MYANMAR
HEALTH SCIENCES RESEARCH JOURNAL

Volume I , Number 3 December 1989

DEPARTMENT OF MEDICAL RESEARCH
No. 5, Ziwaka Road,
Yangon 11191, Myanmar

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Printed at the Publications Division, Department of Medical Research

Study of the effect of single does primaquine on gametocytaemia and infectivity among Amodiaquine–treated *P. falciparum* malaria patients

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One hundred and five patients who attended Thayarwady Civil Hospital during 1987 and 1988 malaria seasons were studied. They were divided into three groups. Among 35 patients who were treated with amodiaquine alone, 23-33% of the patients showed gametocytes in blood during days 14, 21 and 28 of therapy. Among those patients with gametocytes in blood, 50 to 63% of patients showed that they were viable by exflagellation method. Follow-up of one patient showed viable gametocytes in blood till 51st day of drug therapy. Among 45 patients treated with amodiaquine and primaquine (45mg single dose given on day 3), 31% of patients showed gametocytes on day 7, later only 0-10% of patients showed gametocytes on day between 14, 21 and 28. Among the patients with gametocytes in blood 25% of the patients showed that they were viable till day 4. From day 5 they were not viable. Except one patient who showed gametocytes at day 28 was proved to be RII resistant to amodiaquine. Among 25 patients treated with amodiaquine and primaquine (45 mg single dose : given on day 1 of hospital admission) 36% showed gametocytes on day 7 and 42% showed gametocytes on day 28. Of these 26% patients with gametocytes which appeared on day 28 was proved to be viable. It is suggested that primaquine 45mg is effective to control infectivity of gametocytes in blood. It is also suggested that primaquine should be given on 3rd day of hosptital admission to get the most beneficial effect.

INTRODUCTION

To have a significant impact on the transmission of falciparum malaria, Pan American Health Organization in 1982 (1) made the recommendation that Primaquine should always be administered whenever a schizontocidal drug is used.

As a part of an effort to interrupt transmission of drug resistant strain, World Health Organization Scientific Group meeting in 1984 (2) also suggested that a single dose of 45mg primaquine base be given to sterilize the gametocytes. They also noted that research is needed to determine the optimum schedule for such a combination.

In the months and years that have passed since these meeting, there has been little effort to follow these recommendations. A search of the files of the National Medical Library for 1984 and 1985 produced 58 references to primaquine and not one relates to these recommendation (3). Some studies on primaquine was undertaken in Thailand.

Chomcharn et al (4) conducted the study of the effect of single dose primaquine on

P. falciparum gametocytaemia and mosquito infectivity in Thailand. They found that 10 patients with gametocytaemia when given 45mg primaquine were all cleared by day 6, but among the controls gametocytes were positive up to day 17.

Bunnag et al (5) had also conducted field trial of 3 regimens of primaquine (15mg daily X 5 days, 30 mg single dose and 45 mg single dose) on 121 patients with initial gametocytaemia. They reported that the gametocytes disappeared by day 21 in all groups. In the 194 patients without initial gametocytaemia, gametocytes developed in over 25% of them. They cleared again on day 28.

They commented that further work on the infectivity of the gametocytes is required.

But in our experience, with 45mg primaquine given on day 1 of hospital admision to falciparum malaria patients together with 1500mg amodiaquine or 1500mg sulphadoxine and 75mg pyrimethamine gametocytes persisted in blood of 7 out of 23 patients at day 7 and on 1 out of 5 patients at day 28. Thus there may be persistance of gametocytes in Myanmar patients though they were treated with 45mg primaquine. Thus this study is needed.

PATIENTS AND METHODS

One hundred and five patients who attended Thayarwady Civil Hospital during 1987 & 1988 malaria seasons were studied. Thirty five patients with falciparum malaria parasite in blood with parasite counts 1000-200,000/cumm were treated with amodiaquine (total dose of 1500 mg divided in 3 days). The presence and viability of gametocytes in blood were followed at day 1,3,7,14,21 and 28. One patient who had gametocytes in blood throughout this study was followed weekly till his gametocytes were disappeared from circulation.

Second group of 45 patients with P. falciparum malaria were treated with amodiaquine (dose as above)and primaquine 45mg single dose- the later was given on 3rd day of hospital admission. The presence of gametocytes and viability were followed on days mentioned above.

Third group of 25 patients with P. falciparum in blood were treated with amodiaquine (dose as above) and primaquine 45mg single dose given at day 1 of hospital admission. They were also followed as above.

Viability of gametocytes were tested by incubation of blood on glass slide with a drop of normal saline and storing in -20° C for 5-10 minutes. The presence of exflagellation of male microgametocyte was recorded as an indicator of viability.

RESULTS

Among the 35 patients treated with amodiaquine alone, 23-33% of the patients showed gametocytes in blood during days 14, 21 and 28 of therapy. Among those patients with gametocytes in blood, 50-63% of patients showed that they were viable by exflagellation method. Follow-up of one patient showed viable gametocytes in blood till 51st day of drug therapy.

Among 45 patients treated with amodiaquine and primaquine (45mg single dose : given on day 3), 31% of patients showed gametocytes on day 7, later only 0-10% of the patients showed gametocytes on days between 14 and 28. Among the patients with gametocytes in blood 25% of the patients showed that they were viable till day4. From day 5 onwards they were not viable. At day 28, gametocytes reappeared in 1 patient. He was proved to be viable. He was also resistant at RII level to amodiaquine.

Among 25 patients treated with amodiaquine and primaquine (45mg single dose; given on day 1 of hospital admission) 36% showed gametocytes on day 7 and 42% showed gametocytes on day 28. Of these patients with gametocytes which appeared

Table: Percentage of patients with Gametocytes and their viability among patients treated with three groups of drugs

Days	AMQ (1.5G) only N = 35		AMQ (1.5G) with Primaquine 45 mg on Day 3 N = 45		AMQ (1.5G) with Primaquine 45 mg on Day 1 N = 25	
	% of patients with gametocytes	% with viable gametocytes	% of patients with gametocytes	% with viable gametocytes	% of patients with gametocytes	% with viable gametocytes
1	60	90	53	78	50	40%
3	54	90	43	25*	40	0%
7	56	70	31	0	36	10%
14	33	63	7	0	-**	-**
21	23	50	0	0	-**	-**
28	30	50	10	-	42	28%

* Done on Day 4.
** Not Done.

on day 28, 28% was proved to be viable. See Table 1 for details.

DISCUSSION

Mackerras and Ercole (7), Jeffrey et al (8) and Rieckmann et al(9), had reported the action of primaquine on gametocytes as quick action (in 6-12 hours infectivity is lost) and P. falciparum gametocytes are effectively eliminated from the circulation in 2 to 4 days.

Rieckmann et al (9), Burgess and Bray (10) and Chomcharm et al (4) had all reported disappearance of gametocytes when treated with 45mg primaquine by day 6 (4-8 days).

Bunnag et al (5) from their studies in Thailand also reported persistence of gametocytes after various dose of primaquine till day 21. In this study there were persistence of gametocytes after primaquine even at day 28. This delay in the disappearance of gametocytes in the circulation after primaquine in patients from Myanmar and Thailand could be due to development of partial resistance of gametocytes to eliminate from circulation after primaquine.

But as all the gametocytes that are persisting in circulation are not viable (except one patient with RII resistance to amodiaquine) we can assume that this present treatment regimen. Primaquine given on day 3 of patients admission is effective to control viable gametocytes in blood.

Among the patients treated with amodiaquine primaquine given on 1st day of hospital admission, 36-42% of patients had reappearance of gametocytes in blood betwen days 7 and 28. Of these two patients who had reappearance of gametocytes at day 28 was tested for viability. One of them was proved to be viable.

Since the half life of primaquine is very short probably the single dose of primaquine may have the ability to clear the gametocytes already in the circulation but it may have no ability to remove new gametocytes which are released into circulation from the still living trophozoites of RII level resistant patients.

Similar reasons may be given for patients who were treated with primaquine at day 1 instead of day 3.

Thus untimely administration of the drug (primaquine given on day 1 instead of day 3) may be the cause of failure of the action of primaquine. From this study, we support the idea of adding primaquine to schizontocidal drugs but we suggest to give primaquine on day 3 of treatment, when the malaria parasites almost disappeared from circulation instead of day 1.

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Clinical significance of IFAT test on falciparum malaria patients

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The indirect fluorescent antibody technique (IFAT) has been successfully used in sero-epidemiological studies of malaria (1,2). Whether its clinical application to help in diagnosis or as indicator of prognosis was studied on a total of 83 patients (belonging to different categories of clinical severity) and 100 controls (contact of malaria patients) were studied. The findings are shown in Table 1.

Table 1. Seropositive rate of subjects tested

Patient groups	No. of patients studied	Percentage with positive IFAT	Day 0 GMRT
Test groups: (category)			
(1) Patients with no complication	30	86	485
(2) Patients with complications except cerebral signs & symptoms	29	79	86
(3) Patients with cerebral signs & symptoms	24	83	275
a. Pregnant	3		645
b. Severe [illegible]	[illegible]		562
Control group:	100	33	-

There was no relationships between severity of the categories and the IFAT-positivity rate. Geometric Mean Reciprocal Titre (GMRT) of the patients at day 0 of hospital admission varied widely in different groups of patients (Table 1). A time-course profile study on 9 patients from category (3) and 26 patients from category (2) were followed on day 0, 3, 7, 14, 21 and 28. There were no significant alternations in the level of GMRT during the study period.

The study concluded that IFAT test is of no help in diagnosis and GMRT cannot assist the prognosis of P. falciparum malaria patients.

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The Myanmar Health Sciences Research Journal, Vol. 1, No. 3, 1989.

Population mobility and malaria contraction on either side of Bago Yoma in Bago Division

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During August 1983 to February 1984, household surveys were carried out in four rural areas on either side of Bago Yoma in the Bago Division to describe the extent and patterns of population mobility by enquiring about their travelling history with night visits to the forested foothill areas for the past one year. Those people residing close or very close to foothill travelled most (12 to 28%) during the wet season while those residing some distance away spent their nights most (14 to 17%) during the dry season. The closer the village to the foothill, the longer a forest traveller stayed in the forest, the range being 45-170days per year in the foothill and 12-14 days per year in the plain villages. Of those forest travellers, there were a few percentage (5 to 20%) of constant habitual traveller to the nearby forest. In addition, at least one member of 40 to 70% of the households spent one night visit in the forest. The implications of the various migratory groups between the villages and the Bago Yoma were discussed in the context of malaria contraction, and control.

INTRODUCTION

Malaria has been identified as the first or second national priority health problems for control in the three sucessive 4-year terms of the People's Health Plan since its launching in 1978 (1,2,3). Malaria incidence varies according to topographic conditions of Myan-Mar. It constitutes about 20% or more of the total hospital admission in regions with forested mountain ranges such as in the Chin and Kachin States in Northern Myanmar, while the incidence is low in the deltaic region as in the Ayeyarwady Division where malaria admission to hospitals are less than 10% of the total in-patients (4).

The purpose of this paper is to describe the extent and patterns of population mobility of villagers in villages along the eastern and western sides of Bago Yoma in the Bago Division of Myanmar. The present findings were based on the exploratory surveys and geographical reconnaisance for selecting a suitable site to study the dynamics of malaria transmission in a representative foothill and plain areas in the Bago Division.

MATERIALS AND METHODS

Study areas and populations : These study areas were chosen among the villages near or having communication with the forest fringes of the Bago Yoma (mountain range) both on its eastern and western sides (Fig). The Bago Yoma originates just outside the Greater Yangon city and runs northwards for about 450 km. It has about 30 km in its widest width. It is covered with thick forest along its whole length and breath, especially in its southern half.

All together, four sites were visited. The information on each study village with regard to distance from the Yoma, number of households and population, children's spleen and infant parasite rate is shown in the Table.

Household interview: All the households in the villages except random samples (about 40%) in site 4 were visited using a standardized questionnaire. The head or senior adult member of each household was interviewed about the habitual behaviour of all the household members concerning travelling history with night

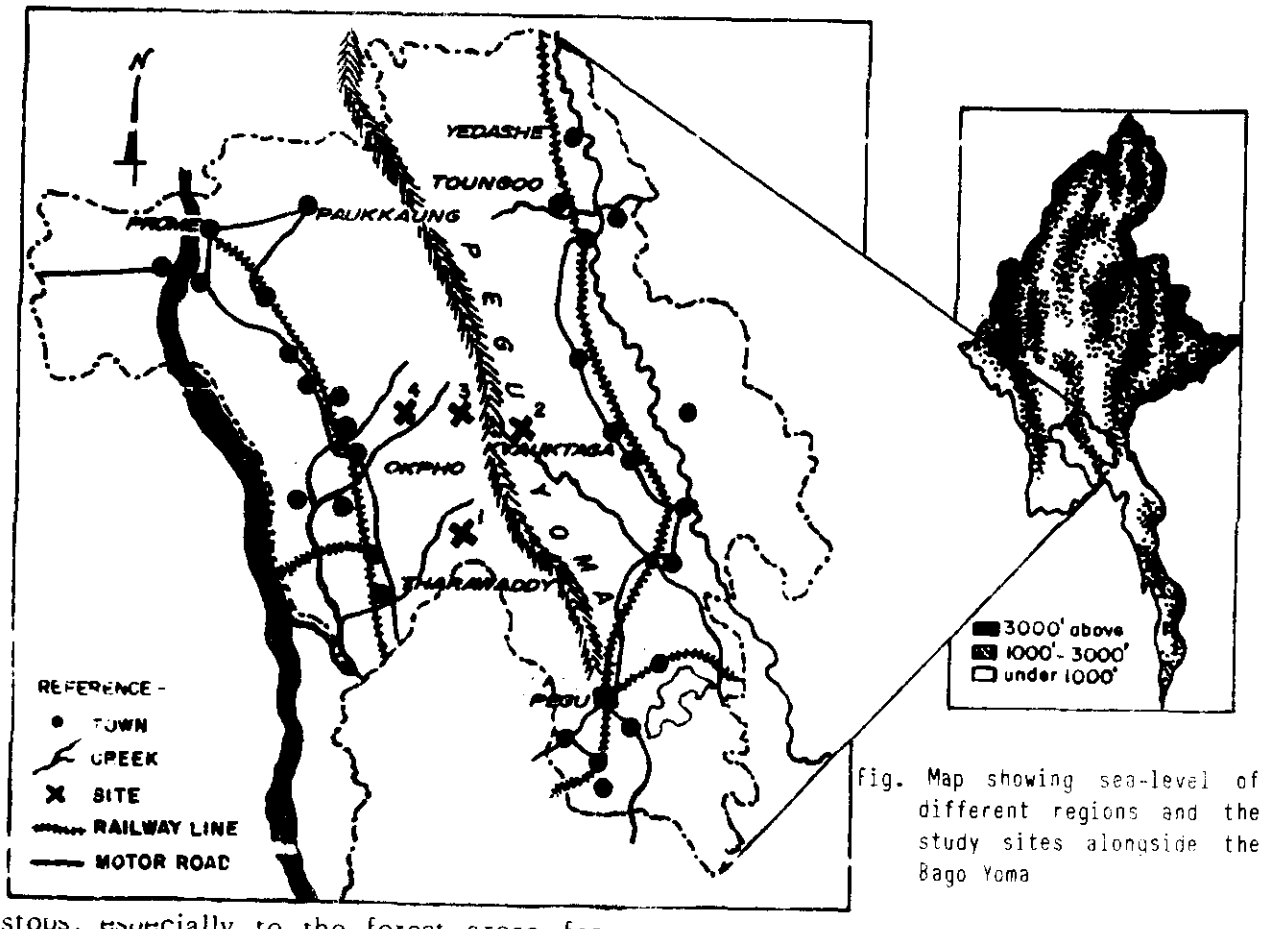

fig. Map showing sea-level of different regions and the study sites alongside the Bago Yoma

stops, especially to the forest areas for the past one year.

RESULTS

Site 1 : On the day of survey, a proportion(about 11%) of the population had moved out temporarily in whole families to work in government agricultural farms near the foothill and another 12% of population had gone out on night-stop visit to the forest in the foothill. As regards travelling to the forest by season, 7.7% of the population travelled in summer, 18.0% in monsoon and 5.8% in winter. Again, about 5% of them were habitual forest travellers in all seasons. In site 1, the numbers of travelling households (which had at least one household member spending one night in the forest) were 71.2%(Table). On the other hand, the average duration of days spent per traveller per year in the forest was 45 days (Table).

Site 2 : On the day of interview, 4.6% of the population from Myochaung and 2.4% from Da-nyin-gone villages had temporarily moved out in whole families to work in the forest area. Also, 9.3% from the former and 21.0% from the latter were travelling out of the villages to the forest. Regarding the travelling status by season, in both the villages, most people travelled out during monsoon, 28% in Myo-chaung and 13% in Da-yin-gone. Moreover, only 18.5% and 4.8% of the people were respectively involved forest travelling in all the seasons. In term of travelling household, 71.4% in the former and 53.3% in the latter villages were involved in work connected with the forest (Table). The average duration of travel per traveller per year was 172 days in Myo-chaung and 83 days in Da-nyin-gone (Table).

Site 3 : By season, most of the forest travelling in Waing occurred in winter

(14.9%) and in Kaing-taw-su in Monsoon (13.4%). By all seasons, only 7.7% of the individuals in Waing and 3.4% in Kaing-taw-su went to the forest for work. On average, the numbers of days travelled in the two villages per traveller per year were 47.8 days and 51.2 days respectively (Table). Similarly, 65.7% and 67.6% of the households were communicated respectively with the forest at least once a year (Table).

Site 4 :Most people in Kyun-net-kone and Sein-gyi-yo travelled more in the dry seasons than wet season, and the average duration of travel per traveller per year was shorter (Table). However, at least a household member of the 40% of the househlds have gone once to the forest in a year (Table).

DISCUSSION

Malaria infections were detected in all villages. The two villages that were very close to the forest fringe (Myo-chaung and Da-nyin-gone) and the other two villages near the foothill (Waing and Kaing-taw-su) were malaria endemic areas because of presence of moderately high children's spleen and infant parasite rates (Table) and the finding of An. dirus from the nearby woodcutting camps situated in the deep forest or An minimus in the villages at the time of survey.(Myo Paing, personal communication). The remaining villages (Hle-lun-gu, Kyun-net-kone and Sein-gyi-yo) may not be malarious as no efficient mosquito vectors were caught at the time of study. The relatively high spleen and infant parasite rates in Hle-lun-gu may be due to moving back to the village of the families who had wotked temporarily in farms near the forest fringe.

The majority of the people residing very close or close to the foothill travelled most during the monsoon while those some distance away spent their nights in the forest during the dry seasons. Similarly, the closer the village to the foothill, the longer a forest traveller stayed in the forest. Thus, population movement to forested foothill from the plain villages might play an important role in contracting malaria, as seen in Hle-lun-gu, kyun-net-kone and Sein-gyi-yo villages.

There are three secondary roads connecting the towns and the respective study foothill villages. From some focus group interviews, it was known that during the summer, many bullock carts and some people on foot from far and near villages converged to the Yoma in a very long procession during day and night for collecting bamboo, thatch leaves and firewood. During the monsoon, the creeks arising from the Bago Yoma are flooded with water, and are used for drifting bamboo in the form of rafts down the stream with a few persons riding on each raft. Thus, there are different types of travel between the forest in the foothill and the far-and-near villages. These different patterns of migration will definitely favour acquisition of malaria infections, transmission and spread of malaria in the region.

In general, the population mobility in the Bago Division is a circulatory type(i.e. population move from place of residence and eventually return to it) like those in Thailand (5) and Bangladesh (6). This pattern is quite different from those movements in many African countries where the mobility usually has spatial characteristics i.e. population move from place to place (7,8).

For effective reduction of morbidity and mortality due ro malaria in the region, it is strongly suggested that operations research on the management and control of malaria at the community level through primary health care approach should be studied in consideration of these various types of high risk group of forest travellers.

Table. Information on study villages by survey period, distance from Bago Yoma, population, malaria endemicity indicators and forest -travelling in Bago Division

Survey site	Survey period	Township	Village	Distance from Bago Yoma(km)	No. of house-holds	No. of popula-tion	Spleen rate* 2-9Yrs	Infant parasite rate*	Annual percentage of forest-travelling household	Annual duration of stay per forest traveller(days)
1	25-28 Aug , 1983	Tharyar-waddy	Hle-lun-gu	16	257	1109	62.0	49.9	71.2	45.2
2	9-14 Nov , 1983	Kyauk-taga	Myo-chaung	0.5	119	570	63.0	37.5	71.4	172.1
			Da-nyin-gone	1.6	107	491	43.0	17.4	53.3	83.1
3	25-29 Jan, 1984	Okpo	Waing	5	105	509	36.4	22.5	65.7	47.8
			Kaing taw-su	5	99	524			67.6	51.2
4	28-29 Feb, 1984	Gyo-pin-kauk	Kyun-net-kone	20	27**	137	0	26.1	40.07	12.5
			Sein-gyiyo	20	33**	187			45.5	13.6

* Data form Parasitology Research Division, Department of Medical Research
** Based on samples

ACKNOWLEDGEMENT

The study was undertaken under the Institution Strengthening Grant from WHO/TDR.

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The Myanmar Health Sciences Research Journal, Vol. 1, No. 3, 1989.

Anopheline Mosquitoes of Myanmar. I.

Anopheles (cellia) dirus Peyton and Harrison, 1979

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Results of the study involving distribution, breeding habitats, biology under laboratory conditions, adult bionomics and its relationship to malaria transmission under different ecological conditions have been studied and presented. Cytogenetics and iso-enzyme studies to differentiate its sibling species were also presented. Of the sibling species of An. dirus, species D was found to predominate in Myanmar, whereas species A was recorded from Central Burma. Susceptibility of An. dirus to DDT and Bacillus sphaericus was also reported.

INTRODUCTION

Malaria workers in Burma previously referred to the leucosphyrus group of species as An. leucosphyrus. Tin (1) and Rao (2). Christophers (3) included Upper and Lower Burma in the distribution fo An. leucosphyrus. Khin-Maung-Kyi(4) examined specimens of An. leucosphyrus from the collections at the Malaria Institute of Myanmar and identified them as An. balabacensis balabacensis Baisas, which he incriminated as an important vector of malaria in Myanmar. When the Department of Medical Research (DMR) initiated studies on malaria vectors in 1983, the prevalence of Anopheles dirus Peyton & Harrison was recognized for the first time in Burma. Subsequent surveys by DMR teams in various parts of the country did not yield any An. balabacensis- balabacensis. In view of the facr that An. dirus is a primary vector of malaria in Burma and that the last detailed study of this species was carried out about 19 years ago, during which period many ecological changes have occurred, DMR endeavoured to investigate into its bionomics and relationship to malaria transmission under varied ecological conditions. In addition to detailed morphological studies, cytogenetics & isoenzyme studies were undertaken to differentiate sibling species. Breeding habitats were surveyed with special ecological studies being carried out in domestic wells of Mudon Town. Adult bionomics with special reference to biting and resting behaviours were critically studied. Other relevant data pertaining to its role as a major vector were also collected.

Identification

Identification of An. dirus was done according to Peyton & Harrison(5). The main morphological characters used for identification being:

(a) Adult wing : Basal Extension fo dark mark on Vein R extending to the level of humeral dark area on Costa.

(b) Pupal chaetotaxy : Length of Seta 9, IV approximately 0.50 of Seta 9,V

Distribution in Burma

Khin Maung Kyi (4) described the distribution of An. balabacensis balabacensis in Burma and stated that this species is mostly associated with forested foot-hills and deep forests (Fig 1).

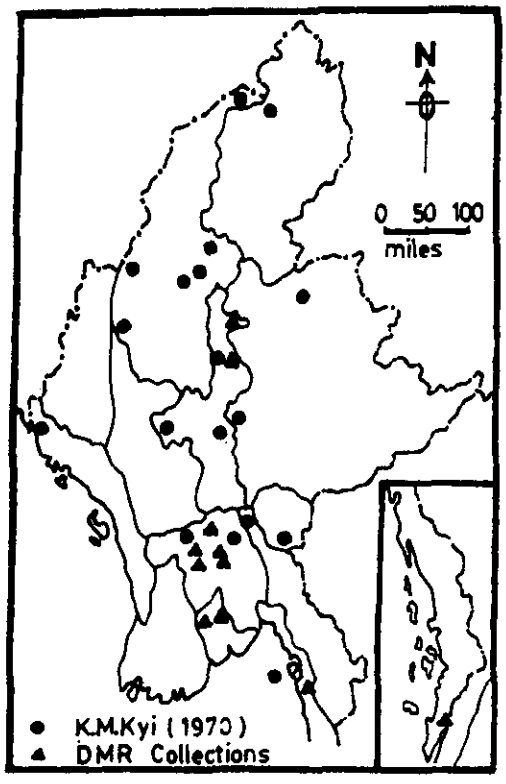

Fig.(1).Map of Burma showing distribution of An. dirus

DMR entomological survey teams collected An. dirus from the following locations Table 1.

Breeding habitats

Khin Maung Kyi (4) quoting various reports on work carried out in Myanmar during the late 1930s and in the 1940s, stated that An. balabacensis balabacensis is essentially a jungle breeder found in rain-filled trenches, puddles and marshes in the forest and in rocky beds of ravines. Based on these observations, Khin Maung Kyi concluded that this species is capable of breeding in a great variety of stagnant waters not exposed to sunlight. Breeding of An. dirus in wells of Mudon was reported by Vector-borne Diseases Control Unit in their 1980 Departmental Annual report (6).

DMR survey teams located An. dirus breeding sites in the following locations.

(1) Mudon : Out of about 900 domestic wells in Mudon Town, An. dirus larvae could be collected from about 50% during the rainy season, especially from Kwan-ka-thaung (Myoma 4 ward). In the dry season, larval density decreases but some larvae can still be located from some wells in Kwan-ka-thaung. The domestic wells, which are in daily use, are lined by laterite rocks and are situated in shaded areas.

(ii) Phado : At Hti-phado wood cutting camp situated in a deep forest area 5 miles from the forest fringe, An. dirus larvae were found breeding in rocky pools along the banks of a stream under dense shade.

(iii) Tha-bye-wa : (a) Rock pools in a tree shaded ravine situated deep inside a teak forest reserve.
(b) Bamboo stumps in the teak forest reserve situated on a hill east of Tha-bye-wa.

Moe Moe (7) investigated into the ecological factors influencing the breeding of An. dirus in the wells. The factors that directly influence breeding were found to be:

(a) Rainfall : Rainfall was found to be an important factor. During the rainy season, the larval density in the wells of Kwan-ka-thaung (Myoma 4) ward, Mudon increased significantly. Similarly, larval density increased in wells of Myoma 3 ward. However, the remaining wards, Myoma 1 and 2 had very little An. dirus breding in the wells, even in the rainy season. In the dry season, An. dirus larvae could be collected only from Kwan-ka thaung (Myoma 4) ward.

(b) Water level of the wells : Distance of water level of the wells was found to be another important factor. Deep wells are more shaded and provide more attractive oviposition sites for An. dirus. The wells in Kwan-ka-thaung (Myoma 4) Wards which were found to be deeper

Table 1. Location where An. dirus was collected by DMR survey teams

Locality	Township	Season	Topography & Remarks on location
YANGON DIVISION			
Ye-sit-kan; Gyo-byu	Taik-kyi	Jun-Jul	Reservoir & catchment area with thick forests. Yangon Water Supply Center.
Lein-hmaw-chan	Taik-kyi	Jun-Jul	Low foot-hills with thick vegetation & forest
Ye-net-kyee, Phaung-Gyi	Hle-gu	June	Low foot-hills with thick forest. Road construction camp
BAGO DIVISION			
Hti-phado, Phado	Kyauk-ta-gar	Jun-Feb	Deep forest wood-cutting camp in Bago Yoma range
Kyar-kyaung-thaik Phado	"	Jun-Jan	Temporary settlement on edge of forested foot-hill
Ngok-to & Gwe-gon,	"	Jun-Jan	Villages within 1½ miles of forested foot-hill
Kat-se-ne Camp,	"	June	Timber extraction camp in Bago Yoma range Deep forest.
Ye-bya Camp	Ok-po	Nov	Timber extraction camp in deep forest of Bago Yoma range.
Waing & Kaing-taw-su villages	Ok-po	Nov	Villages at the foot-hills of Bago Yoma
Tha-bye-wa village	Oktwin	Jul-Jan	Village surounded by Teak forest reserve and teak plantations. Situated in a valley in Bago Yoma range at an altitude of 700' above sea level
MANDALAY DIVISION			
Se-daw-gyi	Madaya	Aug-Sept	Foot-hills & near forest in an irrigation system area
Kabaing	Mogok	Aug-Sept	Hilly area with forest at an altitude of about 4000 feet above sea level
MON STATE			
Kwan-ka-thaung Ward, Mudon Town	Mudon	Throughout the year, but very few during Feb to Jun	One of the four wards comprising Mudon urban area. Edge of town near rubber plantation
TANINTHARYI DIVISION			
Nam-tun Border Camp	Kawthaung	January	Deep forest timber extraction camp adjacent to Isthmus of Kra on Thailand. Hilly topography with many perennial streams in the area

than those in other Wards were harbouring more An. dirus larvae.

(c) Shade , gruss and shrubs on inner walls and debris on water surface : Shade and debris in the wells were also found to be yet another important factor influencing breeding of An. dirus. 70% of the An. dirus positive wells had shade & shrubs on sides and debris on water surface.

(d) Salinity of the water : It was observed that Sodium chloride (NaCl) concentration in the well water had an inverse correlation with the mean density of An. dirus larvae & pupae per dip. In Mudon wells it was observed that at NaCl concentrations above 200 ppm, the larvae and pupae collected per dip decreased to a very low level.

The factors influencing the breeding of An. dirus in the deep forest breeding sources such as rock pools in ravines or stream beds are :

(a) Situated under heavy shade in deep forest. No direct sunlight falling on the water surface.

(b) Breeding water was found to be clear and clean but the pools contain large amounts of decayed leaves at the bottom.

(c) Many species of Culex were found to be co-breeding in these pools.

A few bamboo stumps in the deep forest were found to harbour An. dirus breeding. Water in these bamboo stumps were also found to contain decayed leaves. Co-breeders identified were Aedes (Finlaya) albolateralis (Theobald) and Orthopodomyia albipes Leicesrer, the latter incidentally being recorded for rhe the first time in Myanmar. : in

Adult Bionomics

Seasonal prevalence : Khin Maung Kyi (4) basing on surveys carried out by his staff from 1959 onwards, stated that An. balabacensis balabacensis (= dirus) is prevalent from June to October, with its peak in August and September. DMR survey teams at Phado, during two years of longitudinal surveys, observed that An. dirus was collected regularly fron June till February from foot-hill villages of Bago Yoma. This species was more prevalent in the moonsoon months and the peak density was obtained during September. In the deep forest wood-cutting camp of Hti-phado, 5 miles inside Bago Yoma, high density of An. dirus was still collected in February.

These An. dirus mosquitoes were also presumbaly having a good survival rate at Phado as observed from the Table (2) presenting man-biting rates and vectorial capacities. In comparison to the foot-hill village man-biting rates (mbr), the rate in Hti-phado Camp was found to be about 30 per man night, an excessively high rate.

Table 2. An. dirus vectorial capacities & man-biting rate at Phado (1984-1986)

MONTH	An. dirus				Raintall(mm)	
	1984/1985		1985/1986		1984/85	1985/86
	ma	v.c	ma	v.c		
Sep/Oct	1.14	2.63			346	176
Oct/Nov	1.8	5.68			112	60
Nov/Dec	0.44	0.13	1.27	2.1	-	76
Dec/Jan	0.95	0.60				
Jan/Feb	0.25	0.79				
Feb/Mar	0	0	0.09	0.16		
Mar/Apr	0	0				
Apr/May	0	0			14	33.5
May/Jun	0.45	1.71	0.82	0.11	392	55.5
Jun/Jul	0.91	3.41			871	388.6
Jul/Aug	0.73	2.77			637	642.2
Aug/Sep	1.36	0.21			1039	656.0
Sep/Oct	0.64	0.14	0.55	1.19	176	289.1
Total	0.77		0.68			

ma= man biting rate v.c= vectorial capacity

At Tha-bye-wa village, Oktwin Township, a man-biting rate of 4.6 is obtained during July 1987, Myo Paing et al (8). In January 1988, only 3 An. dirus were caught during the whole trip whereas in February, none could be collected.

In the deep forest timber extractio camps in Kawthaung Township, the man-biting rate of An. dirus recorded during January 1989, was 4.0 per man night.

From the Department of Medical Research (DMR) survey results it can be concluded that An. dirus was prevalent from June to February in the deep forest and foot-hill areas with a peak in the late moonsoon month of September.

Relationship of density to distance from forest : DMR survey team results show that there was definite relationship between An. dirus man-biting rate (mbr) and the distance of the catching station from the forest. Highest (mbr) was observed at Hti-phado wood cutting camp. Next highest rate was at Kyar-Kyaung-thaike (KKT) temporary settlement at the edge of forested foot-hills. At Ngok-to (NGT) village about 1/3 mile from the forest edge, An. dirus was collected at a lower density, while at Gwe-gone village (GG), situated about 1½ miles away from the forest the density of An. dirus recorded was very low. Similar situation exists for An. minimus in the same Phado area, Myo Paing et al (9).

Biting habits

(a) Host preference : Anopheles dirus was collected by DMR survey teams fron both human and animal baited bed-nets and from human baits by direct hand catches. However, no An. dirus has ever been collected by CDC hight traps by our teams. Host preference of An. dirus was studied at Phado by placing two big bed nets (11' X 11' X 6') about 150 feet apart with a house in between and placing two adult humans in one net and a cow in the other. The nets were operated from dusk till just before dawn.

Results obtained showed that of the total An. dirus collected in both the nets, human-baited nets yielded 75-90%. This showed that in Phado, An. dirus was highly anthropophillic. At Tha-bye-wa village the ratio of An. dirus collected by animal baited net to human baited net was 4:46. However, a similar study conducted by one of the authors (W.T.L) at Mudon, showed An.dirus in that area to be more zoophillic although the breeding sites were situated very close to human dwellings.

(b) Biting rhythm : Anopheles dirus biting peak occurred in the 2nd and 3rd quarters of the night(2100 hours to 0300 hours). However, in the cool season its biting activity started earlier. In the early monsoon month of June 1984, An.dirus was caught biting man outdoors from about 0700 hours at Kat-se-ne Forest Extraction camp, Kyauk-ta-gar Township by one of the authors (AAS).

Place of biting

DMR survey teams observed An. dirus to be highly exophilic although not as much as An. minimus. In Phado, the indoor and outdoor biting activities were found to be almost equal during the cold months of November to February. In other areas, An. dirus was observed to be more exophilic in its biting behaviour.

Resting habits

Daytime indoor resting catches carried out longitudinally at Phado, did not yield any An dirus. DMR survey teams collected An. dirus adults resting on under-surfaces of banana leaves at Ye-sit-kan, Taik-kyi Township and in small crevices on the laterite walls of domestic wells in Mudon Town.

Relationship to malaria transmission

Anopheles dirus is regarded as a primary vector of malaria in Myanmar from dissection results and also from epidemio-logical evidences. During the longitudinal

surveys at Phado, out of a total of 147 An. dirus dissected, 2.7% (4/147) was found to be positive for sporozoites.

At Tha-bye-wa, An. dirus collected were not dissected as they were all brought back alive to DMR laboratory for cyto-genetic studies. The total number of An. dirus collected was 46 and the man biting rate was 4.6. At the time of this collection in July 1987, other malaria vectors like An. minimus and An. maculatus were caught in very small numbers. However, blood examination of villagers showed that the malaria incidence was very high, the slide positivity rate in children under 10 years of age being 66.7% and infant parasite rate 50%, Myo Paing et al (8). From this evidence, An. dirus could presumed to be an important vector of malaria in the area. Similar evidences also point out that An. dirus is an important vector in the timber extraction camps of Kawthaung Township.

Colonization of An. dirus in the laboratory

Anopheles dirus has been colonized in DMR laboratory since 1982 using the induced mating technique. The larval food used for the early instars were oatmeal, dry yeast. wheat gum and corn starch or Farex, and for the late instars, a mixed larval food containing Nestum or Lactogen, corn starch of Farex, ox liver powder and Vitamin B complex.

Laboratory biology of An. dirus

Nwe Nwe Yin (10) studied the biology of An. dirus under laboratory condition at the DMR.

Summary of findings are : -

(a) Larval density has a direct relationship to larval mortality. The permissable density for rearing was found to be 200 larvae per tray (size 350 mm long x 240 mm wide x 60 mm deep) and with 500 ml of water.The ideal density is found to be 50 larvae per tray.

(b) At a temperature of 26-32°C pupation starts on Day 7, attains maximum pupation on Day 8 and pupation is completed on day 9, with the females measuring 5.5 mm wing-length. During the cool season when the temperature is between 21-27°C, the larvae take a day longer to pupate, reaching maximum pupation on Day 9 only. However, the adult females obtained were larger, measuring 5.7-5.8 mm wing-length.

(c) Aquatic life span was observed to be as follows:

Larval Stage	Appr: Life span (Days)	
	Monson	Cool
I	2	3
II	1	2
III	1	2
IV	2	3
Pupae	2	3
Total Duration of Aquatic stages	8	13

(d) Highest biting potential is acheived on Day 4 after which the potential falls.

(e) 4 to 5 days old male and female An. dirus give the highest insemination rate. The mating ability of males increases with age till peak is reached on Day 4-5 after which the ability declines.

(f) Ovarian development do not commence within 6 hours of a blood meal. Egg follicles reach Christopher's Stage III-IV after a lapse of 22-26 hours and full development is reached between 44-50 hours after blood ingestion.

(g) Between the temperature 27-31°C, ovipositing females deposit their eggs after a time lapse of 55-56 hours after a blood meal.

(h) Average fecundity of An. dirus was found to be 116-134. About 77% of eggs hatch on day 1 and 90% within day 3 at a temperature of 27-31℃. The average viability of eggs is found to be 91%.

Susceptibility to control agents

D D T : Laboratory-bred An. dirus adult females (unfed) were exposed to 4% DDT discriminating dose at DMR laboratory (temperature 27-31℃). All the adults tested died within the exposure period while there was no control mortality.

Bacillus sphaericus : Anopheles dirus (Mudon strain) laboratory reared 4th instar larvae were exposed to Bacillus sphaericus 2362 and 2297. The LC_{50} & LC_{90} at 24 hours were found to be 0.086 & 0.96 ppm from 2362 and 0.52 & 14.0 from 2297 respectively. Thus, An. dirus was found to be very suspectible to B. sphaericus (Tin Tin Hlaing, personal communications).

Cytogenetic studies for differentiation of sibling species

Myat Myat Thu et al (11) examined two strains of An. dirus having different behaviour & breeding sources. Forest-breeding An. dirus from Taik-kyi was compared with domestic well-breeding An. dirus from Mudon by hybridization, polytene chromosome and iso-enzyme studies. Hybridization tests showed low male fertility in F_1 hybrids which also showed inversion in arm 3R & asynaptic segments in polytene chromosome. A distinct electrophoretic banding pattern difference was also noted, indicating genetic divergence between the two strains. Myat Myat Thu et al (12) compared An. dirus of Mudon with An. dirus of Kanchanaburi, Thailand. The results of hybridization and polytene chromosome studies showed that the two strain are most diverged among the species complex.

Cytogenetic study to differentiate the sibling species of An. dirus have been in progress at DMR. Prof. Visut Baimai of Mahidol University, Bangkok was invited as Consultant and also as a collaborating scientist. Anopheles dirus specimens collected from Tha-bye-wa, Oktwin township and Kwan-ka-thaung, Mudon town were found to be species D according to Baimai et al (13). In early 1989, specimens collected from near the Thailand border in Kawthaung township were also found to be species D, whereas species A was collected from Se-daw-gyi , Madaya township, Mandalay Division, (Myat Myat Thu, personal communications).

Specimens of An. dirus preserved at DMR

The following specimens of An. dirus were preserved at DMR for reference.

(a) Adult specimens
(b) Larval and pupal skin slides
(c) Polytene chromosome slides and photographs.

Colonies of An. dirus maintained at DMR

(a) An. dirus Mudon strain
(b) An. dirus Nam-Tun, Kaw-thaung

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Anopheline Mosquitoes of Myanmar. II.

Anopheles (Cellia) minimus Theobald, 1901

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Department of Medical Research

Anopheles minimus together with Anopheles dirus constitute the primary vectors of malaria in Myanmar. The former has a wider distribution and occurs in higher densities with longer seasonal prevalence and is therefore considered to be the most important malaria vector in the country. Previous studies showed that An. minimus breeds in grassy edges of perennial hill streams, in slow running irrigation channels and terraced rice fields while DMR teams, in addition, found it breeding in small puddles in stream beds together with An. maculatus. At Phado, An. minimus was found to constitute 26.2%, 50.5% and 44.1% in monsoon, cool/dry and hot/dry seasons respectively. Prevalence of An. minimus in relation to distance from forest was presented. Biting, resting behaviour and host preference studies were carried out and the results presented. Man-biting rate and vectorial capacities in relation to rainfall for each month over a year are also presented.

INTRODUCTION

Anopheles minimus and Anopheles dirus are considered to be the two primary vectors of malaria in Myanmar. Of the two primary vectors, An. minimus has a wider distribution in the country as well as higher densities and a longer season of prevalence. Thus, An. minimus could be considered more important as a vector than An. dirus. This species has been studied in Myanmar many years ago by Weeks (1) & Khin Maung Kyi (2) and in the neighbouring countries by Ismail et al (3,4) and by Chow (5). Recently, Myo Paing et al(6) presented the results obtained from a longitudinal study of An. minimus in a forested foot-hill area. The Department of Medical Research (DMR) survey teams have visited many parts of the country for the study of anopheline vectors. During these surveys, facts about An. minimus have been obtained. This paper presents data on An. minimus recorded by DMR teams as compared to previous data available.

MATERIALS AND METHODS

Distribution

Khin Maung Kyi (2) presented the distribution of An. minimus as recorded by his survey teams (Fig 1). It was collected from States & Divisions from all over the country. The location where An. minimus was recorded by DMR teams was presented in (Fig 2) & (Table 1). Anopheles minimus was collected from different ecological situations like (a) plateau areas at Wetwun, Maymyo Township, (b) foot-hill areas like Phado & Tha-bye-wa and Se-daw-gyi (c) deep forest areas like Nam-Tun Camp, Kawthaung Township.

Breeding Habitat

Khin Maung Kyi (2), quoting previous workers, stated that An. minimus larvae were found most frequently breeding in the grassy edges of bags and back waters of parennial hill streams, either unshaded or partially shaded. Other

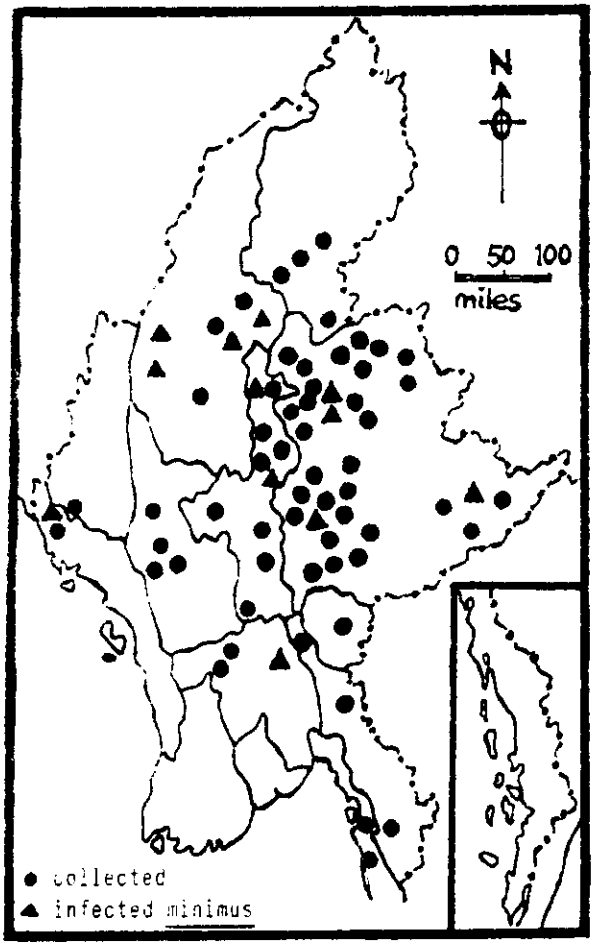

Fig1. An. minimus collected by Khin Maung Kyi

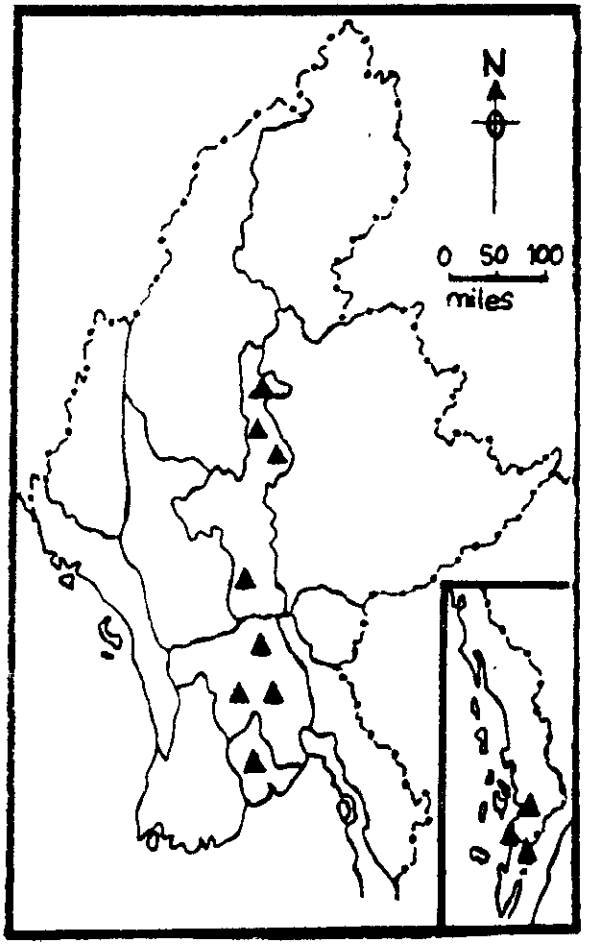

Fig.2. An. minimus collected by DMR teams

breeding places recorded by Khin Maung Kyi were grassy edges of slow-running irrigation channels and terraced rice-fields.

DMR survey teams found An. minimus breeding in the following type of places.

(a) At Phado, Kyauk-ta-gar Township :
  i- edges of slow-running clear streams(together with An. maculatus)
  ii- in small water collections and puddle in the stream bed formed when water flow stopped during the dry season (also together with An. maculatus).
  iii- edges of paddy fields at the end of monsoon
  iv- seepage water collections near paddy fields.

(b) At Wetwun, Maymyo Township, Se-daw-gyi, Madaya Township and Ka-baing, Mogok Township.
  i- Small running streams (sometimes located on the roadside) at points where water flow was extremely sluggish and edges of stream covered with vegetation.

(c) At Ye-net-gyi Road construction Camp, Hle-gu Township.
  i- a stagnent pool contained fairly clear water.

(d) At Tha-bye-wa, Oktwin Township and Shwe-du, Mergui Township.

Table 1. Locations where An. minimus was collected by DMR teams

Locality	Township	Season/Time	Remarks about locality
YANGON DIVISION			
Ye-sit-kan	Taik-kyi	June-July	Near Yangon Water Reservoir with forests.
BAGO DIVISION			
Phado	Kyauk-ta-gar	Dec	Villages within 1½ mile of forested foot-hills near drying stream beds
Tha-bye-wa	Oktwin		
Ye-bya Camp	Okpo	Sept/Oct	Timber extraction camp near Bago Yoma range
MANDALAY DIVISION			
Moe-Swe Camp	Pyinmana	May/June	Adjacent to a thick forest. Timber extraction camp.
Ka-baing	Mogok	Aug/Sept	A hilly area 4000 feet above sea level.
Wet-wun	Maymyo	June/July	Plateau area (3000 feet altitude) with perenial streams
Se-daw-gyi	Mandalay	Sept/Oct	Adjacent to foot-hills & forest. A slow running stream nearby.
Nyaung Thar Yar (larval survey)	Maymyo	Dec	In a plateau area about 3 miles from forested foot-hill. Found in a stream with grassy edges.
TANINTHARYI DIVISION			
Nam Tun Camp	Kawthaung	Jan	Deep forest timber extraction camp
Han-ga-daing camp (larval survey)	Bok-pyin	Jan	Deep forest timber extraction camp near sea
Mon-Su Chaung (larval survey)	Myaik	Jan	Small stream from forested foot-hills flowing through Mon-Su village

i- along grassy edges of small streams in the forest under partial shade. The breeding sources were either inside or very close to deep forest.

(e) Nun-Tun Forest Camp, Kaw-Thaung Township and Han-ga-daing camp, Bok-pyin Township, both situated in deep forest extraction area.

i- small clear-water streams with rocks and grassy edges (together with An. maculatus).

ii- small pools on road side created when building timber extraction trails.

Adult Bionomics

Seasonal prevalence :

Khin Maung Kyi (2) stated that the *maximum prevalence of this species in* all areas was found to be in the post monsoonmonths of October to December, during which the condition of the hill streams were most favourable for breeidng of An. minimus. Khin Maung Kyi also observed that in many areas *this species is abundant from April to* December, although during the monsoon period the larvae were flushed out by rain in many locations.

DMR survey teams found that An. minimus prevalence was throughout the whole year at many locations, although in different densities seasonally.

In Tha-bye-wa, An. minimus was collected in very low numbers during July & August. However, in January and February, hundred of An. minimus could be collected in a single night from one big bed-net (11 x 11 x 6) using a cattle

Table 2. Man-biting rate and vectorial capacities of An.minimus in the forested foot-hill villages of Phado in relation to rainfall

MONTH	Man-biting rate (mbr) Per man night	Vectorial capacity	Rainfall (mm)
1984			
Sept	9.08	4.19	346
Oct	8.69	4.57	112
Nov	9.95	1.65	-
Dec	7.44	1.34	-
1985			
Jan	1.00	0.02	-
Feb	1.61	0.26	-
Mar	3.30	10.42	-
Apr	4.90	1.01	14
May	4.70	14.85	392
Jun	0.36	1.14	871
Jul	2.30	3.99	637
Aug	3.82	0.02	1039
Sep	2.73	8.00	176

as bait. In the deep forest timber Camp of Nun-Tun, Kawthaung Township, An. minimus was collected by human baited nets in middle of January. At Phado, the monthly man-biting rate(mbr) and vectorial capacity of An. minimus in relation to rainfall was presented for 13 consecutive months in Table(2). During the survey at Phado, An. minimus was found to be the predominant anopheline mosquito, especially in the non-monsoon months. During monsoon out of the 17 species of anophelines recorded, 26.2% (321/1226) were An. minimus. In the cool/dry season out of 10 species of anophelines collected, 50.5% (205/406) were An. minimus. In the hot/dry season, An.minimus comprised 44.1% (142/322) out of 11 species of anophelines recorded. From Table 2 it can be observed that An. minimus was collected throughout the year, although peak densities were obtained from September to December. Also, An. minimus density was found to have no relationship to rainfall. Fig.1 shows An.minimus reported by Khin Maung Kyi(2) and Fig 2 shows locations where it was recorded by DMR teams.

Prevalence of An. minimus in relation to distance from forest:

An. minimus is mainly associated with deep forest and forested foot-hill areas. Myo Paing et al (6) showed out of a total of 699 An. minimus collected in the Phado area. 71.4% of (499/699) was collected at Kyar-kyaung-thaik Temporary settlement located at the forest edge,26.2% (183/699) from Ngok-to village situated 0.33 miles from forested foot-hills and only 1.3% (9/699) from Gwe-gon village which is about 1.5 miles from the forest.

Biting behaviour & host preference

Myo Paing et al(6) found An. minimus to be predominantly exophilic, the out-door biting rate being about 3 times that of the indoor biting rate. Host preference studies at Phado also showed that An. minimus was more attracted to humans. Khin Maung Kyi(2) also reported that An.minimus have a high preference for human blood.

Resting habits

Weeks(1) reported from work done at Lashio, North-Eastern Shan States, that An. minimus constituted 90% of the total day-time indoor resting mosquitoes caught. Chow(5) at Yunnan-Myanmar Road found that An.minimus prefers to rest by day-time in human dwellings. Muirhead-Thomson (7) reported from his work at tea garden districts of Assam, that An. minimus was evidently a domestic species sheltering in dark house and coolie houses by day. DMR survey teams, however, failed to collect any An. minimus resting indoors during the daytime.

Gonotrophic cycle

To determine the vectorial capacity, one of its parameters, the gonotrophic cycle was determined under field conditions seasonally at Phado by the authors. During the cool/dry & monsoon seasons the gonotrophic cycle was found to be

on a average of 2.5 days and in the hot/dry season, it was found to average 2.25 days. Field collected An. minimus adult females were used to conduct the test and the mosquitoes were given blood meals from the local volunteers.

Relationship to malaria

Khin Maung Kyi (2) presented an extensive list of An. minimus dissection results carried out by various workers in Myanmar. On the basis of available information, Khin Maung Kyi considered An. minimus as chiefly responsible for intense post-monsoon transmission and in areas where breeding extended throughout the pre-monsoon periods, An. minimus was responsible for pre-monsoon as well as post-monsoon transmission. Myo Paing et al reported that during Phado longitudinal study, the sporozoite rate of An. minimus was found to be 0.43% (3/699). From Table(2) it can be observed that the vectorial capacity of An. minimus in the area was, except for two months, always above the critical level (0.03) required for transmission.

DMR survey teams also found epidemiological evidence to incriminate An. minimus as a major vector of malaria. During February 1989 survey to Tha-bye-wa, An. minimus was collected in large numbers and at the same time the slide positivity rate of villages was found to be 40% and the villagers infant parasite rate 75%. Thus, An. minimus could be considered an important vector in Tha-bye wa. During DMR survey at Tha-bye-wa in July 1987, out of 16 An. minimus caught biting man, one was found to have oocysts.

Some aspects of An. minimus biology under laboratory conditions :

Myo Paing et al (8) observed some aspects of An. minimus biology under laboratory conditions.

(a) Fecundity of An. minimus females was on an average 80, the range observed being 58-102 eggs per female.

(b) The hatching rate of An. minimus eggs was found to be 86.7%.

(c) The peak mating potential of An. minimus, both males & females, was observed on the 3rd day, after which there was a marked reduction on the 4th day. By 5th day successful mating was very difficult to obtain.

(d) Anopheles minimus was found to start taking blood meals on the 2nd day and reached peak biting potential on the 3rd day.

(e) Gonotrophic cycle of An. minimus was studied seasonally in the laboratory under natural conditions after taking human blood meal.
During the cool/dry season the average duration of gonotrophic cycle was found to be 65 hours, while the duration for monsoon and hot/dry seasons were found to be 58 hours and 51-53 hours respectively.

Morphological variations

During the course of this study, the morphology of An. minimus was observed to be very variable. Even off-springs from the same iso-female lines were found to have much morphological differences which could lead to errors in identification. A detailed study is being reported in a separate paper.

DMR Collection

Anopheles minimus adult specimens, morphological variations mounted on slides, pupal & larval skins slides are being preserved as DMR Collections.

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New records of mosquitoes from Myanmar

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INTRODUCTION

Union of Myanmar has a very rich fauna of mosquitoes as recorded by Apiwathnasorn (1) and Knight & Stone (2). Apiwathnasorn recorded 105 species from 14 genera of mosquitoes from Myanmar. However, no recent studies on mosquito taxonomy have been carried out in the country. Medical Entomology Research Division, Department of Medical Research (DMR) started mosquito taxonomy studies with a view to update the mosquito fauna of the country. In the present study, almost all the species collected and recorded were identified from adult specimens, pupal & larval skin slides and male genitalia slides prepared from iso-female lines raised from wild-caught females.

MATERIALS AND METHODS

Specimens were collected from different areas of the country as part of routine ecological study on anopheline vectors of malaria. The method of collection for adult mosquitoes was by hand catches with WHO sucking tubes from human and cattle baits and also from human and cattle baited big bed-nets (11' x 11' x 6'). Some specimens were collected during larval surveys from all potential mosquito breeding sources. This paper presents the new records of mosquito species from Myanmar recorded by the survey teams of Department of Medical Research.

RESULTS & DISCUSSION

(A) Genus: Anopheles Meigen
Sub-genus: Anopheles Meigen, 1918

(i) Anopheles (Anopheles) nitidus Harrison, Scanton and Reid, 1973:

A single male specimen was collected in December 1987 resting on an orchid plant at 0530 hours in the senior author's compound Nga-dat-kyi Pagoda Road, Bahan Township, Yangon. Identification was done on adult male and male terminalia morphologies. Further collections did not yield any more specimens. Male adult specimen and male terminalia slide are preserved in DMR collections.

Sub-genus - Cellia Theobald, 1902

(i) Anopheles (Cellia) dirus Peyton and Harrison, 1979

This species was not recorded previously from Myanmar because it was misidentified as An. balabacensis balabacensis Baisas. Since 1983, DMR scientists have recorded An. dirus from forested areas of the country and from Mudon town, Mon State. Adult specimens, pupal & larval skin slides preserved in DMR collections. Identification was according to Peyton & Harrison (3).

(ii) Anopheles (Cellia) nivipes (Theobald), 1903

This species was identified from specimens collected during July 1987 as An. philippinensis in Tha-bye-wa village. Isofemale lines were raised from wild caught females and adult specimens, pupal & larval skin slides prepared from the offsprings. Anopheles nivipes was identified according to Reid (4) from the adult male characters, pupal & larval skin morphological characters. This species was collected only during the monsoon season. It is mainly zoophilic and collected only by cattle-baited nets.

Anopheles nivipes was found to be associated with forest and foot-hills. Adult specimens, pupal and larval skin slides are preserved in DMR collections.

(iii) Anopheles (Cellia) sawadwongporni Rattanarithikul and Green 1986

A new species of the Anopheles maculatus group. Adult specimens collected from Kyauk-ta-gar Township, Bago Division, were identified by Tathanarithikul as An. sawadwongporni by adult characters. This species was collected from forested foot-hill of Bago Yoma during December 1985.

(B) Genus: Orthopodomyia Theobald

(i) Orthopodomyia albipes Leicester, 1904

This species was collected during February 1989 as larvae from a bamboo stump in a teak forest reserve near Tha-bye-wa village, Oktwin Township, Bago Division. It was identified by its characteristic adult morphology and from male terminalia character. Two male specimens, 3 female specimens, pupal & larval skins and 2 male terminalia slides are preserved in DMR collections.

(C) Genus: Aedes Meigen
Subgenus: Stegomyia Theobald, 1901

(i) Aedes (Stegomyia) craggi (Barraud), 1923

This species was first recorded in July 1987 at a teak forest reserve near Tha-bye-wa, Oktwin Township from day-time humam bait catches. It is a vicious biter of humans and was collected mostly in the early part of the evening about 1500-1600 hours.Wild caught females were given blood meals, isofemale lines raised to obtain pupal & larval skins, male terminalia & adult specimens for DMR collections. Aedes craggi was identified by its male terminalia characters.

(ii) Aedes (Stegomyia) perplexus (Leicester), 1908

This species was collected in January 1988 as larvae from bamboo stumps at Daw-taw-myaung, Tha-bye-wa, Oktwin Township. One adult male, 2 adult females, 1 male terminalia slide and 2 sets of pupal & larval skin slides were preserved in DMR collections. Aedes perplexus was identified from adult & male terminalia morphologies and from its characteristic larval chetotaxy.

(iii) Aedes(Stegomyia) gardnerii imitator (Leicester), 1908

This species was also collected as larvae from bamboo stumps at Tha-bye-wa, Oktwin Township on 14 August 1987. It was identified from its characteristic male terminalia.

Sub-genus Finlaya Theobald, 1903

(i) Aedes (Finlaya) albolateralis (Theobald), 1908

This species was collected from larval surveys at Tha-bye-wa Teak forest area from bamboo stumps in a ravine on 13 February 1989. Two adult males, 4 adult females, 2 male terminalia slides and pupa; & larval skins were preserved. Aedes albolateralis was identified from adult specimen characters and male terminalia characters.

(ii) Aedes (Finlaya) niveoides Barraud, 1934

Collected as larvae from bamboo stumps ar Daw-Taw-Myaung, Tha-bye-wa, Oktwin Township during January 1988. Identified by its male terminalia characters. Male terminalia, pupal and larval skin slide are preserved in DMR collections.

(iii) Aedes (Finlaya) assamensis (Theobald), 1908

Collected as larvae from bamboo stumps at Kwan-ka-thaung Ward, Mudon Town, Mon State during September 1987. Identified by its male terminalia characters. Nine adult males, 7 adult females, 3 male terminalia slides and 2 pupal skin slides are preserved at DMR.

(D) Genus: Heizmannia Ludlow

Subgenus: Heizmannia Ludlow, 1905

(i) Heizmannia (Heizmannia) communis (Leicester), 1908

One adult female of this metallic species was caught from cattle-baited net at Tha-bye-wa village. No offsprings could be obtained as the specimen died during the trip back. The specimen is now preserved in DMR collection.

(E) Genus: Culex Linnaeus
Subgenus: Lophoceraomyia Theobald, 1905

(i) Culex (Lophoceraomyia) traubi Colless, 1965.

This species was collected as a single larva from a stream in Shwe-du Rubber Estate, Myaik Township, Ta[nin-thar-yi Division during May 1988. A male specimen emerged & male terminalia slide was prepared. Culex (Lophoceraomyia traubi was identified from the characters of male terminalia and larval skin chetotaxy. The specimen and its related slides are preserved in DMR collections.

(F) Genus: Armigeres Theobald
Subgenus: Leicesteria Theobald,1904

(i) Armigeres (Leicesteria) omissus (Edwards), 1914

This species was collected as a single larva from a bamboo stump in Daw-Taw Myaung, Tha-bye-wa, Oktwin Township during February 1989. One adult male specimen, one male terminalia, one pupal & one larval skin slides were obtained. Armigeres (Leicesteria) omissus was identified according to Delfinado (5).

For identification of species of the subgenus Finlaya in the genus Aedes, Barraud (6) was used. For identification of other species, except where the authors were specifically mentioned, the Contribution of the American Entomological Institute series were used.

As mosquito taxonomy work progresses at DMR, new records of mosquitoes from Myanmar will be updated.

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Effects of heparin on Russell's Viper Venom envenomated experimental rabbits

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Effects of heparin were studied on 20 Russell's Viper Venom (RVV) envenomated rabbits. After injection with LD 100 dose of RVV, the experimental animals were divided into four groups. Group I rabbits were left untreated; in Group II antivenom treatment was given ; in GroupIII antivenom plus heparin was administered intravenously and in Group IV heparin alone was given. Effectiveness of heparin therapy was assessed by comparing the changes in the blood screening tests of haemostasis such as prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT), survival rate, degree of congestion and haemorrhage in the kidneys, presence or absence of fibrin deposition and acute tubular necrosis in the kidneys of different groups of animals. Results showed no significant differences in the blood tests and fibrin deposition in the kidneys. However, there is an increase in the survival rate, as well as reduction in the severity of congestion, haemorrhage and acute tubular necrosis of the kidneys in the rabbits treated with specific antivenom (ASV) plus heparin (Group III) over that treated with antivenom alone (Group II) and heparin alone (Group IV).

INTRODUCTION

It has been well established that RVV contains activators to coagulation factors V and X (1,2) which may be responsible for the initiation of disseminated intravascular coagulation (DIC) (3,4). Since the action of heparin (5) is to inactivate products of coagulation including activated factor X and thrombin, it may halt or slow down the process of DIC, and preserve organs' function by limiting fibrin deposition in vital organs. Hence it is thought that heparin therapy would be most beneficial in experimental Russell's viper venom envenoming.

The present study is aimed to observe whether heparin confers any added advantage in experimentally envenomated rabbits treated with specific antivenom (ASV).

MATERIALS AND METHODS

Twenty Japanese white rabbits weighing 2.5-3.5 kg were used. Baseline blood samples were taken from the marginal ear veins of all the rabbits. After intramuscular injection with LD 100 dose of RVV (dried crystal obtained from the Biological Laboratories of Myanmar Pharmaceutical Industry, (MPI)), blood samples were collected at 1 hr, 3 hrs, 6 hrs, 12 hrs, and 24 hrs respectively. The experimental animals were then divided into four groups each group comprising of 5 animals, and were treated as follows: -

Group I - The rabbits were left untreated after venom injection.

Group II - Half an hour after venom injection an appropriate dose (ranging from 0.75 ml- 1.05 ml) of specific anti-venom produced by MPI was administered intravenously. This dose is calculated for every rabbit based on the findings (6) that 1 ml of specific antivenom could neutralize 2 mg of RVV.

Group III- Specific antivenom (as for Group

II) plus heparin 20 units per kg was injected intravenously.

Group IV-Treated with heparin only (dosage same as Group III).

All the blood samples were screened for prothombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT)) using standard methods (7).

The experimental animals were observed closely, survival times noted, and autopsy performed immediately after death; those that survived beyond 24 hours were sacrificed. Kidneys from all the animals were fixed in buffered formalin and processed for light microscopy using standard procedure.

Effectiveness of heparin therapy was assessed by comparing among the following different experimental groups.

1. Changes in screening tests of haemostasis
2. Survival rate
3. Histopathological changes in the kidney with special emphasis on fibrin deposition, haemorrhage, congestion and acute tubular necrosis

RESULTS

Results of Screening tests of haemostasis

Results of all the groups are shown in Fig 1. PT, TT and APTT of untreated rabbits (Group1) were non-clotting at 6 hours (clotting times > 120 seconds for PT and TT and > 180 for APTT. Rabbits treated with antisnake venom (Group II) showed slight prolongation of some of the screening tests within 3-6 hours which reverted back to normal at 12 hours. Rabbits treated with both antisnake venom and heparin (Group III) revealed prolongation of screening tests at 6 hours which , however, returned to normal at 12 hours. Rabbits treated with heparin alone (Group IV) displayed earlier prolongation of the screening tests at 1 hour which became non-clotting at 12 hours until 24 hours.

Results of survival rate

The survival rate of the rabbits between the groups were compared (Table 1). In Group I, as expected all rabbits died within 24 hours (4½ - 23hrs). In Group II, where specific antivenom therapy was given, 2 rabbits (40%) survived up to 24 hours. In the group treated with both antivenom and heparin (Group III), 3 rabbits (60%) survived up to 24 hrs. The survival rate of Group IV was similar to that of Group II. Thus the results showed improvement in the survival rate of rabbits treated with antivenom and heparin in comparison to those treated with antivenom alone and heparin alone.

Table 1. Survival Rate (%)

Group	% Survival up to 24 hours
Group I	0%
Group II	40%
Group III	60%
Group IV	40%

Results of histopathological changes

Histopathological changes in the kidneys were examined with emphasis on presence or absence of fibrin deposition, haemorrhage, severity of congestion and morphological features of acute tubular necrosis.

Fibrin deposits were found either in the renal vessels or inside the glomerular tuft in 3 rabbits (60%) of the untreated group (Group I), as well as that of the group treated with heparin only (Group IV). In

the groups treated with ASV (Group II) and ASV plus heparin (Group III) 40% of the rabbits exhibited fibrin deposits in the kidneys. On the other hand it was noted that degree of haemorrhage and congestion was markedly reduced in the group treated with ASV plus heparin in comparison to the group treated with ASV only. Features of acute tubular necrosis (Fig 2-7) were seen in all (100%) the experimental animals of groups I, II and IV while it was found in 4 rabbits (89%) in the Group III treated with ASV plus heparin.

DISCUSSION

The results of the coagulation parameter did not reveal any significant differences between the groups. The earlier development of prolongation of the screening tests and longer duration of non-clotting state in heparin-treated group may be due to the anticoagulant effect of heparin. Increase in the survival rate in Group III may be due to the additive beneficial effect of heparin.

The percentage of fibrin deposits present in Group II and III were the same. This may indicate that heparin in the dosage used in the study may bot be enough to abolish or prevent DIC.

The most striking histological findings of the kidneys were the notable decrease in congestion and haemorrhage in Group III. The reason is not definitely known, however it is speculated that enhanced removal of the activated clotting products under the influence of the heparin may be one of the reasons.

Effects of heparin on DIC due to snake bite (8,9) have been studied by many workers and opinions differ as to its effectiveness. Studies by Warrell et al (8), on cases bitten by Echis carinatus showed no beneficial effects. However, Weiss et al (9) demonstrated beneficial effects of heparin on their cases bitten by Echis carinatus.

Ahuja et al (10) showed neutralizing action of heparin on procoagulant action of RVV, both *in vivo and in vitro* studies. In vitro studies of Malayan pit viper venom by Chan et al(11) showed that high doses of heparin was required to counteract the effects of the venom.

In conclusion, our results showed beneficial effects of heparin in terms of survival rate and histopathological changes in the kidneys. However, the coagulation parameters and fibrin deposits did not reveal any difference. Results of the clinical trial of heparin in Russell's viper bite victims has been reported elsewhere (Myint Lwin et al, Departmental report, 1988).

ACKNOWLEDGEMENT

We thank our Director General for allowing us to publish this paper. We are grateful to U Tin Oo, Photography Unit, for preparation of the photomicrographs and to U Hla Myint of Publication Division for asisting in typing of this manuscript. The invaluable assistance of all our staff in the division is gratefully acknowledged.

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Interrelationship among glomerular filtration rate, fractional sodium excretion and albuminuria in Russell's viper bite patients

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The interrelationship among Ccr, FENa% and Ualb were observed in 38 Russell's viper bite patients for 5-days clinical course. Degree of albuminuria had strong hyperbolic relation with glomerular filtration rate (Ccr) on day 1 after bite but not from day 2 onwards. The extent of albuminuria and fractional sodium excretion (FENa%) level was found to indicate the severity of renal insult. Albuminuria (Ualb) was associated with impaired Ccr and renal sodium handling. Ccr value below 40 ml/min or albuminuria (Ualb) above 2.07 g/24 hr on day 1 after bite was found in oliguric renal failure patients.

INTRODUCTION

Systemic envenoming causes acute renal failure in about 30% of patients following Russell's viper bite in Myanmar (1). A broad spectrum of renal lesions has been reported from histopathological studies, however, there was no information available on the functional changes of the kidneys in these patients. In this present communication the dynamic changes of renal function measured by creatinine clearance (Ccr), fractional sodium excretion (FENa%) and albuminuria (Ualh) are presented and these variables are also constructed in a three dimensional pattern for better inderstanding of interrelationship among these parameters in Russell's viper bite victims.

MATERIALS AND METHODS

Patients - Thirty-eight cases of proved Russell's viper bite victims admitted to Thayarwady township hospital were studied during 1984-85. Russell's viper bite was confirmed by positive venom antigen detected in their sera by enzyme-linked immunosorbant assay (2). Twelve apparently healthy normal subjects were also studied as a reference.

Methods - Twenty min: clotting test (3) was carried out on blood samples obtained from the snake bite patients. Daily serum and urine samples were collected for 5 days and frozen for biochemical measurements. Twenty four hr endogenous creatinine clearance for glomerular function, fractional sodium excretion test for renal handling of sodium and albuminuria were measured. The FENa is the quotient of the urine-plasma sodium and creatinine ratios multiplied by a factor of 100. Its derivation is as follows :

$$FENa = (U/P)_{Na} / (U/P)_{cr} \times 100\%$$

Creatinine by alkaline picrate method, albumin by bromocresol-green specific dye method and sodium by flamephotometry were used.

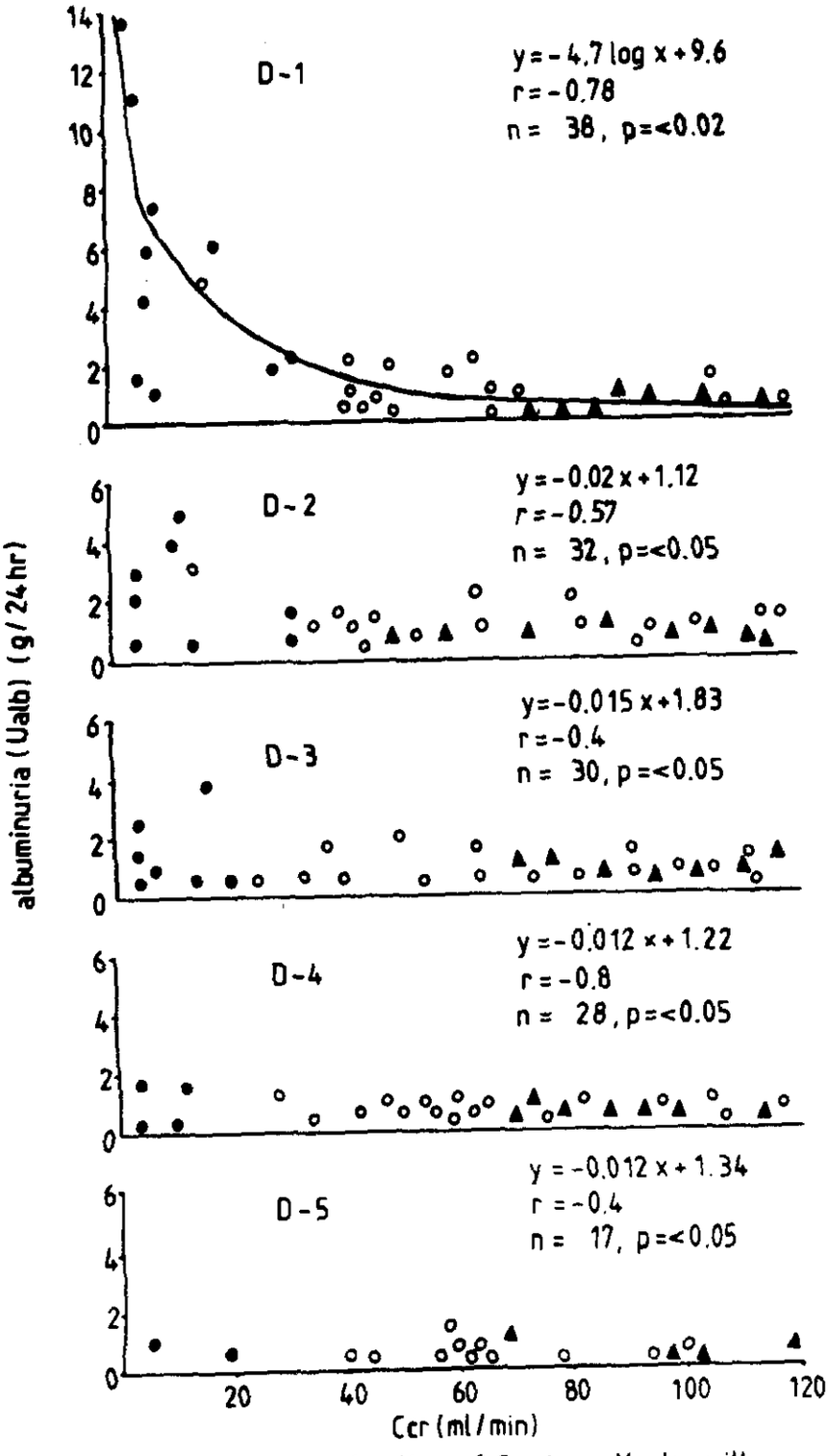
Treatment - Monospecific enzyme refined freeze-dried equine antivenom (BPI) was given as soon as the blood became incoagulable Patients who passed less than 400 ml ot urine per 24 hr after rehydration were defined as oliguric renal failure. Fluid balance was strictly controlled.

Statistical analysis - Mathematical curve fitting was executed on the data by testing linear (y= 1 + bx), exponential

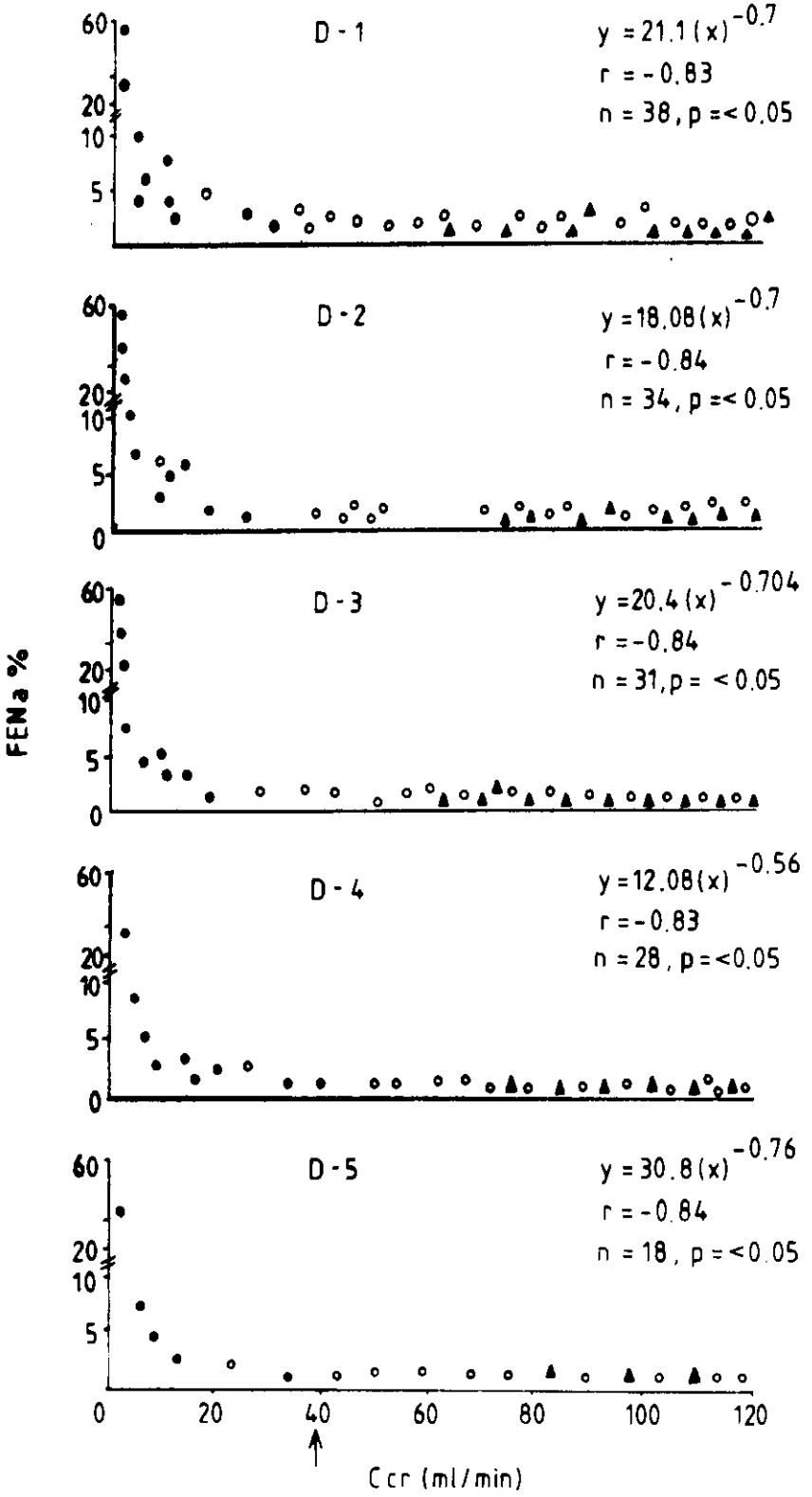
Table 1. Clinical and laboratory data in 38 patients following Russell's viper bite.

RV No.	Bite to admission (hr)	Bite to incoag : blood (hr)	Serum creatinine (mg/dl) adm/max	Oliguria ±	Clinical outcome
87	1.5	2.7	1.46/7.1	+	PD
127	2.0	4.3	1.56/8.0	+	R
151	1.1	2.4	1.1 /2.3	+	Ex
110	2.5	10.7	1.56/8.0	+	R
141	2.5	2.5	1.8 /4.0	+	Ex
157	2.7	2.7	0.86/3.6	+	PD
124	8.2	8.2	2.3 /5.4	+	R
93	2.8	2.8	1.5 /11.7	+	R
143	1.5	2.8	1.3 /6.12	+	R
73	6.5	6.5	1.0 /6.4	+	R
97	4.8	4.8	2.0 /5.3	+	Ex
94	1.8	5.5	2.0 /3.3	-	R
111	1.25	2.6	1.8 /4.3	-	R
123	3.25	8.3	2.7 /3.6	-	R
149	1.0	1.6	1.2 /2.4	-	R
79	2.0	2.0	1.3 /6.3	-	R
134	5.3	5.3	1.0 /6.0	-	R
81	2.2	2.2	1.8 /3.1	-	R
118	6.9	6.9	1.3 /3.0	-	R
78	1.2	4.0	1.3 /1.3	-	R
135	3.4	12.5	1.5 /1.5	-	R
159	1.2	17.2	1.1 /1.5	-	R
98	3.8	3.8	1.2 /1.3	-	R
74	2.7	2.7	1.3 /1.3	-	R
88	3.3	3.3	1.5 /1.8	-	R
130	0.75	16.0	1.1 /1.8	-	R
137	1.0	2.2	1.1 /2.0	-	R
154	0.92	13.4	0.86/1.3	-	R
106	2.7	2.7	1.3 /2.0	-	R
86	2.5	-	0.86/1.0	-	R
91	4.0	-	1.5 /1.8	-	R
92	2.0	-	1.0 /1.0	-	R
100	1.42	-	1.8 /1.8	-	R
107	1.5	-	1.5 /1.5	-	R
108	6.5	-	1.0 /1.0	-	R
115	1.75	-	1.3 /1.3	-	R
117	2.25	-	1.1 /1.1	-	R
145	3.7	-	1.2 /1.2	-	R

incoag : incoagulable, adm : admission, max : maximum,
R : recovered, PD : peritoneal dialysis, Ex : expired



Fig(1). Albuminuria as a function of Ccr in patients with incoagulabe blood[with oliguria(●) and without oliguria(○)]and patients with clotted blood (▲).



Fig(2). FENa % as a function of Ccr.
See figure(1) for symbol definitions.

($y = ae^{bx}$), log($y = 1 + b \log x$), and power($y = ax^b$) functions.

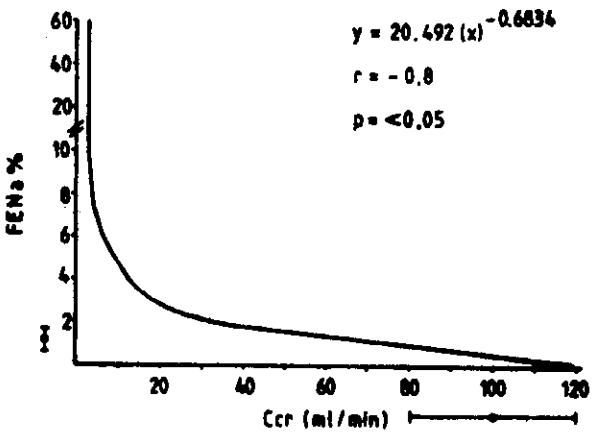
RESULTS

The clinical and laboratory data of all 38 Russell's viper bite patients are in Table (1). Of the 38 proved Russell's viper bite patients, 29(76.3%) developed incoagulable blood at mean 5.6 hr, range 1.6-17.2 hr after bite, their admission time was mean 2.78 hr, range 0.9-8.2 hr after bite and their blood became reclotted after antisera at mean 14.09 hr, range 6.0-25.2 hr after bite. 9(23.7%) had clotted blood throughout the whole clinical course of the study and they were admitted at mean 2.85 hr, range 1.42-6.5 hr after bite. Of 29 patients with incoagulable blood, 11 developed oliguria on day 1 after bite.

Albuminuria as a function of Ccr, Fig (1). Significant hyperbolic relationship between albuminuria and Ccr was observed ($r = -0.78$, $p = 0.02$) on day 1 after bite. Oliguria developed when Ccr fell below 40 ml/min and the corresponding albuminuria was about 2.07g/24hr in these patients. Very poor correlations between albuminuria and Ccr were observed on day 2 to day 5 after bite.

FENa% as a function of Ccr, Fig (2).

Constant hyperbolic function between Ccr and FENa% was observed throughout the whole clinical course of the study ($r=-0.8$ $p=<0.05$). As Ccr values decreased progressively, there was an increasing value of FENa%, the rate of FENa% rise being very gradual at first but became very steep when Ccr reached below 20 ml/min. From the mean relationship between FENa% and Ccr, four arbitrary hypothetical sequential stages of renal functional derangement can be discerned Fig (3).



STAGE	Ccr (ml/min)	FENa%
I	80-120	<1.0
II	30 - 80	1-2
III	16.6 - 30	2-3
IV	<16.6	>3.0

Fig (3). mean relationship between FENa and Ccr. 95% confidence limits of controls (|—●—|)

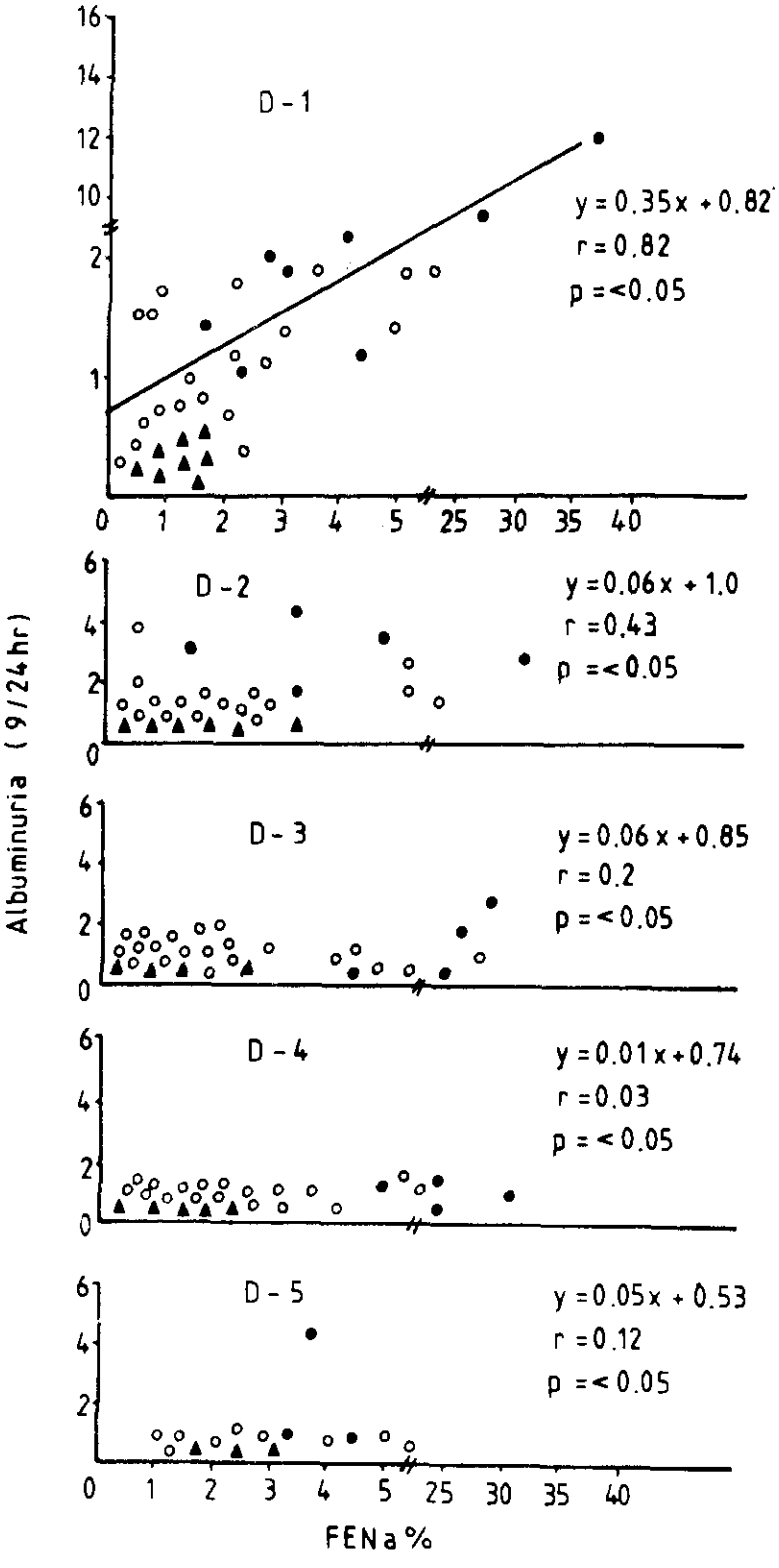
Stage I Ccr ranges within normal limits, ie 80-120 ml/min with FENa% less than 1%.

Stage II Ccr ranges from 30-80 ml/min with FENa 1-2%.

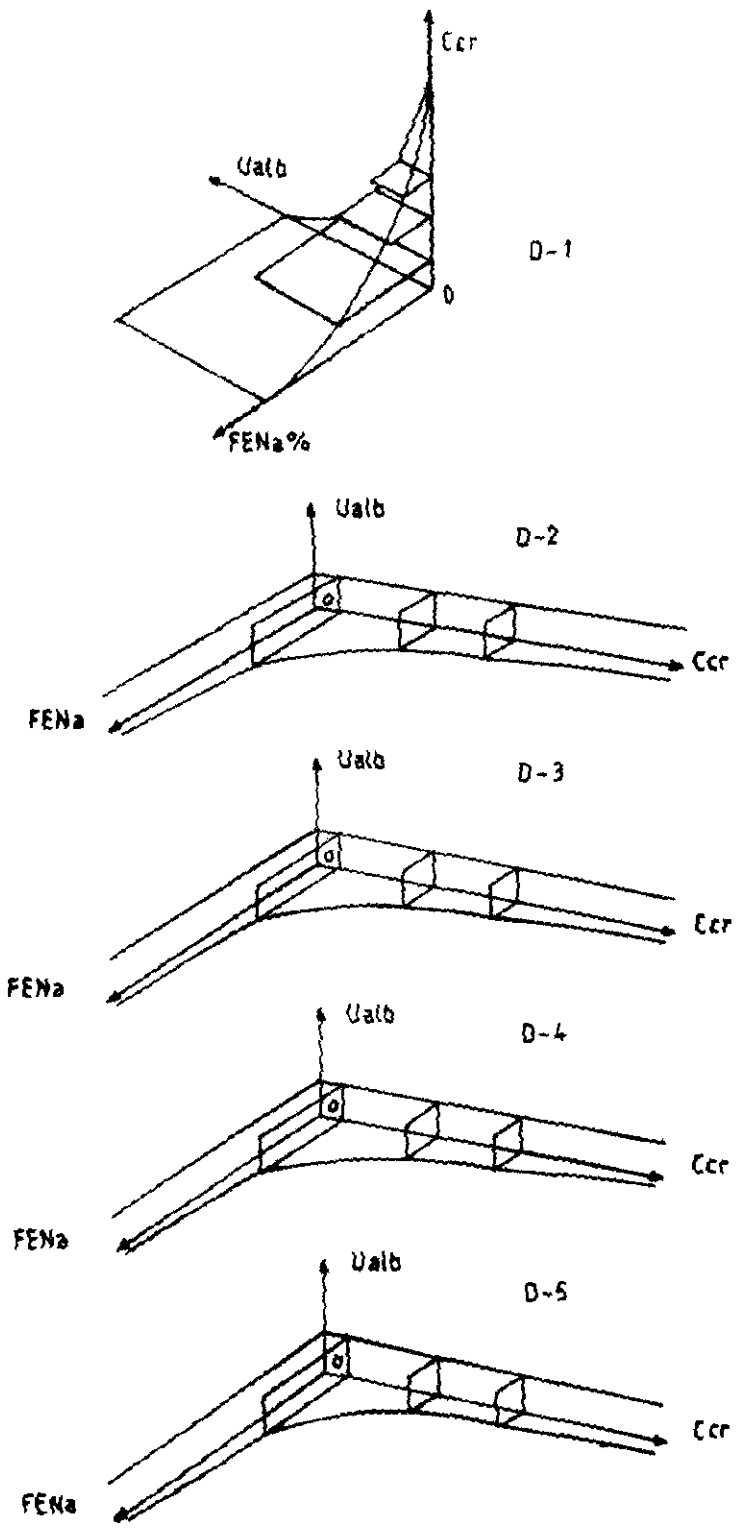
Stage III Ccr ranges from 16.6-30 ml/min with FENa 2-3%.

Stage IV Ccr ranges from 0.0-16.6 ml/min with FENa more than 3%.

Albuminuria as a function of FENa%, Fig (4).



Fig(4) Albuminuria as a function of FENa%
See figure(1) for symbol definations



Fig(5). Albuminuria(Ualb)-Ccr-FENa curves combined in a three dimensional construction, D-1 to D-5 after bite.

Significant positive linear function between albuminuria and FENa% was observed on day 1 after bite ($r=0.82$, $p=<0.05$). Nevertheless, during day 2 to day 5 after bite, there was no significant correlation between these two variables, which shows that impairment of sodium handling was not in parallel with the degree of albuminuria on these days.

Three dimensional pattern of albuminuria (U_{alb})-Ccr-FENa%, Fig (5).

Both FENa% and albuminuria increased expotentially with decreasing Ccr during day 1 after bite. Albuminuria in accordance with arbitary sequential phases of renal function derangement on day 1 was also presented in Table (2).

Table 2. Albuminuria (g/24 hr) calculated from the hyperbolic equation: albuminuria (g/24 hr)= 4.7 log (Ccr)+ 9.6, in accordance with arbitrary sequential stages of renal function derangement on day 1 after bite.

	Ccr ml/min	FENa%	albuminuria(g/24hr)
Controls*	80-120	0.9-1.5	0.0 -0.05
Stage I	80-120	< 1.0	0.2 -0.65
Stage II	30-80	1.0-2.0	0.65-2.66
Stage III	16.6-30	2.0-3.0	2.66-3.86
Stage IV	< 16.6	>3.0	> 3.86

*95% confidence limits

During day 2 to day 5 after bite, the above relationship changed into different pattern. As Ccr reduced progressively, expotential rise in FENa% persisted, but albuminuria no longer correlated with either falling Ccr or rising FENa% values.

DISCUSSION

The most striking biochemical changes found in the present study is the hyperbolic relationship between fall in Ccr and increase in amount of both albuminuria and FENa% during dayl after the bite. This relationship indicates the fact that albuminuria and FENa% levels can be used as a valuable marker in assessing the extent of glomerular filtration failure in early hours after Russell's viper bite. Since oliguria was observed as early as day I after the bite in most of the patients with severe envenoming, the finding of rising albuminuria and FENa% levels before Ccr fell below 40 ml/min, the critical level of GFR, at which oliguria starts to develop, signifies the fact that continuous monitoring of these two variables soon after the bite could detect patients at risk of developing severe renal insufficiency. Moreover, abnormal loss of albumin from the kidneys and FENa% less than 1% occurred before significant reduction of Ccr during stage I further intensifies this value. However, the interrelationship among these variables changed into different patterns on day 2 to 5 after the bite. Albuminuria no longer correlated with the change in both Ccr and FENa% despite constant hyperbolic relationship between Ccr and FENa% levels. The most likely cause of this observation might be either due to a decrease in glomerular capillary permeability to albumin and/or reduction in number of functioning glomeruli as a result of severe renal damage. Therefore, the finding of heavy albuminuria on day I followed by reduced excretion of albumin on subsequent days after the bite should be regarded as a sign of severe and progressive renal damage.

Although the exact pathogenic mechanism(s) that take part in Russell's viper venom nephropathy are not known, the histopathological studies revealed distinct morphological features. The salient feature in the kidney are intraglomerular depostion of fibrin, fibrin degrading products and coagulation(4). Myint Lwin et al(1) also reported that the very rapid development of oliguria supports the idea of massive occlusion of the renal micro-

vasculature with fibrin, resulting in renal ischemia. The other possible factor that should be considered is the direct nephrotoxic action of the venom (5).

The clinical course of acute renal failure may be divided into three stages: (1) stage of renal insult or initiating phase, (2) stage of renal insufficiency, and (3) stage of renal recovery. Stage of renal insult or initiating phase is the period where diagnosis and prevention have major roles(6). From the present study, it is possible that stage I and IV represent initiating and significant renal insufficiency stages respectively. In between these two stages, there is a gray zone which include stage II and III. During stage I, although glomerular injury had already started as indicated by abnormal loss of albumin from the kidneys, there was an intense resorption of sodium from the intact tubules. This might result from renin-angiotensin mechanism. FENa less than 1% indicates, sodium avid state, acute glomerulonephritis, intrarenal hemodynamic alteration and intrarenal obstruction (7). Since our patients received adequate fluid and electrolyte replacement at the time of admission,sodium avid state could not be a contributing factor in lowering FENa level. As glomerular injury advances, both glomerular filtration failure and inpaired renal sodium handling follows, resulting in significant renal insufficiency during stage IV.

It is possible that depending on the magnitute and duration of renal insult, renal failure may be maintained by: a decrease in glomerular capillary permeability, leakage of filtrate across damaged tubular epithelium, tubular obstruction and persistent renal vasoconstriction (8).

In summary the following points were noted:

(1) Albuminuria was associated with impaired Ccr and renal sodium handling.

(2) Degree of albuminuria had a strong correlation with impaired Ccr only on day 1, but not on subsequent days after the bite.

(3) Ccr value below 40ml/min or albuminuria level above 2.07 g/24 hr on day 1 after bite was found in oliguric renal failure patients.

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Acetaminophen (paracetamol) pharmacokinetics in contraceptive pill–using Myanmar Women

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Acetaminophen(paracetamol) pharmacokinetic study was made on a total of 16 healthy adult Myanmar women of reproductive age, half of whom were pretreated chronically for 6 months with an oral contraceptive steroid (OCS), namely, Combination 5. Single oral dose (930 mg) of acetaminophen showed similar extent of maximum plasma acetaminophen concentration in either group. However, the OCS causes significant effect ($p<0.05$) on plasma acetaminophen resulting in shortening of half-life by 21.6% and augmentation of clearance by 5.9%. The OCS affected predominantly the sulphate conjugation of acetaminophen. In regimen requiring repeated dosings, acetaminophen should be administered more frequently in the pill-users.

INTRODUCTION

In this country, one of the most commonly used symptomatic medication as analgesic/ antipyretic is the drug acetaminophen (paracetamol), either by general public on an analogous over-the-counter drug or by health personnel on an essential drug concept. Changes in metabolism of this drug in pill users had been reported (1,2). Incidentally oral contraceptive steroid (OCS) has a growing popularity among Myanmar women. The present study therefore investigated an acetaminophen pharmacokinetics in OCS users among the Myanmar women, aiming them to use this drug rationally.

MATERIALS AND METHODS

The study was conducted on a total of 16 healthy adult Myanmar women, at reproductive age being 17-45 years and body weight of 39-60 kg and equally divided into two groups (i.e., one test and one control) with the individual randomly allocated.

Priming phase : Individual subject in the test group was daily administered for a cycle period of at least 6 months with a popularly used brand OCS, namely, Combination 5 (containing norgestrol 0.05 mg, ethinyloestradiol 0.05 mg and ferrous fumarate). No other drug was administered to any control subject during this phase of study.

Intraction phase : At the end of the priming phase, each volunteer fasted overnight, was orally administered with a single dose of paracetamol 930 mg (MPI : Yangon, Myanmar) in the moring and drinking of water allowed ad libitum during the next 4 hours of trial period. Venous blood sample (5ml each) were collected from the median cubital vein using vacutainer tubes (Becton & Dickerson) at various post-drug time intervals, namely, 15, 30, 60, 120 and 240 minutes. Plasma drug concentrations for either the parent compound acetaminophen or its major metabolities, i.e., sulphate and glucuronide were assayed according to the modified method of Howie et al (3) and Mitchell et al (1) using the Water Associates High Performance Liquid Chromatography system composed of components with Models 204 W/510, U6K and 440 (Millipore; Milford, Massachusetts, USA).

Evaluation : Pharmacokinetic parameters were calculated namely, plasma half-life ($t_{\frac{1}{2}}$), concentration at zero time (C_o),

Table . Plasma pharmacokinetic arameters of acetaminophen and its metabolities of sulprate and glucuronide following single oral dose of acetaminophen 930 mg in Myanmar women chronically taking oral contraceptive steroid Combination 5.

	$t_{\frac{1}{2}}$ (h)	C_o mg ml^{-1}	C_{max} mg ml^{-1}	T_{max} (h)	K_{el}	aVd L kg^{-1}	Cl ml^{-1} min^{-1} kg^{-1}
Acetaminophen							
Control	2.50	31.9	21.4	1.29	0.29	0.64	3.0
	± 0.20	± 3.5	± 2.9	±0.20	±0.02	±0.05	±0.3
	*	n.s	n.s	n.s	n.s	n.s	n.s
OCS	1.96	44.6	23.9	1.55	0.04	0.55	3.2
	±0.05	±7.5	±4.7	±0.20	±0.05	±0.11	±0.4
Acetaminophen sulphate							
Control	2.12	3.3	2.1	1.18	0.37	5.90	35.6
	±0.26	±0.3	±0.1	±0.3	±0.06	±0.60	±5.3
	n.s	*	*	n.s	n.s	n.s	**
OCS	2.78	13.1	8.0	1.44	0.27	3.49	12.5
	±0.29	±4.5	±2.4	±0.16	±0.03	±1.25	±3.8
Acetaminophen glucuronide							
Control	1.96	37.1	18.8	1.79	0.37	0.77	4.9
	±0.20	±7.4	±4.1	±0.05	±0.06	±0.20	±1.5
	*	n.s	n.s	n.s	n.s	n.s	n.s
OCS	2.9	74.5	32.1	1.97	0.27	0.51	2.2
	±0.50	±33.8	±10.5	±0.12	±0.05	±0.10	±0.4

Each value is the means of 8 subjects with its standard errors. Signigicant level of difference statistically between the two treatments are depicted as (*)= P<0.05; (**)= P<0.01; (***)= P<0.0001 and n.s= not significant at the level of P<0.05.

maximum plasma concentration (C_{max}), elimination rate constant (K_{el}), apparent volume of distribution (aVd) and systemic clearance (Cl). Paramount clinical information of any difference in the half-life of acetaminophen between the two groups was scrutinized statistically, using the Student's t test. Metabolising profiles of both the sulphate and gulcuronide conjugations were also vividly portrayed by scanning the time-course concentrations of the metabolites.

RESULTS

Effects of chronic administratiion of the OCS on major pharmacokinetic parameters of acetaminophen and its metabolites of sulphate and glucuronide in Myanmar women are shown in TAble . Whilst the OCS did not alter the plasma acetaminophen maximum concentration,it stimulated the metabolism signi icantly, thereby shortening the half-life of acetaminophen (Fig 1) as much as 21.6 % while augmenting the clearance by as much as 5.9% and thereby delaying the time for the occurence of maximum acetaminophen plasma concentration.

Simultaneous pharmacokinetic determinations of the metabolites denoted the OCS caused the sulphate conjugation to be markedly affected, resulting in high concentration of plasma acetaminophen

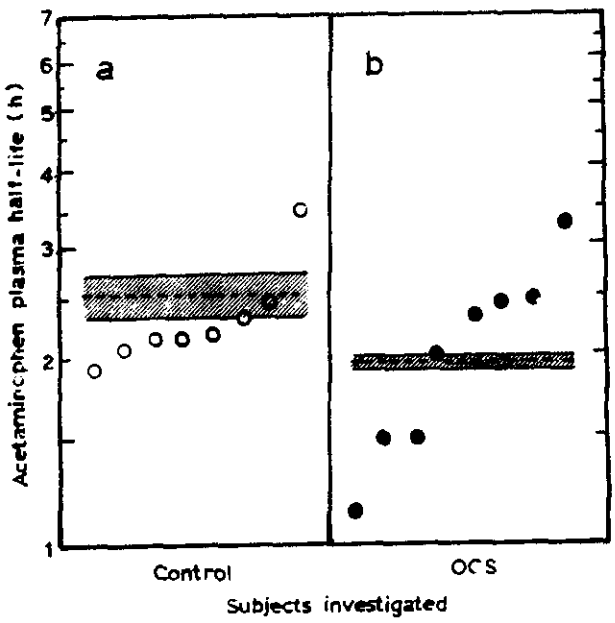

Fig 1. Distribution diagram of subjects in relation to acetaminophen plasma half-life in Myanmar women.

Groups means is denoted as dotted lines, with the respective standard errors of the means coverage as hatched areas.

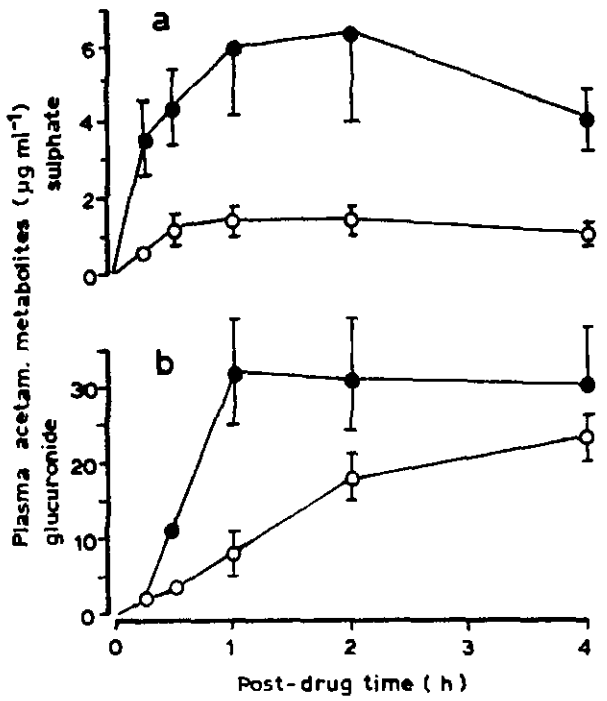

Figure 2. Time course response of acetaminophen metabolites of sulphate and glucuro- following acteaminophen 930 mg oraliy administered to Myanmar women.

Upper panel (a) and lower panel (b) illustrate the responses of the acetaminophen metabolites sulphate and glucuronide, respectively. Each *point represents* the means of 8 observations, together with the standard errors of the means indicated as vertical bars, for the control (o) and the test (o) groups.

sulphate (Fig 2) and reciprocal reduction in both conjugation processes (Table), with respective to *diminished* clearance and prolonged half-life.

DISCUSSION

Acetaminophen, a low intrinsic clearance and binding insensitive drug, showed a pharmacokinetic profile of Myanmar women similar to those of Westerners (4). Chronic administration of the OCS Combination 5 did not alter the maximum plasma acetaminophen concentration in Myanmar women, a finding similar to the Westerners (1). Therefore, the analgesic/ antipyretic activities of acetaminophen will be attained with the usual recommended therapeutic dosage. However, the OCS *delayed the onset* and shortened the duration of pharmacological activities of acetaminophen resulting in shortening the half-life and augmenting the clearance. The increase clearance of 5.9% in Myanmar women was not to the extent seen in those of the Westerners, where Abernathy et al (2) and Mitchell et al (1) reported to be 41% and 64% respectively. In Myanmar women, the OCS mainly affected the sulphation of acetaminophen which is the principal process of metabolism of this drug (5). Decreease in excretion of the metabolites might probably act as 'feed-back' to cause *stimulation of metabolism* of the parent compound acetaminophen.

For the once-off use of acetaminophen, the usual recommended *dose could* produce a full therapeutic effect. In ailment where repeated administrations of the acetaminophen is indicated, those pill-using Myanmar women should require more frequent dosings.

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The Myanmar Health Sciences Research Journal, Vol. 1, No. 3, 1989.

Influence of pH on the growth rates of *Entamoeba histolytica* in cultures

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The growth rates of Entamoeba histolytica isolates were studied in two diphasic, one monophasic, and one synthetic culture media. The effect of varying pH on the growths of E. histolytica was measured. Dobell and Laidlaw's modified medium was found to be the most suitable for the crude cultivation of E. histolytica. The longest survival period and highest growth rates were observed in Hallman, Michaelson and Delamater's synthetic medium. The optimal pH was found to be 6.5. Little evidence of propagation was found at pH 7.5 and none at PH 8.0.

INTRODUCTION

The in vitro cultivation of Entamoeba histolytica was developed by Boeck and Drbohlav (1) using inspissated whole egg slants with serum-glucose-Locke-egg solution. Dobell and Laidlaw (2) conducted a detailed study on the growth of E. histolytica and other amoebae from human in Boeck and Drbohlav's medium (1) substituting rice starch for glucose. Most of the media developed since the work of Dobell and Laidlaw (2) contain rice starch to promote optimal growth of E. histolytica and other amoebae. In 1952, Shaffer (3) studied the nature of some factors that affect the propagation of E. histolytica and finds that pH plays an important role for the growth of amoebae. In the present paper, the rate of growth of different isolates of E. histolytica in different culture media adjusted to specified pH levels has been studied to have a better understanding of pH in the cultivation of E. histolytica.

MATERIALS AND METHODS

Experimental design

The first phase of the experiment was to test the growth rates of five E. histolytica isolates in four different culture media to assess the suitability of the media. Each of the five isolates was inoculated into three tube containing similar culture medium viz. Dobell and Laidaw (2), Hallman et al. (4), Pavlova (5) and Rao (6). Growth aliquots were sampled every 24 hour and the number of amoebae counted in a Fuchs-Rosenthal counting chamber. Triplicate readings taken at each interval were averaged. After the selection of media and isolates for the experiment proper, the rate of ptopagtion was measured at seven pH levels. Amoebae were counted at 12-hour intervals upto 72 hours.

Strains of amoebae

Five E. histolytica isolates of human origin were studied. They were obtained from (i) human cases of amoebic dysentery (Isolates AD1, AD2, AD3, AD4) and (ii) a human case of amoebic vaginitis (Isolate AV1).

Culture methods

Two diphasic media (2,6), one monophasic medium (5) and one synthetic medium (4) were used in the study.

Technique for subculturing

After warming the media at 37°C for 30 min., inoculations were made from the sediment of a positive culture using a Pasteur pipette. Approximately 1 ml aliquots were deposited slowly at the bottom of each fresh medium tube avoiding the transfer of bubbles.

Counting of amoebae

In order to assess the growth rate of amoebe inculture, a method of estimating the number of amoebae at different intervals was necessary. The Fuchs-Rosenthal counting chamber, usually employed for enumerating white blood cells in cerebro-spinal fluid, was used. This method was found by Griffin and McCarten (7) to be more accurate than the original method described by Paulson (8). After inverting the culture three to five times in order to obtain an homogenous suspension of the organisms, a sample was removed from the mid-zone of the liquid and deposited in the two sections of the counting chamber. Each section has 16 squares with a total area of 16 sq. mm and a depth of 0.2 mm. The amoebae in each section were counted separately, and then the chamber was cleaned, refilled and the count repeated. The formula used for the calculation of the amoebae per cubic mm. is shown as follows:

$$A+B+C+D \times \frac{5}{64} = \text{number of amoebae/mm}^3$$

where A,B,C,D = number of organisms counted in each section

$$5 = \frac{1}{0.2} \text{ mm}$$

64 = 16 x 4 squares

Throughout the experiment, the inoculum size, the amount of starch and the age of the stock cultures were kept as constant as possible, for these factors have been found to be the major sources of variation in cultures of entozoic amoebae (7).

Adjustment of pH

The pH of culture media were adjusted to appropriate level with 1N NaOH or 1N HCl using a Beckman pH meter Model 72. One set of media was adjusted to pH 5.0, the second to 5.5, and so on at increments of 0.5, to the seventh set which was adjusted to pH 8.0. The supernatant fluid from each tube was used to make up a series of 9 tubes of culture medium. Each of the resulting 63 tubes of the medium was then inoculated with a standard number of amoebae contained in 1.0 ml of the fluid medium. The inoculum was standardized by the method of Shaffer (3) with one minor modification, that is using a 48-hr culture of the appropriate isolate of amoeba diluted to provide approximately 2,000 amoeba/ml of the inoculated medium. The pH of one culture of each series was check-measured immediately and the remainder layered with vaseline and incubated at 37°C. Counts of the amoebae were done thereafter on the representative cultures of each series at 12-hr intervals upto 72 hours. The pH of each culture tube was also check-measured immediately after the count.

RESULTS AND DISCUSSION

The growth rates of E. histolytica isolates in different culture media are shown in Table 1. The greatest number of organisms was found in Dobell and Laidlaw's modified medium, while Rao's (6) medium gave the lowest number. It was also found that the growth rate differed with the isolates and the medium used. The highest growth rate was found in isolate AD4 with Dobell's medium, the lowest rate in isolate AV1 with Rao's medium and intermediate results with Hallman's (4) synthetic and Pavlova's (5) monophasic media. Harinasuta and Harinasuta (9) grew three strains of E. histolytica in Locke-egg serum (10), liver infusion agar (11), inspissated horse serum (2) and aqueous egg-yolk infusion (12). They observed

Table 1. The rates of growth of E. histolytica in four different culture media

Isolate	Time (hr)	Trophozoites/mm^3			
		Dephasic media		Monophasic Synthetic	
		Dobell & Laidlaw[2]	Rao[6]	Pavlova[5]	Hallman et al.[4]
AD1	0	20	20	20	20
	24	50	30	40	45
	48	130	65	75	95
AD2	0	20	20	20	20
	24	45	30	35	40
	48	125	60	75	80
AD3	0	20	20	20	20
	24	55	35	45	50
	48	135	80	100	115
AD4	0	20	20	20	20
	24	60	40	50	55
	48	160	85	115	125
AV1	0	20	20	20	20
	24	45	35	40	40
	48	120	70	95	100

that all strains grow best in Boeck and Drbohlav's medium, moderately in Dobell and Laidlaw's (2) medium and very poorly in Balamuth's (12) egg-yolk infusion medium. In 1961, Botero(13) studied the rate of growth of E. histolytica (p-A[1] strain) in six different culture media. He also find that the growth cycle differs with the medium used; the greatest number of organisms being obtained with Dobell and Laidlaw's (2) medium, a finding supported by the present work.

The growth curve of E. histolytica isolate AD4 is shown in Fig 1. It is seen that the longest period of survival was obtained in Hallman's (4) synthetic medium and the shortest in Pavlova's(5) monophasic medium. The highest amoeba count was obtained in Dobell's medium (2) after 48hr. The longest survival time of amoebae in Hallman's synthetic

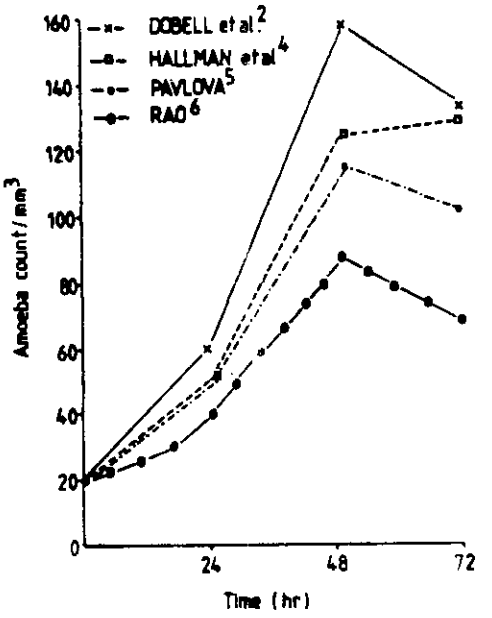

Fig. 1. The growth curves of E. histolytica isolate AD4 in four different culture media

medium might be due to the absence of serum and the substitution of amino acids and vitamins; Amoebae propagate well in this medium with a very good survival time and subcultivations could therefore be made at 96-hr intervals. Pavlova's monophasic medium has been used only effectively to transport material containing Trichomonas vaginalis from women. The growth of amoebae in Pavlova's medium is acceptable but subculturing is required after 48 hrs.

Table 2. Growth of E. histolytica isolate AD4 in two media adjusted to specified pH levels

pH	Amoeba count 10^3/ml			
	Dobell and Laidlaw[2]		Hallman et al.[4]	
	24 hr	48 hr	24 hr	48 hr
5.0	3.0	3.5	2.5	2.6
5.5	3.5	4.5	3.0	3.7
6.0	6.2	10.0	5.8	9.0
6.5	8.5	16.5	7.5	12.0
7.0	7.2	14.0	6.0	10.5
7.5	5.5	9.0	5.5	7.0
8.0	3.0	3.5	2.9	3.0

Table 2 shows the amoeba counts in Dobell's and Hallman's media respectively adjusted to the pH levels specified prior to inoculation. The amoeba counts were performed at 0, 24, and 48 hr respectively. It is seen from the table that the highest counts are at pH 6.5 for these two media.

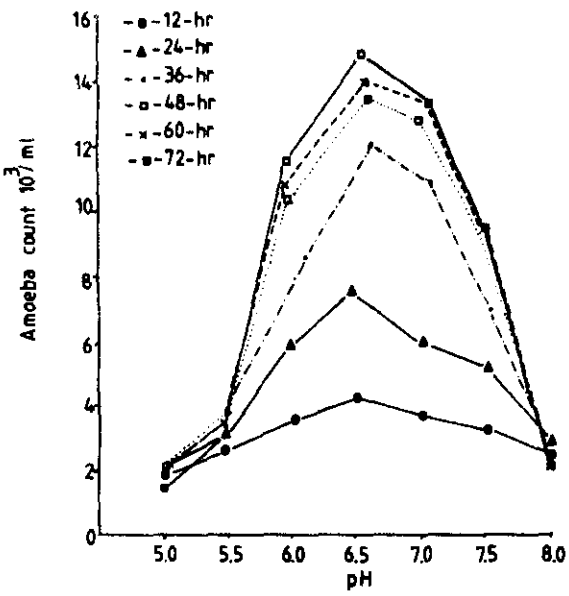

Fig.2-Growth of E.histolytica isolate AD4 in Hallman et al 4 synthetic medium adjusted to specified pH levels prior to inoculation.

Figure 2 shows the growth curves of isolate AD4 in Hallman's (4) synthetic medium. The growth curves reveal that variations in pH had a marked effect on the ability of E. histolytica to propagate. A pH of 6.5 was optimal for isolates of E. histolytica. In all series, the pH remained constant throughout the period of observation, deviating not more than 0.1 from the original tube checked at 0-hr.

Chang (14) reported that, in his hands, E. histolytica grew freely between pH 6.6 and 7.3. He made no comment on the ability of the amoebae to propagate at 6.0, but stated that a minimal growth occurred as the pH levels approached 5.5 and 8.0. The medium he used was not mentioned in his paper.

But in 1952, Shaffer (3) made a study on the effect of varying inocula and of pH on the growth of E. histolytica in Shaffer-Frye (15) medium. He described growth curves of the NIH 200 and the Luna strains(16) of E. histolytica in S-F medium adjusted to specified pH levels. He found that the NIH 200 strain propagate well over the range of pH 6.0 to 6.9 but do so very poorly at pH 5.1, 5.4 and 7.4. He also propagate amoebae in S-F medium at pH 8.0. Growth of E. histolytica has also been reported at pH 5.4, 6.0 and 8.5 (10). Rees, Key and Shaffer (17) again reported that E. histolytica grown in association with penicillin-inhibited streptobacillus propagate well at pH 6.0 to 6.5, a finding in agreement with the present study in which all isolates were found to propagate best at pH 6.5. The differences between the results of Shaffer's (3) and the present study could be attributed to differences in the culture media used. Isolates of E.histolytica tested in the present work propagated well at pH 6.5 and litle evidence of propagation was seen in the two media adjusted to pH 7.5, and none in that adjusted to pH 8.0. It is therefore concluded that higher pH levels did not favour the growth of E. histolytica in any medium.

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The Myanmar Health Sciences Research Journal, Vol. 1, No. 3, 1989.

An Epidemiologic Profile of Laparotomy Cases in Yangon Children's Hospital

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The operating theatre registers of the Yangon Children's Hospital for 1984 through 1986 were studied to determine epidemiologic parameters of interest. Annually, laparotomy cases comprised about 11% of all the major operations in the hospital. Of the laparotomy cases, congenital diseases constituted the highest proportion (42.4%), followed by acute abdominal conditions (37.1%) and cancer (3.0%). 65.1% surgical operations for imperforated anus were on neonates; 44.3% of operations for Hirschsprung's disease on infants under 6 months; 70.6% of operations for intussusception on post-neonates; 41.3% of operations for intestinal obstruction on 1-4 years old; and 51.5% for intestinal perforation, 50% for biliary obstruction, 46.5% for appendicitis and 43.5% for volvulus on 5-9 years old. There was preponderance of males to females for Hirschsprung's disease (3.4 : 1), imperforated anus (2.6 : 1), intestinal obstruction (2.5 : 1) and appendicitis (1.8 : 1). The mean ($\pm$ SD) duration of operation in minutes for biliary obstruction cases was the longest at 129($\pm$ 50), followed by volvulus 114($\pm$ 35), and intestinal obstruction 109($\pm$ 37). The durations, however, varied according to the type of surgical procedures performed. Factors influencing "duration of operation" are discussed from the epidemiologic perspective.

INTRODUCTION

There have been few reports on surgical operations performed in the hospitals of Myanmar. The majority of them provide limited information, mainly on total number of cases operated by single disease or by month according to type of surgery (major or minor)or type of anaesthesia (1). In order to contribute knowledge to the domain of 'surgical epidemiology', we undertook a hospital-based study on laparotomy cases with the following aims: (a) to determine the extent and pattern by disease with respect to age and sex; (b) to determine the duration of surgical operations by disease, and by operational procedure.

MATERIALS AND METHODS

The operating theatre registers of the Yangon Children's Hospital (YCH) were scrutinized. Each laparotomy case was examined for admission number, age, sex, diagnostic condition, time beginning and ending of operation and type of procedure. Data were collected for 3 years from 1984 to 1986. Diagnoses of disease were as stated in the registers. "Intestinal obstruction", however, excludes Hirschsprung's disease, imperforated anus, and other congenital conditions. Data summarized as totals, means, standard deviations, rates and percentage distributions are presented in Table 1 to 4. Student's t-test was used for testing significant difference between two means (2).

RESULTS

Total numbers, rates and patterns of surgical operations Through 1984 to 1986, an annual average of 3406 major

Table 1. Percentage distribution of laparotomy cases by year in Yangon Children's Hospital.

Disease	1984		1985		1986		Total		
	No.	%	No.	%	No.	%	No.	%	
Acute abdominal conditions	141	36.0	121	30.6	147	46.1	409	37.1	
Hirschsprung's disease	36	9.2	65	16.4	32	10.1	133	12.0	42.4
Imperforated anus	39	10.0	54	13.7	36	11.3	129	11.7	
Other congenital abnormalities	97	24.9	73	18.5	37	11.6	207	18.7	
Cancer	13	3.3	9	2.3	11	3.5	33	3.0	
Other diseases	65	16.6	73	18.5	55	17.4	193	17.5	
Total	391	100.0	395	100.0	318	100.0	1104	100.0	

operations were carried out on inpatients in the Yangon Children's Hospital. Of these, 368 cases (11.1%) had laparotomy. Again,of the laparotomy cases, congenital diseases as a group constituted the highest (42.4%). Other common group diseases were acute abdominal conditions (37.1%), and cancer (3.0%) (Table 1). Among the 409 acute abdominal cases operated on in these 3 years, the highest number was for intestinal obstruction (138 cases), followed by appendicitis (114 cases), intussusception (85 cases), intestinal perforation (33 cases), volvulus (23 cases) and biliary obstruction (16 cases) (Table 2).

Age and Sex at operation - In terms of age, 65.1% of surgical operations for imperforated anus were performed in the neonatal period (less than 1 month of life);44.3% of operations for Hirschsprung's disease in the first half of infant life; 70.6% of operations for intussusception in the post-neonatal period (1 month to 12 months); 41.3% of operations for intestinal obstruction and 39.4% of operations for cancer during 1 to 4 years; and 51.5% for intestinal perforation, 50.0% for biliary obstruction, 46.5% for appendicitis and 43.5% for volvulus on 5 to 9 years old (Table 2). For the 10+ year age group, operations were mainly for acute appendicitis, biliary obstruction and intestinal perforation (Table 2).

Regarding sex distribution of common diseases during 1985 and 1986 (where cases were sufficient for the analysis), males had a higher frequency of operations than females particularly for Hirschsprung's disease (3.4:1), imperforated anus (2.6:1), intestinal obstruction (2.5:1), and appendicitis (1.8:1).

Time taken at operation - During 1984, 1985 and 1986, operation time durations for a total of 1067 cases of laparotomy were found to be properly recorded. The overall mean ($\pm$ SD) was 90 ($\pm$ 60) minutes. On the average, operation time for biliary obstruction took the longest, 129($\pm$ 35), followed by cancer, 115 ($\pm$ 56), volvulus, 114 ($\pm$ 35) and intestinal obstruction, 109 ($\pm$ 37) (Table 3).

Duration of operation varied greatly by type of surgical procedure in respect of some common diseases in 1985 and 1986. Except in intestinal obstruction, the operation time by procedure was significantly different in intussusception, volvulus, Hirschsprung's disease and imperforated anus (Table 4).

Table 2. Percentage distribution of laparotomy cases by age in Yangon Children's Hospital during 1984, 1985 & 1986

Disease	Age						Total
	< 1mn	1-5mn	6-11mn	1-4yr	5-9yr	10+yr	
Acute abdominal condition Intestinal obstruction	7.2	13.0	8.0	41.3	22.5	8.0	100.0 (138)*
Appendicitis	0.9			6.1	46.5	46.5	100.0 (114)
Intussusception		31.8	38.8	18.8	9.4	1.2	100.0 (85)
Intestinal perforation		3.0	3.0	18.2	51.5	24.2	100.0 (33)
Volvulus	8.7	8.7	8.7	26.1	43.5	4.3	100.0 (23)
Biliary obstruction				12.5	50.0	37.5	100.0 (16)
Hirschsprung's disease	21.8	22.5	9.8	36.1	8.3	1.5	100.0 (133)
Imperforated anus	65.1	7.7	3.1	18.6	4.6	0.8	100.0 (129)
Cancer	3.0	15.2	12.1	39.4	27.3	3.0	100.0 (33)
Other disease	17.7	10.3	4.5	22.5	30.0	15.0	100.0 (400)
Total	17.9	12.1	7.8	24.4	24.7	13.0	100.0 (1104)

* Number of cases in parentheses

DISCUSSION

In this study, the majority of diseases that required laparotomy were due to congenital diseases, followed by acute abdominal conditions. Some diseases showed patterns of age occurrence and sex preponderance at operation. For instance, majority (70%) of intussusception cases were operated on in the post neonatal period and a good proportion (40%) of intestinal obstruction cases at the age of 1 to 4 years, while around half of the cases of intestinal perforation, biliary obstruction, appendicitis and volvulus had surgery at 5-9 years. More males than females were operated for Hirschsprung's disease, imperforated anus, intestinal obstruction and appendicitis.

A number of factors that influences the duration of surgical operation was identified. They included location of the lesion (e.g. high type of imperforated anus), severity, type of surgical procedure used, ascariasis status, presence or absence of other accompanying disease, whether it was the initial or subsequent operation (e.g. Hirschsprung's disease) etc. The study

Table 3. Duration of operation in minutes taken on laparotomy cases in Yangon Children's Hospital during 1984, 1985 & 1986

Disease	Number of cases	Mean ± S.D.
Intestinal obstruction	131	109 ± 37
Appendicitis	111	60 ± 25
Intussusception	81	85 ± 33
Intestinal perforation	29	95 ± 32
Volvulus	23	114 ± 35
Biliary obstruction	14	129 ± 50
Imperforated anus	125	87 ± 82
Hirschsprung's disease	131	95 ±100
Other congenital abnormalities	204	87 ± 53
Cancer	30	115 ± 56
Other diseases	188	86 ± 52
Total	1067	90 ± 60

Table 4. Duration of operation in minutes by surgical procedure for treatment of some common diseases in 1985 & 1986

Disease Surgical procedure	No.	Mean	S.D.	Statistical test
Intestinal obstruction				
Adhesiotomy	31	109	32	t = 1.05
Resection	13	120	31	p > 0.05
Intussusception				
Manual reduction	32	75	18	t = 8.16
Resection	10	137	29	p < 0.001
Volvulus				
Resection	13	126	29	t = 2.22
Untwisting	4	90	26	p < 0.05
Hirschsprung's disease				
Transverse colostomy	58	54	21	t = 23.92
Pull through	20	248	41	p < 0.001
Imperforated anus				
Transverse colostomy	43	52	20	t = 19.59
Pull through	8	236	42	p < 0.001

showed that, for some diseases, duration of operation depended very much upon the type of operational procedure performed. For instance, in intussusception, while the surgical procedure for resection and anastomosis took 137 minutes, that for manual reduction took only 75 minutes. Also, in our another study described elsewhere (3), duration of operation took longer in cases of Ascaris lumbricoides induced acute abdominal conditions than in cases without the Ascaris. As such, factors which might be associated with the occurrence and treatment of surgical conditions should likewise be identified and explored.

ACKNOWLEDGEMENT

The authors are greatful to the Medical Superintendent for allowing them to obtain the surgical operation data. The help given by the USAID-funded Ascaris Research Project of the Epidemiology Research Division, particularly the project medical officers, Dr Soe Myint Aung and Dr Nwe Ni Tun in collecting and analysing the data was much appreciated. The authors are also grateful to the referee (Professor D. Khin Hlaing) for having carefully gone through the manuscript and providing his useful suggestions.

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Enteric bacterial Contamination of some Myanmar fruit sweets

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Myanmar fruit sweets are widely eaten by our people. In the process of making these sweets, there are many ways by which they can get contaminated. The purpose of the study is to determine the degree of contamination. The percentage isolation of enteric bacteriae was the highest in the sweet-damp types and during the hot-wet season. The enteric bacteriaea were isolated more in tamarinds and plums and the least in marian group. The Myanmar fruit sweets may be one of the potentially important vehicle to transmit diarrhoeal pathogens during the hot-wet season in Myanmar. Improvements in the personal and environmental hygiene are needed to prevent contamination during their production.

INTRODUCTION

Myanmar fruit sweets, packeted in plastics bags are widely eaten by our Myanmar people, especially the girls. A lot of pocket money is often spent on these swets. Different kinds of fruits such as mangoes, plums, tamarinds and marians, etc are generally used in making these sweets. They come in different variety of tastes : sweet, salty, hot sour, etc. In the process of making them, contamination may occur in a variety of different ways such as improper washing of fruits, using of contaminated utensils, drying fruits in the open where they are liable to become contaminated and handling them with contaminated hands. Furthermore, contamination may also occur through prolong storage. The purpose of the present study is to determine the degree of contamination of these fruit sweets.

MATERIALS AND METHODS

Sample Collection

The study was conducted in Yangon between May 1988 and April 1989, during the representative months for the hot-wet, cool-dry and hot-dry seasons. Different brands of Myanmar fruit sweets were bought randomly from whole sale market as well as from small shops. They were bought and tested weekly on Mondays, Tuesdays and Wednesdays for 8-10 weeks during each season. Before being tested, the fruit content of each packet wa noted and were categorized into 4 groups: sweet-damp, sweet-dry, hot-damp and hot-dry. Altogether 162 packets were tested throughout the study.

Determination of faecal coliform(FC)count

Twenty five gram of the sweet was weighed and 10% suspension was made by using 225 ml of Ringer's solution. Then FC count was determined on that suspension by multiple tube methods as described by ICMSF 1968(1) in which different amounts of Ringer's solution were added to tubes containing Mac Conkey broth. In accordance with the numbers isolated and the number of tubes with positive reaction the most probable number of bacteria presents in the original ringer's solution can then be determined.

Isolation of enteric bacterias from samples

Ten gram of sweet samples were mixed with 90 ml of peptone water and preincubated at 37℃ for 18 hrs. Isolation of *Escherichia coli* was carried out by innoculating the suspension onto Mac Conkey agar (MA). For Salmonella

and Shigella spp. enrichment media, selenite-F (SF) was used and incubated at 42°C for 18 hrs and subcultured onto Salmonella Shigella (SS) media. For Vibrio spp. enrichment media, alkaline peptone water (APW-pH 8.4) was used, and incubated at 37°C for 6 hrs and subcultured onto thiosulphate citrate bile sucrose (TCBS) media. All enteric bacteriae were identified by standard procedures. The Chi-square test of significance was used for testing the statistical significance of isolation.

RESULTS

Table: Percentage isolation of enteric bacteria from Myanmar fruit sweets by season, Yangon, 1988-1989

Season	Sweet characteristic				Total
	Sweet*		Hot+		
	Damp	Dry	Damp	Dry	
hot-wet	60.0	20.0	25.0	16.7	31.5
cool-dry	40.0	6.7	41.7	8.3	24.1
hot-dry	46.7	6.7	0.0	8.3	16.7
Total	48.9	11.1	22.2	11.1	

* n = 15 each for damp and dry types each season
\+ n = 12 each for damp and dry types each season

sweet/damp Vs dry in all 3 seasons, $p<0.05$
hot/damp Vs dry in all 3 seasons, $p>0.05$

As shown in the Table, the highest percentage isolation of enteric bacteria was from the sweet-damp types in all the 3 seasons. Likewise, the highest isolation by season was during the hot-wet season. In all 3 seasons the percentage isolation was significantly different between the sweet-damp and the sweet-dry types. However, there was no significant difference between the hot-damp and hot-dry types. There was a non-significant descending trend of isolation percentage from hot-wet to hot-dry season. The pH of the sweet-damp types made of plums, tamarinds, mangoes, lemons and marians was 6.5, 6.3, 4.6, 4.5 and 4.3 respectively. Enteric bacterial isolation was the highest in tamarinds, and the lowest in marians. The FC count ranged from 0-2.4 X 10^{10}/gm of sweet. The isolated enteric bacteriae were E. coli, Enterobacter spp., Klebsiella spp., Pseudomonas spp., Proteus spp. and Citrobacter spp.

DISCUSSION

We found that the highest enteric bacterial isolation was in the sweet-damp varieties. The reasons for this is that in these types, there is carbohydrate and moisture to facilitate the growth of bacterias (2). The highest isolation during the hot-wet season may be due to the relatively high humidity during this season which is favourable for bacterial growth. Although the dry varieties of fruit sweets are less hazardous as far as enteric bacterial isolation are concerned, many people prefer the sweet-damp varieties.

The enteric bacterial isolation was the highest in tamarinds and plums and the least in marians. The low isolation rate from marian may be due to the bacteriostatic and bactericidal affects of its low pH (3). The reverse may be true for the high isolation in tamarinds and plums because their pH were higher.

Specific enteric bacterias such as E. coli, Enterobacter spp., Klebsiella spp., Pseudomonas spp., Proteus spp. and Citrobacter spp. were isolated from the fruit sweets. Since Klebsiella, various Proteus, Enterobacter and Serratia spp. have been isolated from African children with choleriform diarrhoea (4,5), there is a possibility that diarrhoea may result from consuming sweets harbouring such bacteria. Further, because the highest enteric bacterial isolation coincides with peak diarrhoeal incidence during the

hot-wet season in Myanmar (6), the Myanmar fruit sweets may probably be one of the potentially important vehicles for transmitting diarrhoea during this season.

The study suggests improvements in personal and environmental hygiene to prevent this potentially dangerous contamination of Myanmar fruit sweets during their production.

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NOTICE

Please read:

'Myanmar' for 'Burma'

'Yangon' for 'Rangoon'

'Ziwaka Road' for 'Zafar Shah Road'

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Department of Medical Research
Ministry of Health, Yangon, Myanmar

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