrogen excretion of the second fraction and that of 24 hour sample was observed.

INTRODUCTION

Urinary oestrogen assays are widely employed in the monitoring of foeto-placental integrity. Usually 24 hour urine samples are collected but it is rather difficult to get a complete 24 hour urine sample without missing a voiding or two (1). It was hoped that by statistical analysis it would be possible to demonstrate a pattern of circadian rhythm in urinary oestrogen excretion as is found in other endocrine organs. If such a circadian rhythm could be identified it might then be possible to take something less than a 24 hour urine over a measured period of time and extrapolate this value to predict the foetal status.

It was reported that there is a definite circadian rhythms in the urinary excretion of heavy metals, organic substances, electrolytes, trace elements and total solids in adults (2,3). Moreover, a definite circadian rhythm of urinary oestrogen excretion in pregnant American women had been described (4). However, with regard to circadian

rhythm of oestrogen excretion, data on pregnant Myanmar women is still lacking.

The biorhythms of renal activity in healthy persons had been identified (5). Moreover, it had been reported that kidneys of Myanmar handle certain substances quite differently (6,7). Thus the present study is aimed at identifying the presence of circadian rhythm of urinary oestrogen excretion in pregnant Myanmar women and to choose the best correlated fraction which can give the most reliable indication of urinary oestrogen excretion if a 24 hour collection is not possible.

MATERIALS AND METHODS

Fifteen clinically healthy pregnant women (36 weeks onwards) who were attending the antenatal clinic at the Defence Services General Hospital No.1, Mingalardon were selected and the method of urine collection carefully instructed. Six-hourly urine samples were collected for 2 consecutive days. Running time was, 07:00 hour to 13:00

Table 1. Urinary oestrogen contents in six-hourly fractions on the first day

Subjects	Urinary oestrogen content (mg)			
	First fraction 07:00 to 13:00 (hour)	Second fraction 13:00 to 19:00 (hour)	Third fraction 19:00 to 01:00 (hour)	Fourth fraction 01:00 to 07:00 (hour)
1	10.31	9.02	13.94	7.33
2	4.67	3.64	6.28	3.65
3	17.18	9.95	21.17	5.46
4	10.61	9.72	12.24	6.63
5	8.51	4.48	7.48	5.15
6	4.56	3.41	5.92	3.25
7	3.57	2.50	5.24	2.82
8	14.14	8.69	12.26	7.73
9	6.65	4.63	5.74	3.71
10	10.34	8.28	10.12	7.12
11	5.98	4.41	4.67	3.52
12	3.72	3.71	4.12	3.62
13	15.72	10.45	18.84	8.58
14	4.12	5.20	7.72	4.94
15	10.12	10.06	14.32	6.32
Mean	8.68	6.48	9.97	5.36
S.E	1.2	0.74	1.4	0.49

hour (first fraction); 13:00 hour to 19:00 hour (second fraction); 19:00 hour to 01:00 hour (third fraction); and 01:00 hour to 07:00 hour (fourth fraction).

Estimations of fractional urinary oestrogen were carried out by the method recommended by Oakley, Bradshaw, Eccles, Sitch and Heys (1967) based on Kober colour reaction (8). Total 24 hour excretion values for oestrogen were also determined by totalling the amounts found during each 6 hour period.

RESULTS

The urinary oestrogen contents in 6 hourly fractions on the first and second days are shown in table 1 and table 2. It was observed that the urinary oestrogen levels fell in the second fraction (13:00 hour to 19:00 hour), rose

in the third fraction (19:00 hour to 01:00 hour) and declined again in the fourth fraction (01:00 hour to 07:00 hour). Analysis of variance indicated significant variation in urinary oestrogen levels ($p < 0.01$).

Correlations of fractional urinary oestrogen excretion values with 24 hour values are shown in table 3. The highest correlation (and lowest SE) was observed with the second fraction.

DISCUSSION

The present study revealed that there is a definite circadian rhythm of urinary oestrogen excretion in pregnant Myanmar women during late pregnancy. The urinary oestrogen levels fell between 13:00 hour and 19:00 hour, rose between 19:00 hour and 01:00 hour, and declined

Table 2. Urinary oestrogen contents in six-hourly fractions on the second day.

Subjects	Urinary oestrogen content (mg)			
	First fraction 07:00 to 13:00 (hour)	Second fraction 13:00 to 19:00 (hour)	Third fraction 19:00 to 01:00 (hour)	Fourth fraction 01:00 to 07:00 (hour)
1	10.42	7.61	14.92	9.81
2	6.91	4.42	6.14	2.81
3	16.92	10.12	19.86	7.42
4	11.72	10.14	12.74	7.32
5	8.21	6.18	8.32	4.43
6	3.78	2.67	3.52	2.22
7	3.84	2.91	5.41	3.11
8	13.12	9.12	15.04	7.74
9	5.75	3.51	4.39	5.53
10	9.42	7.92	13.45	8.91
11	5.27	3.82	4.76	4.11
12	4.38	4.14	5.31	3.82
13	15.87	11.12	17.72	10.14
14	5.52	3.15	6.71	4.13
15	10.94	11.12	14.14	6.68
Mean	8.80	6.53	10.16	5.81
S.E	1.1	0.82	1.4	0.66

Table 3. Correlation of fractional urinary oestrogen excretion values with 24 hour value.

Fractions	Second (01:00 PM to 07:00PM)	Third (07:00 PM to 01:00AM)	First (07:00 AM to 01:00PM)	Fourth (01:00 AM to 07:00AM)
Correla- tion coeffici- ent	0.9812	0.9324	0.9168	0.8647
Regres- sion Stan- dard error of fracti- onal values with 24 hour value	2.113	3.824	4.721	6.837

again between 01:00 hour and 07:00 hour.

The urinary oestrogen excretions were also studied on 19 normally pregnant American women in 4 hourly urine samples for six days by Duhring, McKean and Green (1973). It fell between 10:00 hour and 14:00 hour, rose between 18:00 hour and 22:00 hour and fell again in the three subsequent 4-hour periods. Another report, after studying 4-hourly intervals for 24 hour periods in 22 women in the third trimester of pregnancy, had shown maximum values of urinary oestrogen excretion between 21 and 24 hours (9). Thus, the pattern of the urinary oestrogen excretion found in the present study more or less agrees with the above findings.

Alteration in C₁₉ steroid precursor availability induced by externally

supplied C₁₉ steroid precursor, particularly dehydroisoandrosterone sulphate (10) and adrenal suppression with glucocorticoid (11) cause corresponding changes in urinary oestrogen level indicating that in normal pregnancy oestrogen synthesis is limited by precursor availability. It was observed that there is a circadian rhythm of oestrogen level and precursor availability, particularly of unconjugated dehydroisoandrosterone, which is preferentially utilized for oestrogen synthesis (12). The adrenals of the newborn respond normally to adrenocorticotrophic hormone but the adult type rhythm of corticoid production is not established until 1 to 3 years of age implying the absence of rhythmic activity during intrauterine life (13). Thus, the observed rhythm in urinary oestrogen level is probably attributable to variation in maternal rather than foetal adrenal function.

Moreover, a slight depression of glomerular filtration rate, and significant decreased creatinine excretion during the night and constant relationship between oestrogen and creatinine excretion throughout the day have been reported (14,15,16). Since urinary oestrogen excretion varies directly with creatinine excretion (17), it can be assumed that the same mechanism is probably responsible for the variation in both. Thus, it seems that the observed variation may be due primarily to variation in renal clearance rather than in the rate of production.

In conclusion, the present study has identified the presence of circadian rhythm in urinary oestrogen excretion of Myanmar pregnant women. Moreover, since a high correlation was observed between the urinary oestrogen excretion of the second fraction (i.e., 13:00 hour to 19:00 hour) and that of 24-hour sample, it is suggested that urine collected between 01:00 PM to 07:00 PM gives the most reliable indication of urinary oestrogen excretion if a 24-hour collection is not possible.

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The effect of "THIRD TRIMESTER" maternal nutrition on the offspring in Wistar rats.

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This research was performed in the Department of Medical Research, Yangon, Myanmar. The experimental work was done during the period of April 1987 to 1988. This paper is part of a thesis submitted in partial fulfillment for the degree of Master of Medical Science (Physiology) by Hla Hla Aye. It was read at the Research Paper Reading Session, Twenty-Fifth Anniversary of the Department of Medical Research, on 31st December, 1988.

INTRODUCTION

It has been reported that maternal malnutrition affects fetal growth and development (1&2). Reduction in food intake or a decrease in protein content from early pregnancy causes reduction in birth weight of offspring in rats (3-6). Some have stressed that the third trimester is the most important period of pregnancy during which supplementation should be given (7-11). But recently some authors have refuted the above concepts that because of homeostatic mechanisms maternal nutrition can vary widely without clinically obvious impairment of reproductive outcome. As pregnancy is a period of lipid accumulation, mothers can store calories and transfer the reserves to the fetus when required (12). Some also assert that the total amount of calories is the main determinant of birth weight and not the trimester at which these calories were consumed (13).

Thus it is important to assess whether growth retardation of fetus can be prevented by maternal supplementation during the third trimester.

It has been difficult to examine these

issues separately and ethically in human populations. Thus rats have been used as animal model.

MATERIALS AND METHODS

Female virgin Wistar rats weighing 180-200g and 140±5 days old were selected. They were placed in a 30±2°C room with a twelve-hour light, twelve hour dark cycle and offered DMR stock diet for three weeks. At the end of the preliminary adaptation period, they were placed in a cage with a proven male breeder at two to one ratio and checked daily for the presence of a vaginal plug. The morning, the rats were found with a plug was designated as day 0 of pregnancy. Rats that failed to mate before reaching 210g were excluded from the study. Pregnant dams were caged in individual aluminium cages. They were divided into three experimental groups and given different dietary regimes, according to table 1.

Maternal food intake and body weight were checked every day between 9-11h. Blood samples were taken from the lateral tail vein on days 0,7,14 & 21 of

Table 1. Design of experiment

Groups	Food intake of dams		
	1st week	2nd week	3rd week
C	AL	AL	AL
R3	AL	AL	60 % AL
R1,2	60 % AL	60 % AL	AL

C = Control.

R3 = Restricted only during the third week of pregnancy.

R1,2 = Restricted during first and second week and given food ad libitum during the third week.

AL = Ad libitum, DMR stock diet.

pregnancy.

The pups were weighed on the day of delivery. Snout-rump length was measured and the average of three readings was recorded. Blood samples were analysed immediately for haemoglobin, plasma protein and PCV. (14-16).

Data was analysed using one-way analysis of variance test. Differences were considered to be significant at $p < 0.05$. Student's 't' test was also performed. Values sharing the same superscript letter in the same column are not significantly different at the 5% level.

RESULTS

Total weight gain of pregnancy:- Control dams gained 53.02 ± 4.23 g for the whole pregnancy while third-week restricted dams gained significantly less weight as shown in table 2, figures 1 and 2. Restriction followed by third week ad libitum fed dams had a higher food intake when refeeding was allowed and the total weight gain was not significantly different from control dams.

Net weight change:- At the end of the

experiment the control dams gained 7.36g and the third week restricted dams lost 7.52g. Restriction followed by third week ad libitum fed dams gained only 5.93g.

Mean litter size:- The three experimental groups produced comparable litter size.

Mean weight of pups:- Birth weight of control pups was 4.61 ± 0.13 g. Third-week restricted dams produced significantly lighter pups, mean being 3.823 ± 0.26 g. Refed dams produced pups which were similar in weight to the control group (table:2).

Snout-rump length:- All three experimental groups had pups of comparable lengths. Thus third week-restriction cause lighter pups which were merely thin but not stunted.

Haemoglobin levels:- Just before parturition haemoglobin level of control dams and those of R1,2 were comparable, mean being 13.51 ± 0.34 g/dl and 12.02 ± 0.59 g/dl respectively. Third-week restricted (R3) dams had significantly lower haemoglobin level, mean being 10.95 ± 0.52 g/dl. All the dams had comparable plasma protein and packed cell volume at the end of gestation.

Haemoglobin, plasma protein and PCV of pups at birth:- Pups born of control dams and R3 dams had comparable haemoglobin, whereas pups born of R1,2 dams produced pups with significantly higher haemoglobin level, the mean being 10.53 ± 0.17 , 10.783 ± 0.19 and 11.86 ± 0.12 g/dl respectively. All pups had similar plasma protein levels. Control and R3 pups had comparable PCV whereas R1,2 pups had higher PCV, mean values being 38.65 ± 0.28 , 38.58 ± 0.61 and 39.2 ± 0.1 g/dl, respectively.

Statistical analysis:- One-way analysis of variance and Student's 't' test were employed.

DISCUSSION

The effect of third trimester restriction was found to cause a significant reduction in birth weight but mean length of

Table 2. Outcome of pregnancy in dams given different dietary regimes

Groups	Total Weight Gain (g)	Net Weight Change (g)	Mean Litter Size	Mean Pup Weight (g)	Snout-Rump Length (mm)
C	53.02 ^a	+7.63	7.9 ^a	4.67 ^a	4.98 ^a
(n=10)	±4.23		±0.49	±0.13	±0.05
R3	38.97 ^b	-7.52	8.5 ^a	3.82 ^b	4.73 ^a
(n=8)	±1.12		±0.26	±0.26	±0.08
R1,2	60.62 ^a	+7.63	8.75 ^a	4.65 ^a	4.89 ^a
(n=8)	±2.67		±0.47	±0.05	±0.04
	F = 5.13 Significant.		F = 1.79 N.S.	F = 0.68 N.S.	F = 1.79 N.S.

Analysis of variance was done and values were significantly different at the 5% level.

C = Control

R3 = Restricted only during the third week of pregnancy

R1,2 = Restricted during first and second week and given ad libitum during the third week.

Student's 't' test was done and values sharing the same superscript letter in the same column are not significantly different at the 5% level.

pups remained normal. There was a 17% reduction in birth weight of pups compared with control.

At the end of gestation third-week restricted dams had significantly lower haemoglobin level than controls. Thus food restriction during third trimester gave rise to lower birth weight pups and lowering of maternal haemoglobin level. Berg (1965) showed in Sprague Dawley rats a 50% overall reduction of food intake or a decrease in protein content of 5 to 6% throughout pregnancy causes a 20-30% reduction in birth weight of offspring. In our experiment a 40% overall reduction in food intake or a reduction in 13% protein during third week of pregnancy causes 17% reduction in birth weight.

In this study we found that weight gain during pregnancy was proportional to maternal dietary intake. There was net loss of weight in dams restricted during

third week. This is in accordance with findings of Rasmussen (1983) in whose model the under-feeding was imposed only during pregnancy.

Last but not the least is that the dams who were feed-restricted for the first two trimesters but refed during the third trimester showed that in every parameter they were comparable with the controls. These dams demonstrated a marked increase in food intake, over and above the control level when refeeding was ensured during the third trimester. This supports Seller's (1987) explanation of embryonic nutrition which states that maternal nutrition does not usually affect preimplantation phase (1st week) but the last week is important. The results emphasized that good maternal nutrition, especially during the third trimester is important not only for the health of the mother but also for the health of her offspring.

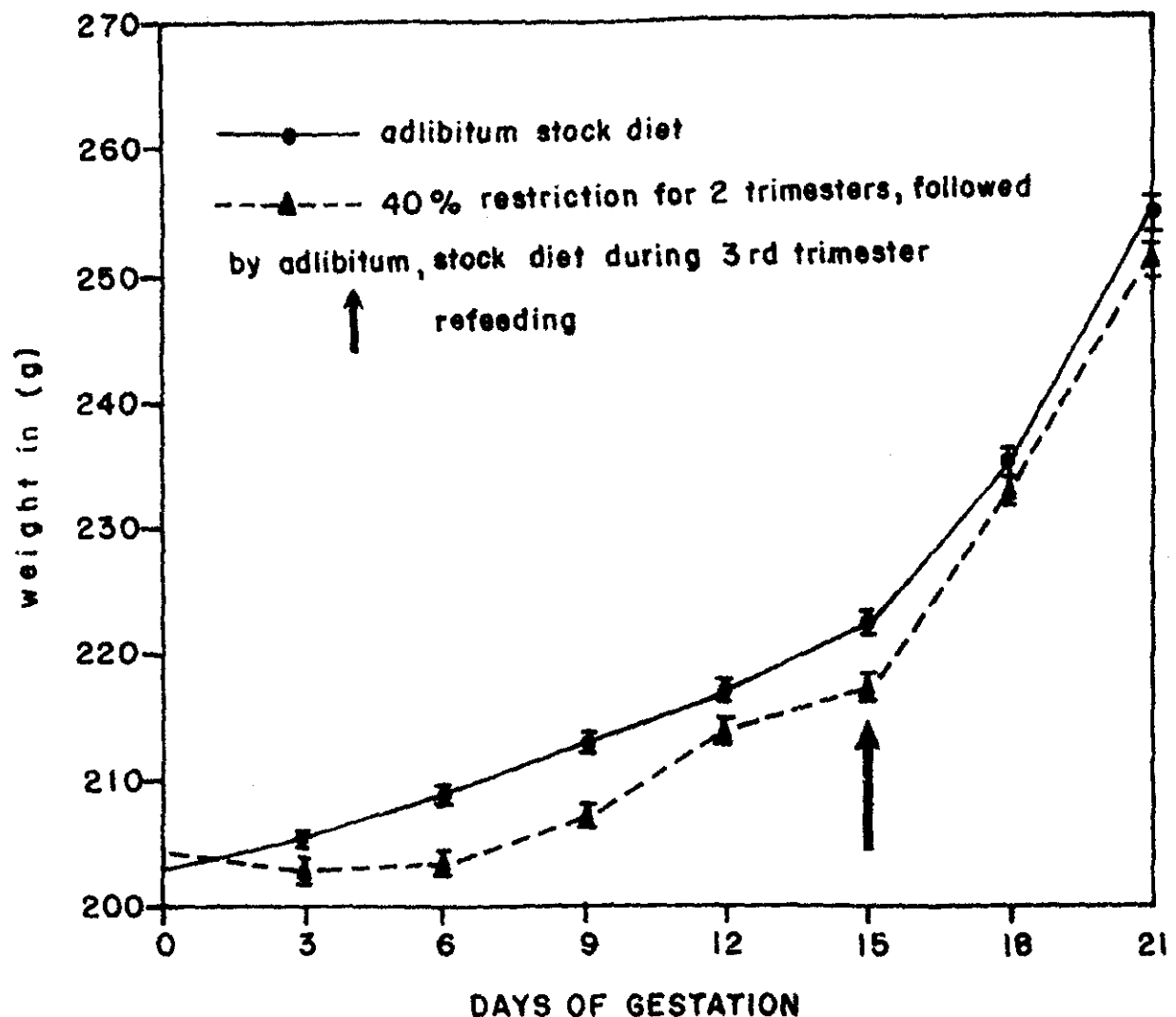


Figure 1. Body weight changes of ad libitum, stock diet-fed dams and dams fed 40% restricted followed by ad libitum stock diet during 3rd trimesters

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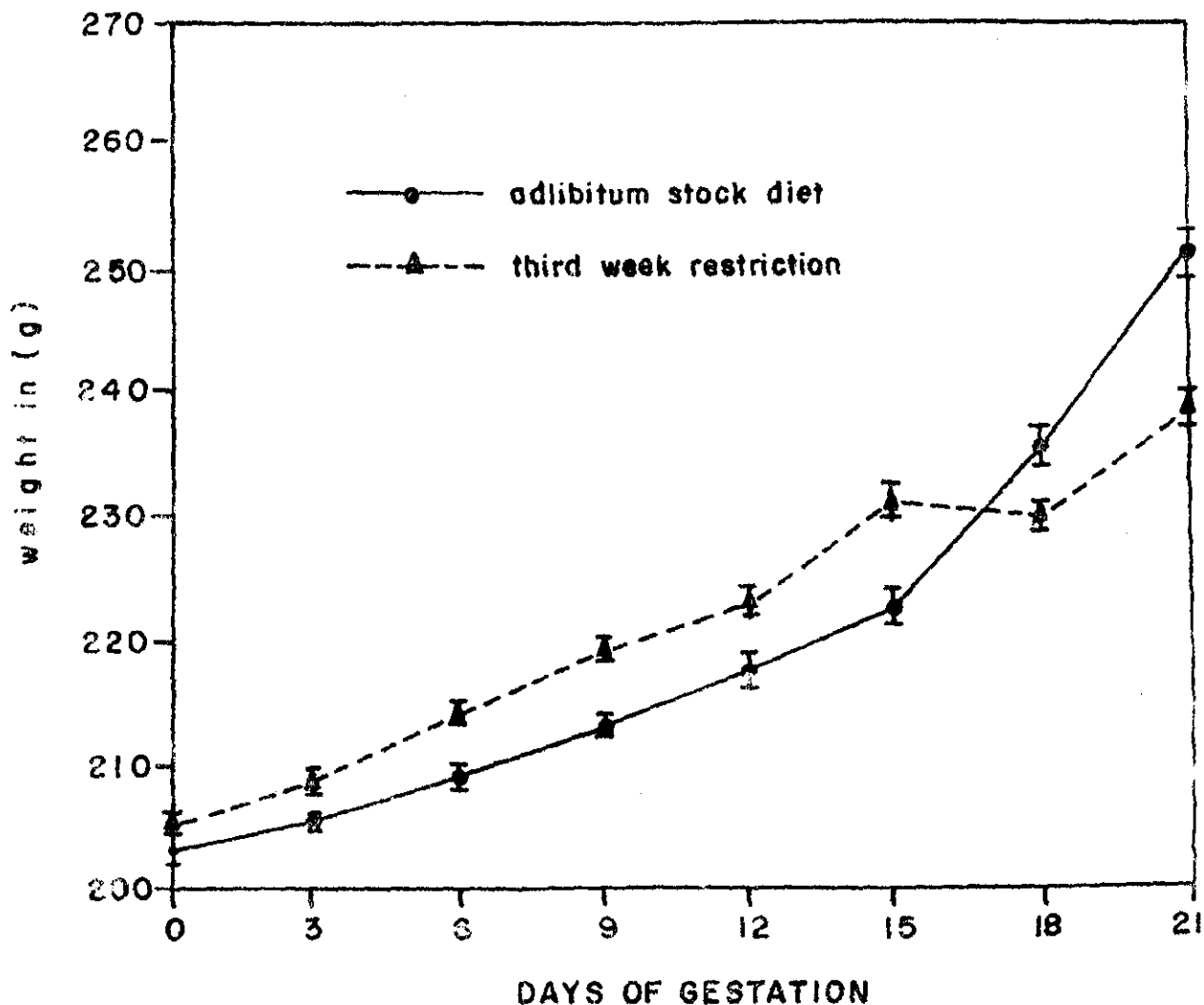


Figure 2. Body weight changes of ad libitum, stock diet-fed and third-week restricted dams

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The efficacy of mosquito fish Trichogaster trichopterus for control of Aedes aegypti in a community, Yangon

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The efficacy of aquarium fish Trichogaster trichopterus as larvivorous fish was tried out in the laboratory and its predatory rate was found to be 1269 larvae per 24-hour exposure in glass bowl with 1.5 litre of water. Based on this finding a small scale field trial was carried out in domestic containers including metal drums, glazed earthen jars, and concrete tanks with Aedes aegypti breeding in Ngadatkyi North Qr., Bahan. The results show that this fish is an efficient biological larval control agent in domestic water containers.

INTRODUCTION

In Myanmar, the major mosquito-borne diseases prevalent are malaria, filariasis, (DHF) and Japanese encephalitis. Out of these four public health problems, dengue haemorrhagic fever seems to be the easiest to eradicate. The vector, Ae. aegypti (Linn) breeds in close proximity to man and its larval habitats could be manipulated by various means. However, in many control programmes in various countries difficulties were encountered. At present vector control is the only major control strategy available for prevention and control of DHF. Much research had been carried out on the biology and control of Ae. aegypti. Periodic manipulation of breeding sources by community participation had been tried many times in Myanmar. Weekly dry days were organised when all water containers are emptied and inspected. This method, although theoretically sound, could not be regularly carried out in practice under conditions prevailing here. The covering of containers placed outdoors has resulted in the covers being lost. Legislation method whereby owners of

houses with positive breeding sources was found to be successful in Singapore and Malaysia. However, its drawback is the demand for manpower required to keep surveillance of houses.

In the search for simple, long-lasting, effective method without the use of insecticides, biological control measures have been tried out. Use of larvivorous fish is reported to be highly effective. People do not accept guppy (Poecilia reticulatus) fish for they know that these fish are natural inhabitants of the roadside drains. However, the attractive aquarium fish Tri. trichopterus was readily accepted by the community. After carefully studying its predatory rates under laboratory conditions, a small scale field trial was conducted to find its efficacy to control Ae. aegypti breeding in domestic water containers.

MATERIALS AND METHODS

Study Area

Study and Check Areas were selected in Shwegondaing, Bahan Township. Ngadatkyi North Ward 1,2,3,4 and 6 of Shwegondaing Road was selected as

Test Area (TA). The area is approximately 45,000 square metres and the approximate population is 1532 with 350 houses. A similar area across the Shwegondaing Road i.e. Middle Shwegondaing was chosen as Check Area (CA) to compare mosquito indices, such as Breteau Index (BI), Container Index (CI), House Index (HI) etc. This CA is approximately 50,000 square metres and the approximate population is 1732 with 420 houses.

Most of the houses in both the TA and CA are single story and built of bamboo or wood with thatch roof. No pipe water supply is present in these areas. People have to carry water from wells present in the areas. There is no regular vector control measure of any kind in operation in these areas.

Recording of metrological data

Daily metrological data for Central Yangon in the "Working People's Daily" was taken as the data for the Study Area. Daily temperature, relative humidity, and rainfall was recorded.

Testing of predatory rates of *Tri. trichopterus* in the laboratory

The predatory rates of *Tri. trichopterus* on the *Ae. aegypti* larvae was conducted in the laboratory using series of testings such as 500,600,700,800,900, 1000,1100,1200,1300,1400,1500 and 2000 *Aedes* third stage larvae and above in each bowls. The tests were conducted in the large round glass bowls (diameter 11.4" x depth 4.6") with approximate 1½ litre of tap water. Each larval density was tested with 6 replicates for 5 times and the exposure period was 24 hours. All the test bowls were covered with nylon netting. The size and body weight of the fish used were ½ inch (approx: 1.5gm), 1 inch (approx: 2gm); 1½ inch (approx: 3gm) and 2" (approx: 4gms). The

mean water temperature during testing was around 27±2°C. Before testing, the fishes were starved for 6 hours.

Field trial

After series of laboratory testings and having determined the predatory rates of *Tri. trichopterus*, the fish were released into the domestic water containers on 1st May 1986. All the water containers in both the test and check areas were counted and numbered. Pre-control data were taken before the release of fishes. One-and-half inch size *Tri. trichopterus* fishes were released at the rate of one fish per container irrespective of the size of the container. The houseowners, especially the children were told to look after the fish and asked them not to remove it from the containers. Larval surveys were conducted again after two days of releasing the fishes and the adult collections also continued only after two weeks of the fishes released.

RESULTS

Table 1 and figure 1 showed the predatory rate of *Tri. trichopterus* under laboratory condition. From the table 1½ inch (ca 3gm body weight) fish consumed double or triple mosquito larvae than ½" and 1" size fish per 24 hours. The predatory rate of fish over 2 inches was not significantly different from that of 1½ inch size fish. It was clearly seen that a single fish of 1½ inch can consume an average of 1269 *Aedes* larvae in 24-hour period which was considered a very good predatory rate. Thus 1½ inch size fish was chosen for the trial.

Table 2 showed the effect of *Tri. trichopterus* fish on *Ae. aegypti* mosquito indices in (TA) and (CA). Prior to control the Breteau Index (BI) was found to be very high (not less than 70 even in the hot months). But after application of fish, Breteau and

Table 1. Predatory rate of Tri. trichopterus fish on Ae. aegypti larvae

No. of prey larvae tested	No. of fish released	No of prey consumed fish in 24 hours				F-test	
		$\frac{3}{4}$ "	1"	$1\frac{1}{2}$ "	2"	$\frac{3}{4}$ ", 1, $1\frac{1}{2}$ "	$1\frac{1}{2}$ ", 2"
2000	1	29	360	1300	1291	Highly significant with size of fish	Not significant with size of fish
2000	1	32	400	1275	1275		
2000	1	28	370	1280	1260		
2000	1	30	400	1250	1285		
2000	1	31	500	1240	1289		
Summation	5	150	2030	6345	6400		
Mean X	1	30	406	1269	1280		
SD ±		1.41	49.63	21.54	11.41		

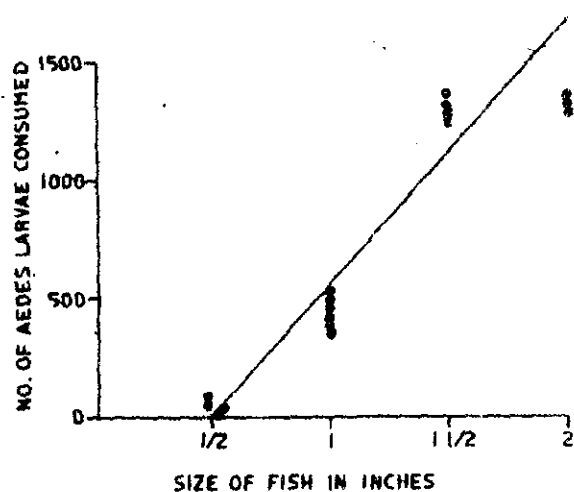


Figure 1. Regression of average consumption of third stage and above Ae. aegypti prey by the $\frac{3}{4}$ ", 1", $1\frac{1}{2}$ " Tri. trichopterus on prey density

Container Indices in the (TA) even during the rainy season was observed to be zero. It was only in the 9th month (cool season) some of the containers were absent with fish. Some of the fishes were lost and killed during washing the containers. Some children also wanted to rear the fish and transferred it into their aquariums. It was only those containers without fish that were reinfested with Aedes larvae. After releasing of fish, adult Aedes density also decreased and did not rise any further than 3.7 female mosquito per man per hour (Table 2). The existing mosquitoes were also possibly infiltrators from nearby (outside the TA) areas.

DISCUSSION

The predatory rate of Tri. trichopterus fish varied according to their sizes (weights). The chosen size $1\frac{1}{2}$ inch (3gm weight) of a Tri. trichopterus fish consumed 1269 Aedes larvae (third

Table 2. Effect of Trichogaster trichopterus on Aedes aegypti mosquito indices in Test Area and Check Area

Period	Test Area (TA)			Check Area (CA)		
	Breteau Index	Container Index	Adult Density	Breteau Index	Container Index	Adult Density
Prior to control						
Rainy season	118.01	47.58	25.97	117.84	46.76	15.87
Cool season	82.68	30.64	13.66	72.37	30.96	3.93
Hot season	72.02	29.04	4.1	66.83	28.52	3.52
(Fish released on 1st May 1986)						
Post control data						
Premonsoon	0.0	0.0	3.2	127.74	54.94	19.99
Midmonsoon	0.0	0.0	2.65	165.02	70.97	14.87
Postmonsoon	0.0	0.0	2.65	128.04	55.06	24.07
Cool season	2.2	.89	3.675	124.99	53.76	12.75
Hot season	70.2	4.77	3.675	80.46	34.6	10.63

stage and above) per 24 hours. In comparison with other predators such as Toxorhynchites splendens consumed (60 ± 3) immature stages of Ae. aegypti per 24 hours (Myint Myint Kyi (1)). Sebastian, et al. (2) also mentioned that a medium size labellulied nymph devour (133 ± 21) all immature stages of Ae. aegypti per 24 hours.

The mosquito indices such as larval indices and adult densities were almost similar in both (TA) and (CA). In the (TA), it was only after releasing of Tri. trichopterus fish that the entomological indices of Ae. aegypti was found to decrease abruptly. The fishes were released on 1-5-86 (premonsoon period). The larval indices went down from BI (103.19) and CI (41.61) to BI (0) and CI (0) respectively. These zero larval indices remained throughout the rainy season (Dengue epidemic period)

till December. During this period, fishes from 2 or 3 containers were lost while changing the water and were replaced with new fishes on routine inspection visits. Epidemics of dengue haemorrhagic fever (DHF) occur typically during wet season and the apparent correlation with rainfall has led to the conclusion that the mosquito population increases at that time and the epidemic of DHF therefore correlated with presumed rise in number of the vector Ae. aegypti (3-6).

Normal trend of the Ae. aegypti mosquito population density is higher in monsoon rather than that of cool and hot seasons. But, in the (TA), the larval indices did not rise, instead markedly fell in monsoon. Also, the post control results of adult density was found to be decreased throughout the year.

In the (CA) the larval indices such as average Breteau Index (BI) and average Container Index (CI) during rainy, cool and hot months were found to be 140.27, 124.99 and 88.46 respectively and those for CI were 53-76 and 34.6 respectively. Also, the adult densities were found to be 19.64, 12.75 and 10.63 during rainy, cool and hot months. Thus, in the (CA) the trend of the mosquito population was found to be normal. there were peaks and troughs in both larval and adult indices. The peak months were observed in rainy season, highest in July and August, whereas in the (TA) the mosquito indices were maintained at zero throughout the rainy season (DHF epidemic period) after releasing of the Tri. trichopterus fish. The Ae. aegypti female landing rates were also found to be very low throughout the study.

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The effect of cigarette smoking on nasal mucociliary activity

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Nasal mucociliary transport rates of healthy twenty male non-smokers and twenty cigarette smokers (20-39 years) were measured using Saccharin Crystal Technique by Proctor (1973). The mean nasal mucociliary transport rate of non-smokers was found to be 10.17 mm/min. with a range of 8.78 to 12.22 mm/min. and that of cigarette smokers was 7.95 mm/min ranging from 7.04 to 8.74 mm/min. Significant decrease ($p < 0.001$) in nasal mucociliary transport rate was observed in cigarette smokers.

INTRODUCTION

Normal mucociliary function is one of the protective functions of the nose and 80-90 percent of the inhaled particulate matter of 5-6 μ or greater are retained in the nose and nasopharynx by adhering to the mucus film (1). It had been stated that ciliary functional defects and structural abnormalities appear to have a direct causal relationship to lower and upper respiratory tract infections (2) which are the national health problems in Myanmar and smokers especially are found to have respiratory tract troubles.

Our fundamental knowledge of the nasal mucociliary function especially in relation to the respiratory diseases is not well documented. In partial fulfillment to verify the problem, the present study was undertaken to reveal the effect of smoking on nasal mucociliary activity.

MATERIALS AND METHODS

Subjects:

To study the nasal mucociliary transport rate in normal adults, (20) reliable

male non-smokers aged between 20 to 39 years, were studied. They were thoroughly investigated to be assumed normal subjects for this study from the history and clinical examinations. Those with history of sinusitis, known case of nasal allergy, vasomotor imbalance and respiratory diseases were excluded.

To study the effect of cigarette smoke, (20) cigarette smokers, aged 20 to 39 years, all males were studied. Those who smoke at least one cigarette (standard size with filter) per day for a minimum of one year were regarded as smoker (3).

Sampling

Since, the effect of previous exposure to dust and smokes is very important in selection of the subjects, they were randomly chosen from sedentary workers (all clerks) from non-exposed groups i.e from central office of People Construction Corporation.

Method:

The nasal mucociliary transport rate was measured by saccharin crystal technique (4). Three measurements of the nasal mucociliary transport rates of

each subject were measured on separate days under the same conditions of temperature and humidity and the average value was taken as the mean nasal mucociliary transport rate of the subject.

The subject was allowed to sit on a stool and a saccharin crystal (same size) was placed on the mucosa of the floor of left side of the nose, 20 mm away from anterior nares. The choice of this site to place the saccharin crystal was to avoid anterior dead spot and the nasal vibrissae. It was quite easy to place the crystal over the moist nasal mucosa with the help of the nasal speculum. The crystal stayed in its position even when the subject sneezed. Almost all subjects could tolerate the placement of crystal without irritation.

The exact time was recorded as soon as the crystal was placed with the help of a stop-watch. Then he was allowed to sit in a chair. The subjects were requested not to blow, sniff, rub or pinch the nose. If the subject cannot refrain from sneezing he was asked to do so with open mouth. They were instructed to inform immediately whenever a sweet or strange taste was felt. The exact time was recorded when the subject reported that he had sense of sweet taste. The duration of the saccharin crystal from start of the experiment to the time of sweet taste was calculated.

The distance from anterior nares to the posterior wall of the nasopharynx was measured with a small catheter calibrated with millimeter marks.

The measurement was done at the end of the experiment. The catheter was lubricated at the tip and slowly introduced along the floor of the nose. With the help of a tongue depressor, the tip of catheter coming down to nasopharynx from above soft palate was visualized. Then the subject was asked to say 'Ah' and the tip of the catheter was adjusted to touch the posterior wall of nasopharynx.

The distance from anterior nares to nasopharynx was noted. Most of the subjects refused a probe to be introduced into their noses. So the small rubber catheter was used for measuring without injury to the nasal walls. The recorded distance minus 20 mm was assumed to be the distance of the nasal mucociliary pathway by which saccharin crystal itself or mucous blanket dissolved with saccharin was carried.

The rate of mucociliary activity was calculated by the distance of mucociliary pathway in millimetres divided by duration in minutes. The result was shown as millimetres per minute (mm/min).

RESULTS

General characteristics of the subjects are shown in table 1. The two groups in this study are quite comparable in all parameters.

Nasal mucociliary transport rates of healthy, non-smokers and cigarette smokers are shown in table 2. Measurements were done on twenty male non-smokers and the mean age being 25.8 years. The mean distance and duration for a saccharin crystal to be moved is 80.7 mm and 7.95 minutes. The mean nasal mucociliary transport rate was found to be 10.17 mm/min with a range from 8.78 to 12.22 mm/min. In study on cigarette smokers, the mean distance and duration for a saccharin crystal to be moved is 80.9 mm and 10.2 minutes. The mean nasal mucociliary transport rate was found to be 7.95 mm/min with a range from 7.04 to 8.74 mm/min.

Comparison and statistical values of nasal mucociliary transport rate for non-smokers and cigarette smokers are shown in table 3. Significant decrease in nasal mucociliary transport rate was observed in cigarette smokers ($p < 0.001$).

DISCUSSION

In order to investigate nasal mucociliary

Table 1. General characteristics of the subjects

GROUP	N	SEX	OCCUPATION		AGE (yrs)	HEIGHT (cm)	WT (kgm)	CHEST CIRCUM (cm)
Non-smoker	20	Male	Sedentary workers	M	26.8	157.7	58.5	26.7
				S.D	±4.8	±2.9	±4.6	±1.8
				R	(20-39)	(151.2-163.4)	(47.9-63.7)	(23.1-28.4)
Smoker	20	Male	Sedentary workers	M	28.2	155.5	57.9	25.9
				S.D	5.4	2.3	5.1	1.9
				R	(20-39)	(150.9-159.7)	(48.1-62.9)	(23.9-28.2)

N = number of subjects

S.D = Standard deviation

M = mean

R = Range

Table 2. Nasal mucociliary transport rates of non-smokers and cigarette smokers
(temp. bet. 32 and 36°C & humidity between 62 and 65 per cent)

GROUP	No.	SEX	OCCUPATION		AGE (yrs)	DISTANCE (mm)	DURATION (min.)	RATE (mm/min)
Non-smoker	20	Male	Sedentary	M	26.8	80.7	7.95	10.17
			Workers	S.D	±4.81	±2.92	±0.85	±1.04
			(clerks)	R	(20-39)	(76.00-86.00)	(6.42-9.22)	(8.78-12.22)
Ciga- rette smokers	20	Male	Sedentary	M	28.2	80.9	10.21	7.95
			Workers	S.D	±5.43	±2.71	±0.62	±0.47
			(clerks)	R	(20-39)	(76.00-86.00)	(8.82-11.34)	(7.04-8.74)

M = Mean

R = Range

S.D = Standard Deviation

Table 3. The comparison and statistical values of nasal mucociliary transport rates for non-smokers and cigarette smokers.

	Non-smokers (mm/min)	Cigarette Smokers (mm/min)
Mean	10.17	7.95
Standard Deviation	±1.04	±0.47
Range	(8.78 - 12.22)	(7.04 - 8.74)
t = 8.67 (20 non - smokers Vs 20 cigarette smokers)		
p < 0.001		

functions in man, radioactive particles were first used as a tracer elements and was determined externally. Proctor and Wagner (4) used human serum albumin with I^{131} and Karja and Nuutinen (5) used a droplet of Technician 99 marked human serum albumin. It was quite difficult and sophisticated to monitor the tracer particles in the above mentioned methods.

Proctor (6) had developed simpler technique without involving radioactive materials. In this method, harmless simple saccharin crystal was used as a tracer particle. Since there are circumstances, in which a simpler technique without involving radioactive material is desirable, the saccharin crystal technique was used in the present study. The reproducibility test was done and the use of saccharin crystal as a tracer material to measure the mucociliary transport rate was found to be quite applicable and the results obtained are reliable.

The mean nasal mucociliary transport rate of non-smoker is 10.17 mm/min, with a range from 8.78 to 12.22 mm/min. The mean transport rate observed in the present study is faster than those of Proctor (1973) (i.e 7.7 mm/min), and Karja and Nuutinen (1982) (i.e 9.00 mm/min). The reason for this faster rate in Myanmar subjects may probably be comparatively high temperature in our environment as the results of Proctor *et al* (1965) and Karja *et al* (1982) were conducted in the temperate zone.

Comparison and statistical values of nasal mucociliary transport rate for non-smokers and cigarette smokers had clearly shown the hazardous effect on nasal mucociliary activity after exposure to cigarette smoke. Some studies in tissue culture have indicated a total cessation of activity of the cilia on exposure to cigarette smoke (8,9). Change in ciliary activity after exposure to cigarette smoke shown in the present study may be produced by the particulates or the dust and the gases contained

in the cigarette smokes. However, the present study could not differentiate whether the effect of cigarette smoke is due to particulate matters or gaseous phase.

In short, the present study had clearly revealed the mucociliary transport rate in apparently normal Myanmar non-smoker and the hazardous effect of cigarette smoking. The authors are left with some important questions in need of answers that can only come from further investigations. The questions are; whether the effect of cigarette smoking on nasal mucociliary activity is permanent or not?, is it direct (local) or indirect (systemic) effect?, is there any relationship between nasal mucociliary activity and susceptibility to respiratory diseases?

If further research can be explored and after getting more informations with regard to clinical significance, this test may be used as an indicator of early diagnosis and severity of respiratory diseases. Moreover, the test used in the present study is cheap, simple and non-invasive, this will be of great values to our scientists and physicians. But, at present, the above study is expected to provide a valuable data for further scientific studies on respiratory diseases which are important leading problems in the community health care sector of people health plan in Union of Myanmar.

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The effect of cloth filters on contaminated drinking water

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The effect of cloth filters on removing faecal coliforms from contaminated water was determined in Yangon by isolating faecal coliform using the multiple tube method. Three different sources of drinking water (tap, shallow well and pond) samples were subjected to filtration using one layer, two layers and a soap and water washed single layer cloth filters. The commonly used cloth filters for filtering drinking water in Myanmar homes were not effective in removing faecal coliforms from contaminated drinking water.

INTRODUCTION

Diarrhoeal diseases which are a major cause of morbidity and mortality in infants and young children in developing countries are easily spread by contaminated water (1). Contamination of drinking water may result from collecting drinking water with contaminated buckets, filtering it into the storage pots with dirty cloth filters and getting drinking water from the storage containers with unclean contaminated hands (2). Most of the people in Myanmar usually drink water from the storage pots after it is filtered with cloth filters. To date, no studies can be located that show the effectiveness of the cloth filters in removing bacteria from contaminated drinking water. Here, we report the effect of cloth filters on removing faecal coliforms from contaminated drinking water.

MATERIALS AND METHODS

The study was conducted in Yangon between August to October 1990. The natural water samples were collected from a tube well, a shallow well and a

pond from Thingangyun Township which represents many of the peri-urban communities in Greater Yangon area. Water used for drinking from these sources are usually contaminated. Six samples from a tube well and seven samples each from a shallow well and a pond were tested during this study. The samples were collected in sterile containers by standard methods of collection (3). They were transported to the laboratory without ice and processed within one hour after arrival.

Faecal coliform count (FCC) of water was determined on water samples before and after filtration with cloth filters by the multiple tube method as described by International Commission on Microbiological Specifications for Foods (ICMSF) (4). Filtration was done in three different ways on all the samples: (a) using only one layer of filter; (b) using 2 layers of filters and (c) using one layer of filter that had been washed with soap and the respective water. The cloth filters which were used during this study were the new

ones which were bought from the local market in Thingangyun Township.

RESULTS

As shown in the Table, the FCC of water was not reduced after Filtration in 3 different ways. When the water was not contaminated, the Filtrated water remained clean. However, with the contaminated water samples, the FCC generally increased or remained more or less the same after Filtration, regardless of how they were filtered (one layer, two layers or after washing with soap and water). This was particularly so with the more contaminated shallow well and pond water sources.

DISCUSSION

We found that the often used cloth filters were not effective in removing faecal coliforms from the contaminated drinking water. In some samples, FCC increased after filtration possibly because the cloth filters themselves were contaminated. The faecal coliforms increased even after the cloth

filters were washed with soap and respective water because these filters were washed with contaminated water. This study shows the fact that the ubiquitous cloth filters that are used in the majority of Myanmar homes are not effective in removing faecal coliforms (and possibly any other bacteria as well including pathogens) from contaminated drinking water.

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Table. Faecal coliform (FC) isolations from contaminated drinking water sources before filtration (BF) and after filtration with one layer (AF₁), two layers (AF₂) and a single layer soap washed (AF₃) cloth filters, Yangon, 1990.

Water sample type	Median (range) of FC counts (MPN ^a /100 ml)			
	BF	AF ₁	AF ₂	AF ₃
Tap (N = 6)	32.0 (0 - 93)	78.0 (0 - 460)	20.5 (0 - 93)	7.0 (0 - 9)
Shallow well (N = 7)	110.0 (93 - 11000)	93.0 (75 - 4300)	150.0 (43 - 2400)	43.0 (43 - 460)
Pond (N = 7)	240.0 (93 - 46000)	460.0 (23 - 46000)	460.0 (43 - 46000)	350.0 (23 - 11000)

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CASE REPORT

Glomus tumour of the stomach

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INTRODUCTION

The presence of glomus tumours in parts of the body other than at the cutaneous-subcutaneous junction is infrequent. Such tumours have been identified in the joint space of the knee (1), in the trachea (2), in the mediastinum, and several have been noted in the stomach (3,4). This report is occasioned by an experience with what appears to be the rare instance of gastric glomus tumour. As glomus tumours of the stomach are very rare, the following case report may be of interest.

Clinical history

A 32 year old house-wife was admitted to the Surgical Unit, New Yangon General Hospital on 5-5-86. Her main complaints were extreme weakness and fainting attacks of one day duration following an attack of massive melaena. She neither gave history of peptic ulcer before nor recent ingestion of drugs. Pulse rate was 120 per minute, and blood pressure was 87/60 mmHg. As she was very pale and anaemic, two units of blood were given immediately. Post transfusional haemoglobin was 6 gm%. White blood cell count was 6700 per c.mm. with a normal differential count. Platelets were adequate. Routine per rectal examination did not reveal significant abnormality.

Gastroscopy

Gastroscopy was done on 13-5-86. A

tumour 4 cm. in diameter was found in the anterior wall of the antral region of the stomach, just proximal to the pylorus. An ulcer was also seen at the surface of the tumour. The gastroscopic diagnosis was leiomyoma of the stomach with superficial ulceration leading to gastrointestinal haemorrhage.

Radiology

Barium meal showed a well defined circumscribed filling defect in the anterior wall of the gastric antrum measuring about 4 cm x 4 cm. Bridging mucosal folds were noted. A small barium depot in the filling defect was suggestive of an ulcer (Figure 1). The radiological impression was submucosal tumour with ulceration.

Surgery

The patient had another four units of blood transfused, and planned for elective surgery. Operation was carried out on May 21, 1986. A tumour mass about 4 cm. in diameter was found occupying the anterior wall of the antrum. At the apex of the tumour, towards the gastric lumen, a pea-sized ulcer was seen, with a dark central spot. Because of the unknown nature of the tumour, a lower partial gastrectomy and gastrojejunostomy was performed. Examination of the rest of the stomach, the intestines, liver and spleen did not reveal any other pathology. Two units of blood were given during the operation.



Figure 1. Barium meal X-ray of the stomach showing antral filling defect.

Pathology

Grossly, the specimen consisted of a small segment of the pyloric end of the stomach. The anterior wall of the stomach contained a submucosal tumour with a diameter of 4 cm. At the apex of this tumour, the mucosa was ulcerated (Figure 2). No enlarged lymph nodes were detected in the perigastric lymph nodes.

Histologically, the tumour was enveloped for the most part in the muscularis and lamina propria. A distinct fibrous tissue capsule was not present, but surrounded by a mass of hypertrophic partly hyalinized smooth muscle. Contained within this was a vascular tumour. The vessels were all lined with normal endothelial cells. Their



Figure 2. Resected gastric specimen with glomus tumour.

walls were thickened by the presence of many small round cells with large, centrally placed, prominent nuclei and an inconspicuous, slightly acidophilic, nongranular cytoplasm. A few cells had clear cytoplasmic spaces partly surrounding the nucleus. A few of the vessels had some smooth muscle in their coats with the tumour cells outside it (Figures 3&4). With reticulin fibre staining by Gomori's method it was found that the vascular lumina were surrounded by a narrow zone of reticulin fibres of varying density. These fibres defined small nests of two to four glomocytes and occasionally surrounded individual cells (Figure 5), same as a characteristic pattern, described by Appelman and Helwig (5). Final pathologic diagnosis was glomus tumour of the stomach.

DISCUSSION

The first gastric glomus tumour was identified in 1947 and reported with two other examples in 1951 by Kay



Figure 3. Angiomatous pattern of tumour showing vessel walls thickened with glomocytes, H&E stain.

et al. (3) In 1969 Appelman and Helwig summarized 17 previously reported cases and 12 cases from the Armed Forces Institute of Pathology (5). Kanwar and Manaligot had documented and summarized 67 cases from the world literature and reported another case of glomus tumour of the stomach in 1975. Analysis of the reported cases showed that the tumour occurred in any age group (18 to 89 years) and had no predilection for either sex (6). Our case is in conformity with the age, symptoms, site, submucosal location and characteristic histologic features of a glomus tumour of the stomach as documented by Kanwar and Manaligot. Because there are no specific clinical or radiologic features associated with the glomus

tumour, it can be recognized only by its histologic features. Available data are inadequate for determining the histogenesis of the tumour. It may be a neoplasm, a hamartoma, or a functional hyperplasia (5). Diagnosis from a frozen section is often difficult. The glomus cells are small and round and are often densely packed together. Tissue from solid area prepared by the frozen techniques having many of the resultant artifacts might be easily confused with other round cell lesions, notably lymphomas and carcinoid tumours. Therefore it is necessary to recognize that such a lesion as the glomus tumour does occur in the stomach, and with the proper diagnosis on frozen section could prevent unnecessary extensive stomach resection.

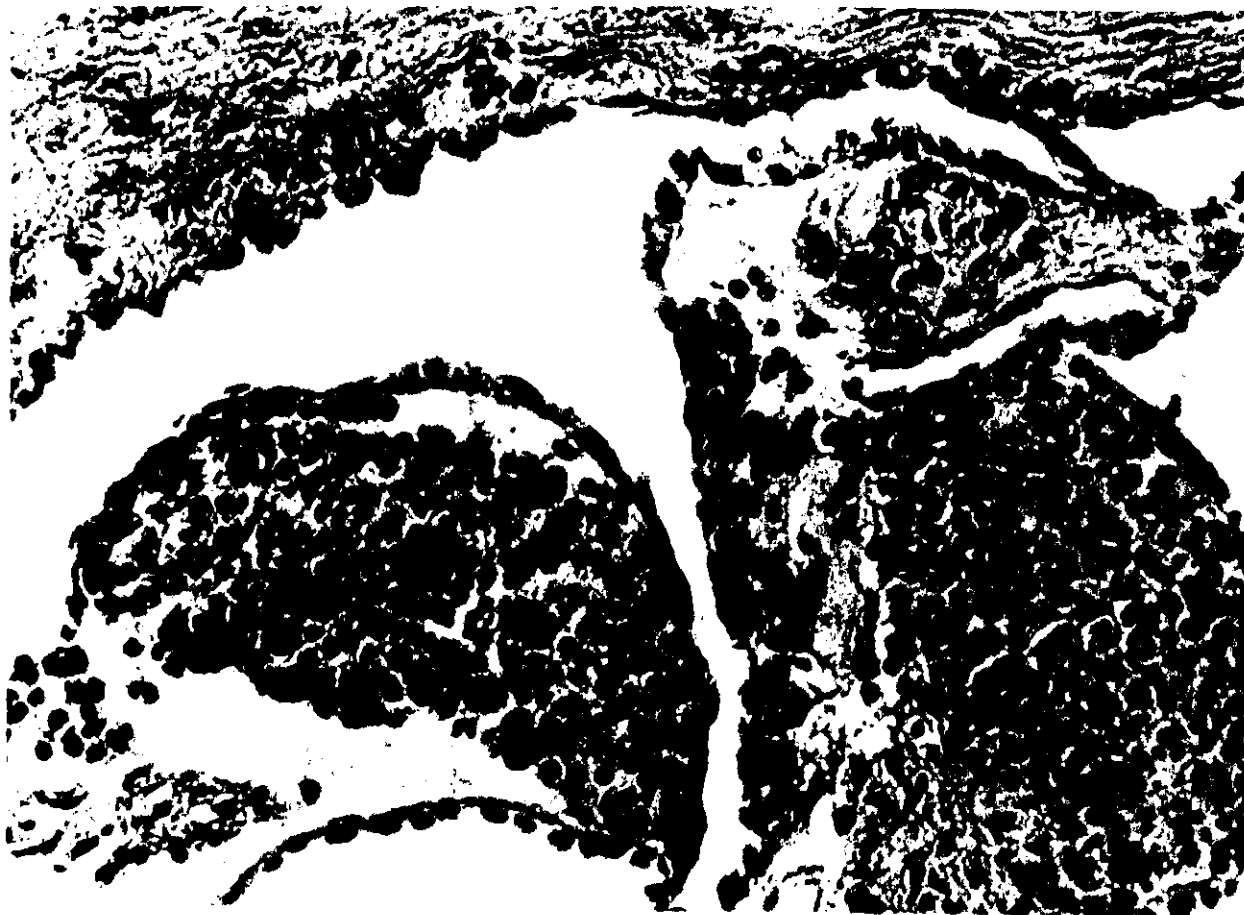


Figure 4. Small round glomocytes with large centrally placed prominent nuclei and fairly clear cytoplasm, H & E stain

Most gastric glomus tumours are benign but a case of malignant glomus tumour of the stomach had been described which showed metastasis to the liver, lymph node and peritoneum (Shtern R D and Vasnetsov N S 1974), (7). Despite evidence of tumour spread, however the patient lived more than 15 years after surgical resection. Hence for all practical purpose, glomus tumour of the stomach should be considered a benign lesion that is best treated by conservative surgical resection (Almagro 1981), (8).

SUMMARY

The clinical symptoms and the patho-

logical anatomical findings associated with a glomus tumour in the gastric wall, proximal to the pylorus, in a woman, aged 32, are described. Review of the literature discloses an apparent propensity of these tumours to occur in any age group and had no predilection for either sex and to be associated with upper gastrointestinal bleeding.

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Figure 5. The reticulin fibres surround the vascular lumen and small nests of glomocytes. Gomori's silver stain

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