

MYANMAR HEALTH SCIENCES RESEARCH JOURNAL

Volume 2 , Number 2 August 1990

DEPARTMENT OF MEDICAL RESEARCH

No.5, Ziwaka Road,

Yangon 11191, Myanmar

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Printed at the Publications Division, Department of Medical Research

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Clinical, ultrasonic and pathologic analysis of ovarian tumours at the Central Women Hospital

(A prospective study)

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During an 18 month period from 1st January 1988 to 30th June, 1989 90 cases diagnosed as ovarian tumours were admitted to Unit 11, Central Women Hospital. Pre-operative data collection on clinical features and ultrasonic findings were made. Following laparotomy, operative findings and histopathologic diagnosis were noted. 87 cases had a laparotomy. Out of 82 cases of ovarian tumours, 21 were malignant (25.6 per cent) and 61 (74.4 per cent) benign. The clinical and ultrasonic diagnostic accuracy of ovarian tumours were 87.35 per cent and 91.18 per cent. There was no statistical difference between the two methods in diagnosing ovarian tumours ($P>0.05$). The accuracy of differentiating malignant from benign ovarian tumours clinically, by ultrasound or by laparotomy using gross findings and histopathology as the definitive diagnosis was also calculated and there was no significant difference ($P>0.05$). The sensitivity, specificity, predictive values for positive and negative tests were calculated in differentiating benign from malignant ovarian tumours. It was found that clinical diagnosis had the highest sensitivity rate (73.68 per cent) whereas operative findings had the highest specificity rate (96.72 per cent).

INTRODUCTION

During the study period from 1st January 1988 to 30th June 1989, there were 237 cases of ovarian tumours out of a total of 2451 gynaecological admissions to the Central Women Hospital. The number of cases with a histological diagnosis of benign ovarian tumours were 190 and malignant tumours were 47.

Although benign ovarian tumours may be asymptomatic or give rise to symptomatology such as presence of a mass, abdominal discomfort or pain, the overall prognosis of benign ovarian

tumours is good. However, malignant ovarian tumours tend to present with an altogether different picture. They tend to be asymptomatic. The presenting symptoms are usually of short duration and may be due to pressure alone or increased abdominal girth which may be attributed to middle age spread. These symptoms signify advanced growth and the overall 5 year survival is poor at 30% (1).

The traditional and time-honoured method of arriving at a diagnosis would be by taking a careful history and performing a thorough clinical examination. In recent years diagnostic imaging

techniques, namely ultrasound scanning, nuclear magnetic resonance imaging and computerised tomographical imaging have emerged as ancillary aids in the diagnostic work-up. Indeed, there seems to be a tendency to rely heavily on these methods. One of the objectives of the study was to compare the accuracy of clinical skills to that of ultrasound imaging in the diagnosis of ovarian tumours. Another objective was to compare the diagnostic accuracy of various methods, viz clinical assessment, ultrasound examination and operative findings in the differentiation of benign from malignant ovarian tumours using pathological diagnosis as the definitive diagnosis.

The cornerstone of treatment for ovarian tumours and more so for malignant ones is a staging laparotomy and surgery. In a country like ours where we have to put much reliance on operative findings to decide whether radical surgery is required, the correct diagnosis of malignancy at laparotomy is vital. Thus, testing the degree to which operative findings correlate with the definitive diagnosis would be helpful in determining the extent of surgery performed.

The third objective of determining the sensitivity and specificity of clinical, ultrasound and operative findings would indicate the degree of accuracy of diagnosing malignant ovarian tumours and the safe exclusion of benign ovarian tumours.

PATIENTS AND METHODS

This prospective study covered an 18 month period from 1st January 1988 to 30th June 1989. Included in the study were patients admitted to Unit 11, Central Women Hospital who were diagnosed as ovarian tumours either clinically or by ultrasound and patients with other clinical or ultrasound diagnoses who were found to be ovarian tumours at laparotomy. At first

encounter with the patient, history taking and clinical examination were performed. This was followed by an ultrasound examination in most of the patients - 68 (75.55%) as circumstances permitted. At laparotomy, operative findings were systematically noted and recorded. In most of the patients 82- (89.13%), a histopathological diagnosis was obtained. The clinical examination and laparotomy were performed either by three persons of consultant status or 4 assistant surgeons each with at least 8 years experience in Gynaecology. In 9 cases which were emergencies, initial clinical examination or laparotomy were performed by 4 assistant surgeons of the Central Women Hospital. 64 (94.12%) of the ultrasound examinations were performed by the consultant radiologist of the Central Women Hospital and 4 (5.88%) others were performed by two other radiologists of consultant status. All the histopathological specimens were examined by the consultant pathologist of the Central Women Hospital.

RESULTS

Ninety cases diagnosed clinically as ovarian tumours were admitted to Unit 11, Central Women Hospital. 87 cases had a laparotomy.

The accuracy of clinical and ultrasound examination in the diagnosis of ovarian tumours is shown in Table (1). Statistical tests indicated that there was no significant difference between the two methods in diagnosing ovarian tumours ($P>0.05$). Therefore, ultrasound scanning is not superior to clinical diagnosis in the accurate diagnosis of ovarian tumours.

The accuracy of differentiating malignant from benign ovarian tumours clinically, by ultrasound or by laparotomy using gross findings and histopathology as the definitive diagnosis is shown in Table (2). There was no significant

difference between clinical diagnosis, ultrasound diagnosis and operative findings in differentiating benign from malignant tumours ($P>0.05$).

Table 1. The accurate diagnosis of clinical and ultrasound examination in the diagnosis of ovarian tumours

	Number	Per cent
<u>Clinical Diagnosis</u>		
Ovarian tumours correctly diagnosed -	76	87.35
Ovarian tumours diagnosed as other pelvic tumours -	6	6.89
Other pelvic tumours diagnosed as ovarian tumours -	5	5.76
	87	100.00
<u>Ultrasound Diagnosis</u>		
Ovarian tumours correctly diagnosed -	62	91.18
Ovarian tumours diagnosed as other pelvic tumours -	5	7.35
Other pelvic tumours diagnosed as ovarian tumours -	1	1.47
	68	100.00

The sensitivity, specificity and predictive values for positive and negative tests were calculated in differentiating malignant from benign tumours. (Table 3) Statistical tests showed that there was no significant difference in the sensitivity of clinical assessment and operative findings in diagnosing ovarian tumours ($P>0.05$). However, there was a significant difference between clinical assessment and ultrasound examination ($P<0.05$) and operative findings and ultrasound examination ($P<0.05$) indicating that both clinical and operative findings were superior to ultrasound in predicting malignancy. With regard to specificity, there was no significant

Table 3. Sensitivity, specificity, positive predictive value and negative predictive value in differentiating malignant from benign ovarian tumours

	Clinical	Ultrasound	Operative
Sensitivity	73.68	50.00	66.66
Specificity	89.47	90.47	96.72
Predictive value (positive)	70.00	71.42	87.50
Predictive value (negative)	91.07	79.16	89.39

Table 2. Accuracy in differentiating malignant from benign ovarian tumours by clinical diagnosis, ultrasound diagnosis and operative findings

	Correct		Incorrect		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Clinical Diagnosis	68	85.53	11	14.47	76	100
Ultrasound Diagnosis	48	77.42	14	22.58	62	100
Operative Findings	73	89.02	9	10.98	82	100

difference between the three methods in diagnosing ovarian tumours ($P>0.05$).

In the predictive value of a positive test, operative finding was of the highest score whereas in the predictive value of a negative test, clinical examination scored the highest value.

DISCUSSION

The distinction of ovarian tumours from other pelvic tumours including benign ovarian tumours become increasingly important as ovarian cancer is now the commonest malignancy of the female genital tract in most western countries (2). In a retrospective study on genital cancer at the Central Women Hospital, ovarian cancer was second to carcinoma cervix, carcinoma cervix being 52.1 per cent and carcinoma ovaries 36.1% (3). Although ovarian cancer incidence is only slightly higher than the incidence of cancer of the uterine cervix and corpus, survival is considerably worse (4). Ovarian cancer causes more deaths than the combined deaths of cancer of the uterine cervix and corpus (5). Therefore, accuracy in diagnosis becomes imperative. It has been estimated that 15 per cent are in correctly thought to be fibroids and another 35 per cent to be benign cysts while the remaining 50 per cent are correctly diagnosed pre-operatively (6). In this study, ovarian tumours were correctly diagnosed in 76 out of 87 (87.35 per cent) patients by clinical examination. Ultrasound diagnosis was accurate in 62 out of 68 (91.18 per cent) patients. However, significance testing showed that ultrasound scanning was not superior to clinical diagnosis. In a country like ours where ancillary aids might not be available in all state and divisional hospitals, this is a welcome fact. However, the clinicians involved had at least 8 years experience in gynaecology and this may have been responsible for the high rate of accuracy.

In the differentiation of benign from malignant ovarian tumours, the diagnostic accuracy for clinical, ultrasound and operative findings were 85.53 per cent, 77.42 per cent and 89.02 per cent respectively. Significance tests showed that all three methods were at par in differentiating benign from malignant ovarian tumours. In the clinical work-up of a case, one would take into consideration the behaviour of the tumour, the general condition of the patient and the local extent of the disease.

Ovarian carcinoma is best regarded as a peritoneal disease.

Ultrasound examination would include assessment of pelvic masses, the presence of ascites (either minimal or clinically evident), involvement of the liver with secondaries and the condition of other abdominal viscera, e.g. kidneys. In studies in the west, it has been found that ultrasound is poor in the detection of ascites, omental and peritoneal metastases and lymphadenopathy (7). Thus, attempts have been made to correlate echo pattern with benign and malignant tumours (8). In that study, it was found that the percentage of accuracy of diagnosing malignant ovarian tumour was 89.36 per cent with a low rate (0.66 per cent) of false negative diagnosis. Histologic and echo pattern correlation was also high. We have not correlated echo pattern with malignancy or histology in our study. At staging laparotomy, the assessment would include details regarding the presence of ascites, the extent of the tumour at the ovary (extra-capsular tumour or capsule ruptured), and involvement of the fallopian tubes, uterus, visceral peritoneum, omentum, diaphragm, liver and lymph nodes.

In the differentiation of malignant from benign tumours, it was found that clinical assessment and operative findings were more sensitive parameters compared to ultrasound examination.

This indicates that they would pick-up all positive cases i.e. malignancy. Regarding specificity, there was no significant difference between the three parameters indicating that the exclusion of malignancy by these three methods is reliable.

Operative findings scored highest in the predictive value of a positive test indicating that it could detect the highest number of patients with malignant disease. Clinical examination, with the highest predictive value of a negative test could correctly exclude malignancy.

In this study sensitivity has been defined as true positives/true positives + false negatives and specificity as true negatives/true negatives + false positives. Sensitivity indicates the number of malignant cases correctly identified. On the other hand specificity is the number of benign cases correctly identified or malignant cases confidently excluded.

Predictive value of a positive test means percentage of cases who are clinically malignant and are truly malignant whereas the predictive value of a negative test is the percentage of cases who are clinically benign and are benign (i.e. not malignant).

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Accepted for publication 13 June 1990.

Renal lithium clearance in essential hypertension

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There is considerable interest in whether a defect in renal sodium handling is important in the development of essential hypertension. A recent report indicated that the proximal tubule might be involved, since fractional lithium clearance, a measure of the fraction of filtered sodium reaching the end of the proximal tubule, was reduced in hypertensive patients. However, there were a number of objectionable points in that paper. In the present study, we measured clearances of creatinine (C_{Cr}), lithium (C_{Li}) and sodium (C_{Na}) in 21 hypertensive patients and 21 controls matched for age, sex and ethnic background. Clearances were measured over a 4-h period during which the subjects were seated. The results provide no evidence for altered proximal tubular sodium handling in essential hypertension under the conditions of the study.

INTRODUCTION

The kidneys have long been implicated as a cause of raised blood pressure. Goldblatt showed in the early 1930s that blood pressure was raised when the renal artery was clipped in the absence of a contralateral kidney (Goldblatt one-kidney, one-clip hypertension; 1K1C). Sodium retention is also known to be associated with 1K1C hypertension, whilst it does not seem to operate in the 2K1C model(1). More intriguing is the link between unclipped kidneys and the development of high blood pressure. Cross-transplantation experiments between models of rat hypertension and normal rats have shown that hypertension develops only when the "culpable" kidney is present (2,3).

Recently, the lithium clearance technique has been proposed as a tool for assessing sodium handling by the

proximal tubules(4). Two studies(5,6) have claimed that fractional lithium clearance is reduced in patients with essential hypertension, perhaps reflecting enhanced reabsorption of sodium in the proximal tubules. However, there are a number of criticisms that can be made on these studies:(1) posture, which is an important determinant of renal function(7,8,9) was uncontrolled; (2) the subjects were not matched for age and ethnic background; (3) the hypertensives in Weder's study were very young, (mean age 25 years), so renal hypertension is a possibility; (4) overall renal function cannot be gauged, because only fractional values are given, and values for glomerular filtration rate were not given.

In view of the limitations of these studies, we studied hypertensives with normal renal function and matched control subjects, in whom posture was

controlled during the measurement of lithium clearance.

MATERIALS AND METHODS

Subjects

The subjects were 21 patients with essential hypertension (10 males), aged 51.0 ± 3.0 years (mean $\pm$ SEM), off treatment and on their customary sodium intake. The normotensive controls were matched for age and ethnic background.

Experimental protocol

Patients/subjects were instructed to abstain from drugs and alcohol from 24 h preceding the start of the experiments. Coffee, tea and other xanthine and/or caffeine-containing food and drink were prohibited from the morning of the experiment till the end of the experiment. A single dose of lithium carbonate (600 mg; 16.2 mmol lithium) was taken orally 10-12 hours before the clearance period. On the morning of the experiment, a normal light breakfast was permitted and the consumption of fluids (other than those prohibited) was encouraged. Immediately before the start of the clearance period, blood pressure was measured by ultrasound sphygmomanometry (arteriosonde with attached recorder) with subjects in the sitting position; an average of 5 readings was taken.

A sample of blood (60 ml) was taken for measurement of packed cell volume and plasma sodium, potassium, urea, glucose, creatinine, lithium, aldosterone, renin activity and atrial natriuretic peptide. An intravenous injection of $^{51}\text{CrEDTA}$ (70 μCi ; Amersham International) was then administered, after which subjects were given 250 ml of tap water to drink. Urine was then collected for 4 h, during which 100 ml of water was drunk per hour. The subjects remained seated throughout the 4 hours. Further blood samples were

taken after 2 h (20 ml; for measurement of ^{51}Cr radioactivity) and 4 h (22 ml; for measurement of ^{51}Cr radioactivity and plasma lithium and creatinine concentrations).

A 24-h urine collection, incorporating the 4-h clearance period, was obtained from all subjects.

Analyses

Plasma and urine creatinine were measured using the Jaffe reaction (10,11). Sodium and potassium concentration in plasma and urine were measured using flame photometry (Instrumentation Laboratory, Model 543). Plasma and urinary lithium concentrations were measured by atomic absorption spectrophotometry using the Instrumentation Laboratory aa/ae spectrophotometer, model 151(12). Due to the exponential nature of the fall in plasma lithium concentration after taking a test dose, a representative plasma lithium concentration (P_L) was calculated from the formula:

$$P_{Li} = \frac{P_1 - P_2}{2.3 \log_{10} P_1/P_2}$$

where P_1 and P_2 are the plasma concentrations of ^{51}Cr lithium at the beginning and end of the urine collection period, respectively(4). Plasma aldosterone, ANP and PRA were measured by radioimmunoassay(13,14,15). The clearance of $^{51}\text{CrEDTA}$ was measured using the single injection technique(16).

Calculations

Clearances were calculated using the standard clearance formula and standardised for 1.73 sq. m body surface area. Fractional excretion of lithium and sodium were calculated as: -

$$C_{Li} \text{ or } C_{Na} / C_{Cr} \times 100$$

Statistics

Student's paired "t" test and Wilcoxon's matched-pairs signed-ranks test were used to test the significance of differences between the matched groups of normotensive and hypertensive subjects. Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (r_s) were used for testing the relationship between the variables. Since the choice of statistics did not influence the conclusions arrived at, only the results of the parametric tests have been presented.

(Informed consent was obtained from all subjects. The study was approved by the Ethical Committee of Charing Cross Hospital, London, U.K).

RESULTS

Table 1 shows the age, blood pressures, 24-h sodium and potassium excretions (reflecting 24-h intakes), overall renal function, and hormonal profile in the hypertensives as compared to normotensives.

Table 1. Age, sitting blood pressures, 24-h sodium and potassium excretions, renal clearance data and plasma hormone levels in 21 pairs of hypertensive and normotensive subjects. Clearances were measured over a 4-h period.

Values are means $\pm$ SEM. Statistical analysis of differences between the two groups was made using Student's paired "t" test.

NS: not significant

Variable	Hypertensives	P	Normotensives
Age (years)	51.0 $\pm$ 3.0	NS	51.5 $\pm$ 2.5
Sitting SBP (mm Hg)	166 $\pm$ 5	< 0.001	115 $\pm$ 3
Sitting DBP (mm Hg)	107 $\pm$ 2	< 0.001	78 $\pm$ 1
Sitting MAP (mm Hg)	126 $\pm$ 3	< 0.001	91 $\pm$ 2
24-h sodium excretion (mmol)	327 $\pm$ 12	NS	135 $\pm$ 12
24-h potassium excretion (mmol)	62 $\pm$ 5	NS	70 $\pm$ 6
Creatinine clearance (ml/min per 1.73 sq.m)	192 $\pm$ 5	NS	94 $\pm$ 6
51-CrEDTA clearance (ml/min per 1.73 sq.m)	88 $\pm$ 7	NS	87 $\pm$ 5
Sodium clearance (ml/min per 1.73 sq.m)	0.78 $\pm$ 0.09	NS	0.81 $\pm$ 0.08
Plasma aldosterone (pmol/l)*	587 $\pm$ 64	NS	536 $\pm$ 65
PRA (ng of ANG 1/h per ml)*	1.70 $\pm$ 0.24	NS	2.59 $\pm$ 0.45
Plasma ANP (pg/ml)*	20.8 $\pm$ 2.9	NS	14.7 $\pm$ 1.5
Plasma creatinine (μ mol/l)	83 $\pm$ 4	NS	77 $\pm$ 3
Plasma lithium (μ mol/l)	301 $\pm$ 24	NS	285 $\pm$ 17

N.B. SBP = systolic blood pressure; DBP = diastolic blood pressure;

MAP = mean arterial blood pressure; PRA = plasma renin activity;

ANP = atrial natriuretic peptide; ANG 1 = angiotensin 1.

* n = 20 pairs

Apart from the blood pressure, there is no significant difference in any of the variables between the two groups. Plasma renin activity is slightly lower, and ANP is slightly higher, in the hypertensive group.

Figure 1 shows the comparison between the two groups regarding lithium clearance and fractional lithium clearance. Values are means $\pm$ SEM. There were no significant differences between the two.

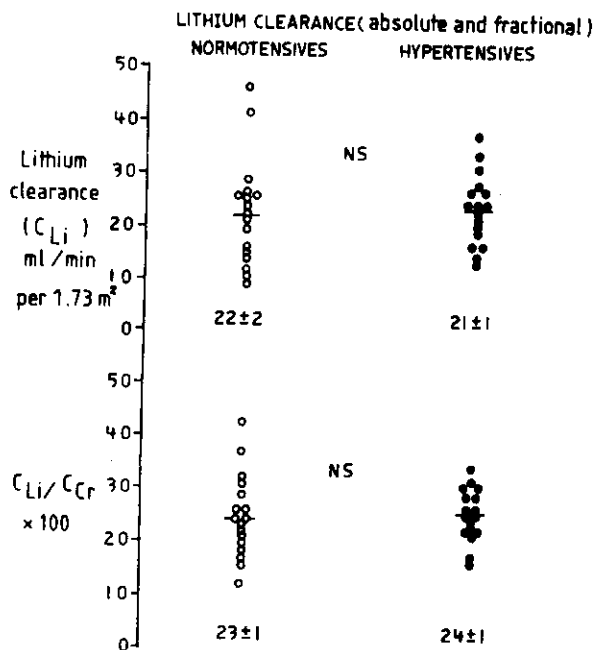


Figure 1. Lithium clearance and fractional excretion of lithium in 21 hypertensive patients compared with 21 matched normotensive controls. The clearance of creatinine was used as a measure of glomerular filtration rate. Mean ($\pm$ SEM) are also given. NS: not significant.

Figure 2 shows the correlation between mean arterial pressure and lithium clearance/fractional lithium clearance. There is no correlation at all between MAP and C_{Li}, though there is a slight trend towards a positive correlation between MAP and fractional excretion

of lithium (C_{Li}/C_{Cr} x 100) in the hypertensives.

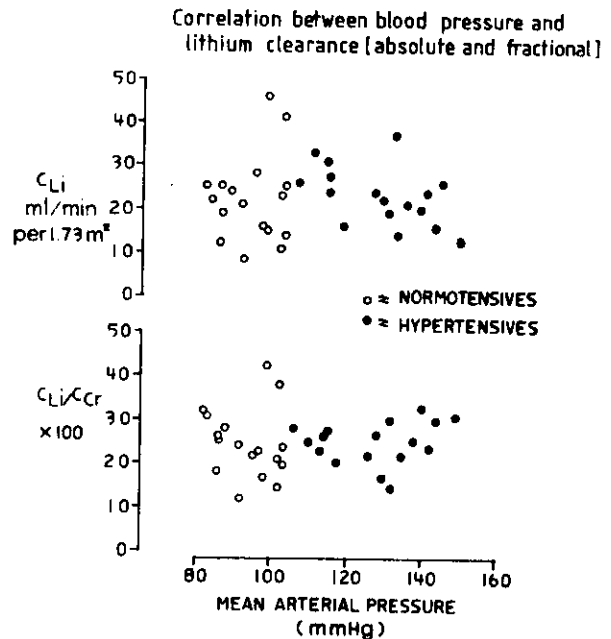


Figure 2. Correlation between sitting mean arterial pressure and lithium clearance (upper panel) and fractional excretion of lithium (lower panel).

DISCUSSION

Despite proper matching of the subjects, no differences could be detected regarding overall renal function or renal handling of sodium (as assessed by the lithium clearance technique). If the hypothesis that an enhanced renal sodium reabsorption in the proximal tubules (and hence lowered C_{Li}) leads to an increase in blood pressure is true, there should be a negative correlation between MAP and C_{Li} or C_{Li}/C_{Cr}. However, there was no correlation between the two; in fact, a positive trend (r=0.18) was seen between C_{Li}/C_{Cr} and MAP in the hypertensives. These results therefore provide no evidence to support previous reports that fractional reabsorption of sodium

in the proximal tubule, as assessed by lithium clearance, is enhanced in patients with essential hypertension. The apparent discrepancy between our findings and those of Weder(5) and Langford(6) could be due to the fact that posture was not controlled in these studies. An increase in sympathetic activity may play a role in the development of hypertension(17), and assumption of the upright posture could have accentuated a difference in sympathetic activity, resulting in lowered C_{Li} and FE_{Li} in the patients with essential hypertension. Another group of investigators, who also controlled posture, could not detect any difference in C_{Li} between hypertensive patients and normotensive controls(18). Further research is required to elucidate this point e.g. either by studying renal function in hypertensive patients and normotensive controls after sympathetic blockade, or after stimulation of the sympathetic system by changes in posture.

There is also a possibility that differences in renal handling could represent transient phases in the development of hypertension, as seen in rat models(19,20). Weder, using young hypertensives, could have inadvertently "caught" that transient phase. However, since, unlike rat models, the exact age of development of hypertension is not defined, it is impossible to duplicate these findings.

Thus, the results taken alone, would suggest that (a) an increase in reabsorption of sodium in the proximal tubule does not play a role in the pathogenesis or maintenance of established essential hypertension; (b) C_{Li} is not a sufficiently specific or sensitive technique for assessment of proximal tubular sodium handling; (c) homeostatic mechanisms may compensate for genetic defects and therefore studies under stimulated conditions may be required to detect abnormalities of tubular sodium handling.

ACKNOWLEDGEMENTS

Hla-Yee-Yee was supported by the British Council. D.R.J. Singer is a British Heart Foundation Research Fellow. The authors wish to thank Ms. B.E. Jones of the Department of Nuclear Medicine, for measuring the clearances of $^{51}CrEDTA$; Dr. K.D. MacRae, Reader in Medical Statistics, for statistical advice; Mrs. M. Miller, Mr. M. Buckley and Dr. A.L. Sugden of the Blood Pressure Unit, St. George's Hospital, London for measurements of aldosterone, ANP and PRA, respectively. (This paper was presented in poster form at the Medical Research Society Seminar Meeting, Leicester, July 1988 & read at the 36th MMA Conference.)

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Accepted for publication 14 March 1990.

Renal function, plasma sodium and osmolality during the menstrual cycle

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In order to assess whether hormonal changes occurring during the menstrual cycle affect renal function, glomerular filtration rate (C_{Cr}), and proximal tubular reabsorption of sodium and water (lithium clearance; C_{Li}) were assessed in 9 healthy women in 2 phases of the menstrual cycle. Plasma sodium and osmolality were also measured. Each subject was studied three times ("basal", "proliferative" and "luteal" phases), while on her customary sodium intake, and in a controlled (sitting) posture. There were no significant differences between the phases with respect to any of the renal variables studied. There was a significantly lower plasma sodium in the luteal phase, and a trend towards lower plasma osmolality.

INTRODUCTION

The possibility that hormonal changes occurring during the menstrual cycle might affect renal function has deterred a number of investigators from using female subjects in renal studies. Indeed, small variations in glomerular filtration rate during the menstrual cycle have been reported(1,2). The reproductive hormones could also have an effect on sodium and water handling. Progesterone is known to have a natriuretic effect, at least in acute doses(3,4), whilst oestrogen has been demonstrated to retain sodium and water(4). These hormones could therefore have an effect on renal tubular handling of sodium, which would result in differences in sodium and water homeostasis between the proliferative phase (when oestrogens predominate) and the luteal phase (when progesterone effects as well as oestrogen effects may be expected).

MATERIALS AND METHODS

Subjects

The subjects were 9 healthy normotensive women, 19-40 years, who were on their customary sodium intake (110-150 mmol/day), had no known renal disease, and who had not taken hormonal contraceptives for at least three months. They all had regular cycles (3-5 days bleeding, 26-30 day cycles).

Study design

Each subject was studied at the same time of day on three occasions: phase 1 (day 5,6 or 7 of the cycle); phase 2 (day 13,14 or 15); and phase 3 (day 20, 21 or 24). The subjects abstained from drugs and alcohol for at least 24 h before the start of the experiment. Tea, coffee, and caffeine- or xanthine-containing food and drink were withheld from the morning of the experiment till

its completion. Lithium carbonate (600 mg; 16.2 mmol lithium) was taken 10-12 h before the start of the clearance period. Approximately 15 minutes before the clearance period, weight and height were measured and 28 ml of blood was taken by venepuncture for measurement of packed cell volume (PCV) and plasma progesterone, β -oestradiol, sodium, potassium, osmolality, creatinine and lithium. An oral water load of 250 ml, followed by 100 ml/hour was given to ensure adequate urine flow. Subjects remained seated through the clearance period, which lasted 4 hours. Blood pressure was measured at 2 hours by auscultation sphygmomanometry by the same person, using the same arm on each occasion. Blood (8 ml) was again taken at the end of the 4 hours, for measurement of plasma creatinine and lithium. Urine collection was continued for 20 hours following the clearance period, in order to measure 24-h sodium and potassium excretions.

Analyses

Plasma and urine creatinine were measured by the Jaffe' reaction, sodium and potassium by flame photometry (Instrumentation Laboratory, model 543); lithium concentrations were measured by atomic absorption spectrophotometry (Instrumentation Laboratory aa/ae spectrophotometer model 151) (5). Due to the exponential nature of the fall of plasma lithium concentration after taking the test dose, a representative plasma lithium concentration (P_{Li}) was calculated from the formula:

$$P_{Li} = \frac{P_1 - P_2}{2.3 \log_{10} P_1/P_2}$$

where P_1 and P_2 are the plasma concentrations of lithium at the beginning and end of the urine collection period, respectively (6). Plasma osmolality was measured using the method of depression of freezing point

(Camlab Automatic Micro-osmometer).

Serum progesterone and β -oestradiol concentrations were measured by radio-immunoassay, using kits.

Calculations

Standard formulae were used for calculation of clearances and fractional excretions of sodium and water were calculated from the formula: -

C_{Na} or $C_{Li} / C_{Cr} \times 100$, where C_{Na} , C_{Li} and C_{Cr} represent clearances of sodium, lithium and creatinine, respectively.

Fractional distal excretion of sodium was calculated as $C_{Na}/C_{Li} \times 100$, and fractional distal excretion of water as $V/C_{Li} \times 100$, where V = urine flow rate.

Statistics

Statistical analysis of differences in values for the three phases was made using analysis of variance (ANOVA) with repeated measures. Differences between means of observations were analysed by the method of the least significant difference (LSD) wherever a significant difference was detected with ANOVA. Pearson's and Spearman's correlation coefficients were used to analyse relationships between variables in each phase separately. Fisher's 'Z' transformation was applied to Pearson's 'r' values to analyse relationships for all phases combined.

RESULTS

Table 1 shows the body weight, PCV and plasma concentration of electrolytes, osmolality, creatinine and lithium. There are no significant differences in most of the variables between the 3 phases; however, plasma sodium concentration was significantly lower in the luteal phase.

Table 2 compares the clearances of creatinine, lithium, sodium and potassium as well as 24-h sodium and potassium excretion between the three phases of the cycle. No significant differences were detected. Calculated distal tubular function also revealed no significant differences.

The β -oestradiol levels were 171^{+43} , 486^{+121} and 421^{+67} pmol/l (mean $\pm$ SEM); progesterone levels were < 5 , 11^{+3} and 55^{+11} nmol/l (mean $\pm$ SEM) for phase 1, 2 and 3, respectively.

DISCUSSION

All the 9 subjects ovulated, as adjudged by the significantly higher progesterone levels in phase 3, and β -oestradiol levels were highest in phase 2. The hormone profile was therefore normal, oestradiol being predominant in the proliferative phase (phase 2) and both progesterone and a secondary oestrogen peak being seen in the mid-luteal phase.

Table 1. Body weight, packed cell volume(PCV) and plasma data in 3 phases of the menstrual cycle in 9 normal women in a single ovulatory cycle
Values are means $\pm$ SEM. NS: not significant

Variable	Phase 1	Phase 2	Phase 3	ANOVA
Body weight (kg)	56.7 ± 3.4	57.2 ± 3.6	57.2 ± 3.5	NS
PCV (%)	40 ± 1	40 ± 1	41 ± 1	NS
Plasma sodium (mmol/l)	141 ± 1	140 ± 1	138 ± 1	$< 0.01^*$
Plasma potassium (mmol/l)	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	NS
Plasma osmolality (mosm/kg H ₂ O)	290 ± 2	288 ± 2	285 ± 2	NS
Plasma creatinine (μ mol/l)	87 ± 3	86 ± 3	85 ± 3	NS
Plasma lithium (μ mol/l)	241 ± 22	247 ± 19	247 ± 17	NS

*Phase 1 vs. Phase 2: NS; Phase 2 vs. Phase 3: $p < 0.025$; Phase 1 vs. Phase 3: $p < 0.001$

Table 2. The clearances of creatinine, lithium, sodium and potassium and 24-h sodium and potassium excretions in the 3 phases of the menstrual cycle

Variable	Phase 1	Phase 2	Phase 3	ANOVA
Creatinine clearance (ml/min)	97 ± 5	94 ± 5	98 ± 6	NS
Lithium clearance (ml/min)	24 ± 2	25 ± 2	26 ± 2	NS
Fractional excretion of lithium(FE_{Li}) ($C_{Li}/C_{Cr} \times 100$) (%)	25 ± 1	27 ± 1	27 ± 2	NS
Sodium clearance (ml/min)	1.25 ± 0.12	1.38 ± 0.13	1.29 ± 0.19	NS
Potassium clearance (ml/min)	14.2 ± 2.3	15.4 ± 1.2	15.9 ± 1.2	NS
24-h sodium excretion (mmol/d)	118 ± 14	113 ± 14	147 ± 17	NS
24-h potassium excretion (mmol/d)	37 ± 5	36 ± 4	49 ± 5	NS

Though other investigators have reported an increase in glomerular filtration rate in the luteal phase of the menstrual cycle, we could only detect a tendency towards a rise in creatinine clearance in phase 3; nor was there a correlation between progesterone concentrations (which were greatly increased in phase 3) and creatinine clearance.

There are no published data of C_{Li} during the menstrual cycle for comparisons to be made with our results. It is of interest, however, to note that C_{Li} is increased in association with increased GFR in pregnant rats and women; fractional excretion of lithium was, however, unchanged(7).

Thus, if the lithium clearance method is a valid marker of end-proximal delivery, these results would indicate that proximal sodium and water handling is unaltered during the menstrual cycle at least in women who do not experience premenstrual tension or significant weight changes.

Of interest also was the finding that plasma sodium fell significantly in phase 3, and that plasma osmolality showed a tendency towards a fall. These could result from (a) volume expansion (b) "resetting" of osmoreceptors regulating vasopressin release or (c) progesterone-induced natriuresis. Possibility (a) is not favoured by the absence of body weight and PCV changes. More probable are the latter two possibilities and these will be considered in more detail.

Natriuresis could also produce the fall of plasma osmolality and sodium. However, sodium clearance and excretion rates for both the 4-h clearance period and 24-h were not significantly different between the 3 phases; nor was there a correlation between progesterone or oestradiol levels and C_{Li} or FE_{Li} . If, as is widely supposed, progesterone inhibits the action of aldosterone, natriuresis and antikaliuresis may be expected in phase 3. The negative

correlation between plasma progesterone concentration and potassium clearance during phase 3 provides circumstantial evidence that progesterone might indeed have been inhibiting the action of aldosterone; a possibility which receives some support from the observation that during this phase plasma progesterone levels were positively correlated with values for C_{Na}/C_{Li} ($r=0.79$; $p < 0.01$).

It is believed that during pregnancy the sensitivity of the osmoreceptors regulating vasopressin release is disturbed(6), and it is conceivable that the same explanation might apply to the luteal phase of the menstrual cycle. Further support for this hypothesis comes from the finding of others that the fall in plasma osmolality is not associated with an increase in total body weight, extracellular fluid volume or PCV (8, 9, 1 respectively).

ACKNOWLEDGEMENTS

Hla-Yee-Yee was supported by the British Council. Hormone assays were done by Mr. G. Carter, Department of Chemical Pathology, Charing Cross Hospital, London, U.K. The authors thank Dr. K.D. MacRae, Reader in Medical Statistics, CXH, for statistical advice. (This paper was presented in part at the Physiological Society Cambridge meeting, July 1988, and in poster form at the 36th Myanmar Medical Conference, Yangon, January 1990).

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Accepted for publication 14 March 1990.

Haemolytic effect of Russell's viper venom on red blood cells

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The red blood cells (RBC) of human and Wistar rats were incubated with 300 ng of Russell's viper venom (RVV) at 37°C. At different incubation times, the haemolytic effect of RVV was tested by estimating plasma haemoglobin (Hb) level and by performing osmotic fragility test. Five cases of human Russell's viper bite (RVB) victims with non-clotted blood were also studied. No significant haemolytic effect was found on human RBC whereas significant haemolytic effect was observed on RBC obtained from Wistar rats. These results suggested that action of RVV may be species specific.

INTRODUCTION

The Russell's viper venom (RVV) contains a number of components and among them, haemolysin is one of the least important components for causing death(1). But frank haemoglobinuria which is one of the sequelae of intravascular haemolysis is found to be a presenting symptom in some of the Russell's viper bite (RVB) victims(2). It has also been reported that renal failure can result from intravascular haemolysis due to diverse clinical conditions of Russell's viper envenomation(3).

The purpose of this study is to clarify whether intravascular haemolysis (IVH) in Russell's viper envenomation is due to direct lytic action of venom or secondary to disseminated intravascular coagulation (DIC) where the trapped red blood cells are traumatized by the fibrin strands deposited in the vessel walls.

MATERIALS AND METHODS

The venom used was pooled crude venom (dried crystals) of *Vipera russelli*, obtained from the Biological Laboratory of the Myanmar Pharmaceutical Industry. It was freshly dissolved in 0.9% saline before use to get 300 ng per 0.05 ml of saline.

IN VITRO STUDY

(a) Human RBC:

Blood samples from 5 normal healthy subjects were collected separately into the heparinized tubes. Each sample was then divided into 2 tubes (1 ml each), one for the test and the other for control. For the test, 300 ng of venom in 0.05 ml of normal saline and for control only 0.05 ml of saline was added. The tubes were mixed thoroughly and incubated at 37°C. Osmotic fragility test and plasma haemoglobin estimation(4) were done at half hour and at hourly intervals up to 4 hours.

(b) Rat RBC

Pooled heart blood samples from Wistar rats were collected in the heparinized tubes. These samples were incubated at 37°C with venom and normal saline and the osmotic fragility and plasma haemoglobin level were tested as above.

IN VIVO STUDY

Five confirmed RVB victims with non-clotting blood who were admitted to Tharyawady Township Hospital were selected for the study. Blood samples were collected in the heparinized tubes on admission and again when blood became re-clotted. The osmotic fragility test and plasma haemoglobin estimation were done on these blood samples together with control samples from patient's relatives.

Statistical significance was tested by Student "t" test for paired samples.

RESULTS

Human RBC: *In vitro* experiments showed that plasma haemoglobin levels and per cent lysis of test samples were not significantly different from control samples at all incubation periods. Figures 1 and 2 show the results obtained at 2 hours incubation period. The results for all incubation times are also identical. Similar results are also obtained in *in vivo* experiment.

Rat RBC: Plasma haemoglobin levels of tests were significantly higher than the controls at all incubation times (Figure 3). Likewise, osmotic fragility tests done at saline concentrations of 5 g/l to 6.5 g/l showed that per cent lysis differences between tests and controls were statistically significant at all incubation periods. At concentration of 7.5 g/l, the differences were only significant at 1 hour, 2 hours and 3 hours incubation periods and not at 4 hours (Figure 4).

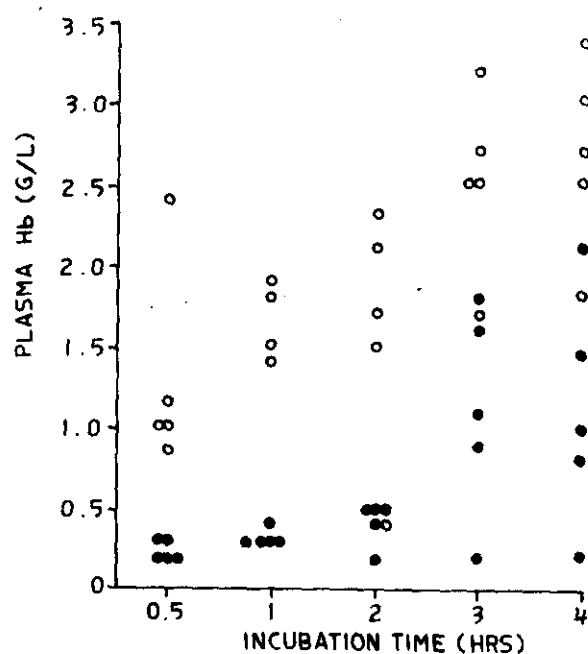


Figure 1. Plasma haemoglobin levels of human blood after incubation with venom (test 0) and saline (control ●)

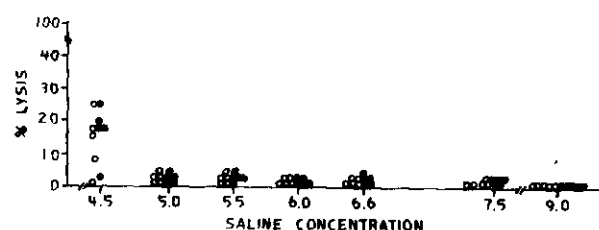


Figure 2. Per cent lysis of human RBC after 2 hours incubation with RVV in test (0) and saline control (●)

DISCUSSION

In the present study, both *in vivo* & *in vitro* experiments showed that RVV has no significant direct lytic effect on human RBC, as demonstrated by osmotic fragility test and plasma haemoglobin estimation.

Although there is a report of a RVB victim presenting with frank haemoglobinuria(2), the lytic effect of venom on

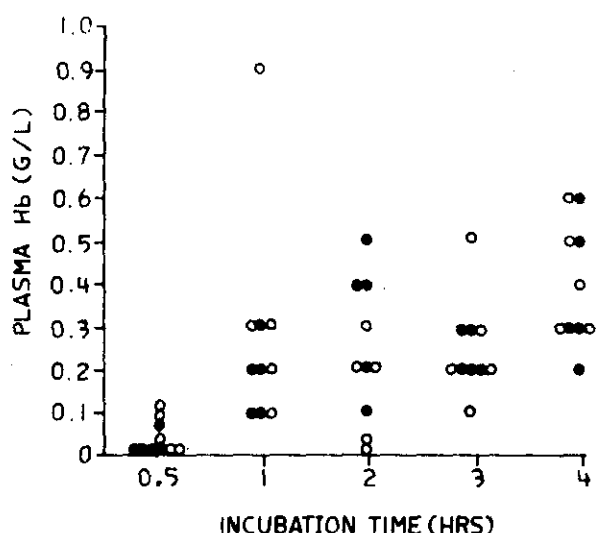


Figure 3. Plasma haemoglobin levels of rat blood after incubation with venom (test ○) and saline (control ●)

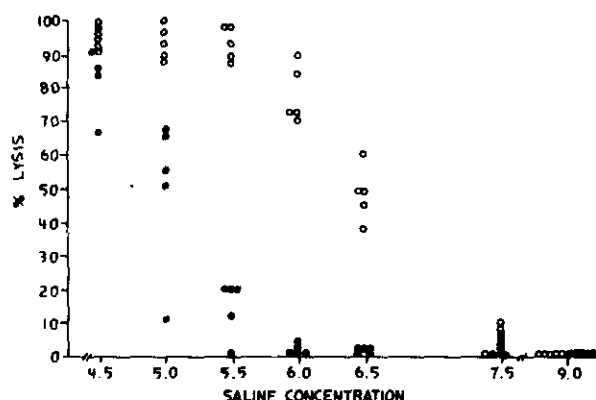


Figure 4. Per cent lysis of rat RBC 2 hours after incubation with RVV in test (○) and saline in control (●)

Myanmar RVB victims is very rarely seen. The occurrence of intravascular haemolysis (IVH) in RVB victims(5) may probably be due to mechanical traumatization of RBC by the intravascular clots or fibrin strands that have formed already by the procoagulant action of venom. Hence the contribution of the direct lytic effect of venom may not play a major role in causation of IVH in human RVB victims.

On the other hand, *in vitro* experiment of rat RBCs showed that RVV had

statistically significant lytic effect on rat RBC as shown by osmotic fragility test and plasma haemoglobin levels. Therefore, the lytic effect of RVV may be a species specific phenomenon(6).

In the present study, although similar experimental conditions were used, lytic effect was significantly higher in the rat RBCs, as compared to that of the humans. Our findings are not congruent with Thein-Than et al., 1989(7) who did not find haemolytic effect of RVV. This might be due to the fact that they used a lesser amount of venom and the washed red cells or a rat plasma-factor may be a prerequisite in the lysis mechanism. Actually the haemoglobinuria so observed might have been due to the strong lytic effect of RVV on RBC, together with the effect of DIC in which very fragile RBCs were traumatized by the fibrin clot.

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Accepted for publication 14 March 1990.

Serum haptoglobin levels in Russell's viper bite victims

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Serum haptoglobin levels of proven Russell's viper bite victims were studied at various time intervals after the bite. Although some degree of hemolysis was observed, there was no complete saturation of haptoglobin with hemoglobin in these patients. This indicates that hemoglobinuria induced renal damage can be excluded as a major cause of Russell's viper venom nephropathy in systemic envenoming patients.

INTRODUCTION

Haptoglobins are the marker proteins in the body circulation which are markedly reduced when free hemoglobin appears in the circulation due to hemolysis(1).

The spectrum of renal manifestations after Russell's viper bites include renal failure, proteinuria, hematuria and sometimes hemoglobinuria(2). Some workers even reported that severe intravascular hemolysis may occasionally occur in Russell's viper bite and exchange transfusion has been reported to be fruitful in the management(3).

This study was carried out in order to study the extent of hemolysis in Russell's viper bite patients throughout the clinical course of management, by following the serum haptoglobin levels at various time intervals after the bite.

MATERIALS AND METHODS

Twelve patients with proved Russell's viper bite confirmed by positive venom antigen(4) detected in their sera were studied from November to January 1986-87 in Tharyarwady Township. Apparently healthy controls from the same area were also studied as reference. Systemic envenoming was

defined by the presence of incoagulable blood by 20 min. clotting test(5). Blood samples were taken on admission and six hourly from time of bite up to 5 days. Samples were separated and stored at -25°C until analysis. Serum haptoglobins were quantified by the method of Owen et al.(6) and expressed as mg/dl hemoglobin binding capacity.

Statistical analysis: Statistical analysis was done by non-parametric Mann-Whitney test.

RESULTS

Admission serum haptoglobin levels (mean 64.3 mg/dl, range 35.5 to 168 mg/dl) fell significantly to the lowest level (mean 39 mg/dl, range 28 to 67 mg/dl) at median 27.3 hr after the bite but gradually rose significantly ($p=0.02$) from the lowest level to mean 62.8 mg/dl, (range 35.5 to 154.5 mg/dl) at 4.7 days (range 4 to 5 days) after the bite, but not reached significantly to controls ($p=0.02$), Table 1 and Figure 1.

DISCUSSION

This study confirms the fact that some degree of hemolysis do occurred in Russell's viper envenoming soon after

Table 1. Clinical and Laboratory data

R.V.No.	Admission		Lowest		Discharge (or) Transferred		Clinical Manifestations	
	Time After bite (hr)	Hp mg/dl	Time After bite (hr)	Hp mg/dl	Time After bite (hr)	Hp mg/dl	Local	Systemic
110	3.0	168.0	81.0	35.5	165.0	65.5	P,S	Lp,RF
143	2.8	73.0	30.0	67.0	99.0	154.5	P,S	RF
149	1.2	72.5	26.8	43.0	111.0	51.5	P	
111	1.8	43.5	94.0	37.0	125.0	65.5	P,S,N,B	
123	4.0	51.0	17.3	29.5	120.0	65.5	P,S,B	
94	6.0	43.5	6.0	43.5	94.0	90.0	S	
130	3.7	65.5	18.8	35.5	91.0	43.5	P,S,H	
154	2.08	51.0	27.8	35.5	108.0	35.5	P,S	Lp,H
137	2.0	51.0	77.5	36.0	120.0	43.5	P,S	
78	1.7	52.0	11.5	28.0	124.5	43.0	P	
134	4.7	35.5	4.5	35.5	95.0	44.0	P	
159	2.3	65.5	89.0	43.5	112.0	51.0	P,S	Lp,H
mean	2.94	64.3	27.3 (median)	39.0	113.7	62.8		
SD	1.4	34.7	4.5-94.0 (range)	10.0	20.2	32.6		

Control Hp level (mean = 115.36, range 65.5 to 190 mg/dl)

B, bleeding; N, necrosis; P, Local pain; S, swelling; RF, renal failure; Lp, loin pain; H, hypotension.

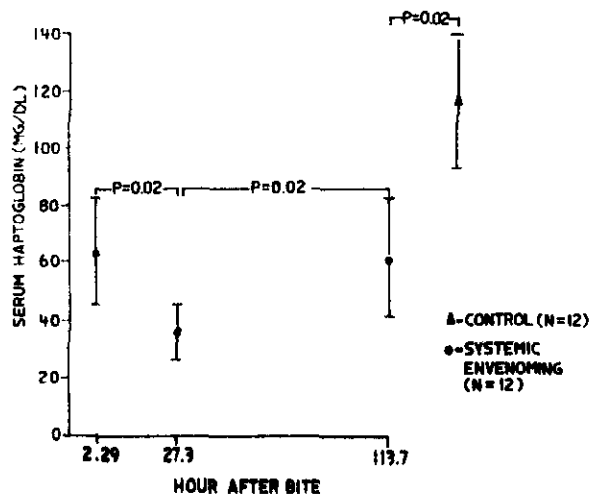


Fig. 1. Serum haptoglobin levels in systemic envenoming patients with time course after bite. Controls (Δ); Systemic envenoming patients (●); Bars describe SD

the bite. However, the levels of serum haptoglobin measured in these patients showed there was no complete saturation of haptoglobin with hemoglobin at any time after the bite. Thus, the possibility of hemoglobinuria occurring in Russell's viper bite cases is negligible. It has been reported that tubular obstruction by hemoglobin casts may cause renal failure following hemoglobinuria (7). Although the exact pathophysiology of acute renal failure in Russell's viper bites is still unknown, this study exclude hemoglobinuria induced renal damage as a major cause in these cases.

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Accepted for publication 14 March 1990.

**Diagnosis of resistant strains of plasmodium falciparum
after chloroquine and amodiaquine therapy from
42 to 72 hours of drug administration**

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The effect of chloroquine and amodiaquine on the *in vivo* development of asexual parasites (trophozoite form) during the treatment of falciparum malaria was studied on 48 patients. They consisted of 6 patients who were sensitive, 8 patients with R1 and 6 patients with R11 resistance to chloroquine, and 5 patients who were sensitive, 13 patients with R1 and 10 patients with R11 resistance to amodiaquine. Percentage distribution of tiny, small and large forms of trophozoites were counted every 6 hours till blood smears were negative. From the results we may be able to predict the presence of resistant strains of parasite between 48-72 hours of treatment.

INTRODUCTION

Decreased sensitivity of *Plasmodium falciparum* to an ever increasing number of antimalarial drugs is being reported from various parts of the world. The problem of drug resistance in Myanmar is recently reviewed by Myint Lwin(1). During 1986 drug monitoring study from Tharrawaddy (Pe Than Myint et al. (2), reported as much as 80% and 58% of cases are resistant in various degrees to chloroquine and amodiaquine. Hence, early diagnosis of drug resistant strains of parasites is essential to help in the management of patients. This study is attempted to aid in the early diagnosis of resistant strains of parasites after chloroquine or amodiaquine therapy.

PATIENTS AND METHODS

Forty eight patients who attended the Thayarwady Hospital during 1986 and 1987 malaria seasons were chosen for

study. They were febrile and had clinical signs and symptoms of malaria, positive blood film for falciparum asexual parasites with counts between 1,000 to 100,000 per cu. mm and no history of antimalarial treatment for 2 weeks before admission.

Urine was tested for chloroquine, amodiaquine, sulfonamide and quinine. Patients with positive reaction to above drugs were not included in the study. Patients with complications such as jaundice, severe anaemia (PCV less than 30%) and those with cerebral signs and symptoms were also not included in the study. Of these 20 were treated with chloroquine (1500 mg base divided into 3 days) and 28 were treated with amodiaquine (1500 mg base divided into 3 days). They were admitted to hospital for 7 days and were requested to return for follow up examination on days 14, 21 and 28.

A detailed record of signs and symptoms were kept and body temperature was measured 6 hourly till the patient was

afebrile for 24 hours and then once a day. Thick and thin films were examined every 6 hours in the hospital and once at weekly follow up visit. Quantitative parasite count was done according to the method described in WHO technical report [WHO (3)]. The percentage proportion of detail shape and form of asexual trophozoites of *Plasmodium falciparum* as seen under the microscope such as . . .

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

Treatment results were evaluated according to WHO criteria suggested for chloroquine resistance. From thin blood smears stages of parasite development were assessed from 200 asexual parasites. Number of tiny, small and large ring forms were then expressed as a percentage of the total parasites seen.

RESULTS

Out of 20 patients treated with chloroquine 6 patients were sensitive, 8 patients were RI resistant and 6 patients were RII resistant. Of 28 patients treated with amodiaquine 5 patients were sensitive, 13 patients were RI resistant and 10 patients were RII resistant.

Among the patients who were sensitive to chloroquine the mean percentage of tiny forms at 0 hour was 74% and the large forms 10%. After 30 hours the proportion of tiny forms fell gradually to 10% and the large forms increased to 62%. There were not much changes in the level of small ring forms. All the parasites disappeared after 48 hours of the drug therapy in all the patients. There was reverse correlation between the fall in tiny forms and the increase in large forms.

Among the patients who were RII resistant to chloroquine the mean percentage of tiny forms fell slowly to 54% in 24 hours and rose again to 76% at 30 hours. The level dropped slowly but rose again to 70% at 48 hours. It dropped slowly again and rose to 60% at 72 hours. The trophozoites failed to disappear from circulation even after 7 days.

Thus, the patients who were sensitive to chloroquine had percentage of tiny forms continuously broke down to 0 level in 36 hours in most of the patients and all trophozoites forms disappeared from blood after 48 hours.

Among the patients who were RII resistant to chloroquine the percentage of tiny forms fell gradually but increased again between 30 to 48 hours and again at 72 hours according to the cycle of maturation of trophozoites. Out of 6 RII resistant cases trophozoites persisted in the circulation until day 5 in 3 patients and reappeared at day 7. In the remaining 3 patients the trophozoites were present throughout till day 7 or more.

Among the patients who were sensitive to amodiaquine the mean percentage of tiny forms at 0 hour was 72% and large forms 8%. At 42 hours the tiny forms fell gradually to 11% and large forms increased to 50%.

All the parasites disappeared after 48 hours during therapy in all patients.

There was a reverse correlation between the fall in tiny forms and increase in large forms. No significant change was observed in percentage distribution of small forms.

Among RII resistances to amodiaquine at 0 hour the mean percentage of tiny forms was 78% and large forms 6%. The level of tiny forms decreased slowly in 24 hours and increased to 74% and 76% at 30 and 42 hours. Trophozoites failed to disappear from circulation till day 7 in 7 patients. In another 3 patients the trophozoites disappeared on day 7.

Thus, in patients who were sensitive to amodiaquine the percentage of tiny forms fell continuously towards the 0 level within 48 hours and in all patients trophozoite forms disappeared from the blood after 72 hours.

In RII resistant patients to amodiaquine the percentage of tiny forms fell in very slow speed but rose again at 54 hours according to the maturation of the trophozoites. The trophozoite forms persisted in blood on day 7 in all RII patients.

DISCUSSION

During last 2 decades, there were very few papers which reported on the effect of antimalarial on the morphology of trophozoites. Jing Bo Jiang et al. (4) had reported the effect of mefloquine and qinghaosu on the different stages of parasites. Guo Qiao Li et al. (5) reported the effect of mefloquine, qinghaosu and pyrimethamine - sulfadoxine on morphology of malaria trophozoites. They stated that the drugs which destroy tiny and small ring forms, and act at early in the asexual cycle. By this method they claimed that artemether (qinghaosu) act early in the asexual development cycle when compared to mefloquine and sulfadoxine-pyrimethamine (drugs with long half life).

Recently Mu-Mu-Sein-Myint et al. (6) has studied the effect of antimalarial on

the development of *P. falciparum* trophozoites. Their study threw some light on the action of artemether, quinine, sulfadoxine-pyrimethamine, chloroquine and amodiaquine on 3 different ring forms of parasite but definite conclusion cannot be drawn because of not being able to include enough sensitive and resistant cases. They suggested that the study should be extended.

In our study on the patients who were sensitive to antimalarials, the action of chloroquine and amodiaquine were similar. They prevent the release of new tiny forms into the circulation after 24 hours of drug administration by not reducing the number of small and large ring forms; their action can be claimed to be on the schizonts stage. Hence, although chloroquine, amodiaquine and mefloquine have similar action, these are different from qinghaosu.

In this study, in the patients who were resistant to drug tiny forms were found to be released into circulation in bouts and the wavy pattern of increase in total and relative count. This is probably due to the release of new generation. It is of no doubt that our sample was still small but as all the patients showed similar pattern we had attempted to report our findings.

In conclusion if the asexual parasite count does not fall significantly after 48 to 72 hours and if the proportion of tiny ring forms do not fall after 48 hours, or if new crops of tiny forms reappear in circulation after 48 hours of treatment with chloroquine and amodiaquine, it is almost sure to be a case of resistance even if the initial blood film is not available and the initial level of parasite is not known. In case of RII resistance further study is still needed to be able to describe clearly in getting diagnosis in early days of treatment.

ACKNOWLEDGEMENTS

We would like to thank the former and present Director General of Department of Medical Research, Dr. Maung-Maung-Lay (Township Medical Officer), doctors and staffs from Thayarwady Hospital and the colleagues from Clinical Research Unit (malaria). We also would like to extend our thanks to UNDP/WORLD BANK/WHO, Special Programme for Research and Training in Tropical Diseases (TDR) for their help.

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Accepted for publication 22 Jan. 1990.

Comparison of malaria situation in a forested foot-hill area of Bago Division between monsoon and cool-dry season

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During exploratory surveys by Medical Entomology Research Division, Department of Medical Research to select areas for anopheline ecology studies, Tha-bye-wa village, Oktwin Township, Bago Division was found to have a very high malaria parasite rate and also high vector density as well as a rich mosquito fauna. The results of survey carried out in monsoon season and in the cool/dry season were compared and the epidemiological situation discussed.

INTRODUCTION

The results of exploratory survey carried out by the Medical Entomology Research Division, Department of Medical Research (DMR) during July 1987 at Tha-bye-wa village, Oktwin Township, Bago Division have been reported by Myo-Paing et al.(1). The village is situated in a valley among the foot-hills of Bago Yoma at an altitude of 700 feet and is on the Youngoo-Prome Road, 17 miles away from Oktwin. The village is completely surrounded by Teak forest reserves and teak plantations and many perennial streams were found nearby. Similar survey was again carried out in the Tha-bye-wa village by the same team during the month of February 1989, to compare the malaria situation during monsoon and cool-dry seasons.

MATERIALS AND METHODS

Entomological surveys: Mosquito catches were carried out as in the previous survey by human bait hand catches and baited big bed-net catches.

Blood examination: Thick and thin films were taken from all persons

present in the village during the time of survey. All blood films were stained with giemsa and examined personally by one of the authors (SM).

Spleen examination: All children between the age of 2-9 years were examined for spleen enlargement according to Bruce-Chwatt(2).

Results of February 1989 survey

Entomological survey:

Table 1. Anopheline fauna and densities recorded

Species	Number caught from		Man-biting rate (mbr)
	Human	Cattle	
<i>An. maculatus</i>	30	120	15.0
<i>An. minimus</i>	5	96	2.5
<i>An. culicifacies</i>	8	17	4.0
<i>An. annularis</i>	1	-	0.5
<i>An. vagus</i>	12	35	6.0
<i>An. splendidus</i>	-	7	-

Larval survey results: Larval survey was carried out for 3 days in the teak

forest surrounding the village and in the streams nearby. Larvae were collected from different sites and brought back to the DMR laboratory and on attaining late 4th instar, separated in individual vials to get sets of larval skin, pupal skin and adult specimens.

Mosquitoes that emerge from larval collections according to types of breeding sources are as follows:

(a) Small pools on the bed of Ka-baung Chaung

- (i) *Anopheles culicifaces*
- (ii) *Anopheles stephensi*

(b) Small rock pools under dense shade in deep forest

- (i) *Anopheles dirus*

(c) Bamboo stumps in deep forest

- (i) *Anopheles dirus*
- (ii) *Aedes (Stegomyia) subalbopictus*
- (iii) *Aedes (Stegomyia) perplexus*
- (iv) *Armigeres (Leicesteria) omissus*
- (v) *Orthopodomyia albipes*

(d) Small jungle streams

- (i) *Anopheles minimus*
- (ii) *Anopheles maculatus*

Identification of the mosquito fauna was done according to the following authors:-

- (i) Barraud(3), Harrison(4), Harrison & Scanlon(5), Peyton & Harrison(6), Reid(7), Harang(8), Zavortink(9) & Delfinado(10).

Anopheline specimens collected were not dissected for malaria infection but taken back alive to DMR laboratory for cytogenetic and taxonomical research work.

New records of mosquitoes from Myanmar:

From larval survey collections from bamboo stumps in the deep forest, 3 males and 2 females of *Orthopodomyia albipes* Leicester, 1904 emerged. This is the first time that this genus was recorded from Myanmar. The adult specimens, pupal and larval skins and

male terminalia slides were preserved in DMR collections.

Blood examination results

Table 2. Comparison of parasitaemia rates between July 1987 and February 1989

PERIOD	JULY 1987			FEBRUARY 1989		
Age group	No. examined	No. +ve	% +ve	No. examined	No. +ve	% +ve
1	4	2	50.0	4	3	75.0
1- 4	12	9	75.0	6	5	83.3
5- 9	8	5	62.5	12	4	33.3
10-14	11	7	63.6	10	8	80.0
15-19	10	8	80.0	6	3	50.0
20	28	10	35.7	39	13	33.3
Total all age	73	41	56.2	77	36	46.8
Under 10 years	24	16	66.7	22	12	54.5

Plasmodium falciparum rate was found to be 82.9% (34/41), *Plasmodium vivax* rate 7.3% (3/41) and mixed infections 9.8% (4/41). The gametocyte rate was found to be 2.7% (3/113). Spleen examination in the 2-9 years age group showed that splenomegaly rate was 84.6% (22/26).

DISCUSSION

Malaria infection rate in the village was found to be high during the monsoon season (July) as well as in the cool dry season (February). From the infant parasite rates obtained it is evident that local transmission is occurring in the village. Slide positivity rate in children under 10 years of age was also high on both occasions. Thus, malaria transmission could be assumed to occur throughout the year at Tha-bye-wa village

without much seasonal difference.

From the entomological aspect, *An. dirus* was found in high numbers during the rainy season, when the slide positivity rate was high. Thus, *An. dirus* could be assumed to be the main vector transmitting malaria during the monsoon season, with *An. minimus* playing a secondary role due to its low density.

In the cool dry season, *An. dirus* adults could not be collected from human or cattle baits but only very few larvae could be collected from isolated breeding sites. During the cool dry season, *An. minimus* and *An. maculatus* were the two anophelines that predominate the total anopheline fauna of the area and they could be assumed as playing the major role in transmission during the non-monsoon season in the absence of *An. dirus*, as *An. minimus* is a confirmed vector and *An. maculatus* is a possible vector.

In comparing the malaria situation of Tha-bye-wa village between the monsoon and cool dry season, the slide positivity rates, infant parasite rates and under 10 years parasite rates were found to be high in both seasons. The spleen rate in 2-9 year age group during February 1989 was 84.6% (22/26). This spleen rate was similar to the rate observed during the July 1987 5th survey when 88.2% (15/17) children were found to have splenomegaly. From the spleen rates also the high endemicity of malaria was evident for both seasons. Local malaria transmission was also found to be high in both seasons. The main difference in the malaria transmission situation is in the role played by different vectors in different seasons.

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Accepted for publication 22 Jan. 1990.

Laboratory studies on the larvicidal activity of
Bacillus sphaericus - 2362 and 2297 against
Anopheles dirus and Anopheles annularis

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The larvicidal efficacy of *Bacillus sphaericus* -2362 and 2297 was tested against larvae of *Anopheles dirus* (Mudon strain) and *Anopheles annularis* (DDT-resistant strain from Sittwe), using the method of Dubitskij(1) with some modifications. The results showed that although both the strains of *B. sphaericus* were effective larvicides, strain 2362 was found to be much more potent than strain 2297. *Anopheles dirus* was also found to be more susceptible than *An. annularis*. Larval cadavers were found to play an important role in the maintenance and recycling of *B. sphaericus*-2362.

INTRODUCTION

The use of microbial larvicides such as *Bacillus sphaericus* and *Bacillus thuringiensis* H-14 have proved to be excellent biological control agents against several mosquito species in the laboratory as well as in field trials. These biological control agents could be used to control many mosquitoes which were found to be resistant to insecticides. In Myanmar, *Anopheles annularis* from Sittwe was found to be highly resistant to DDT(2,3). *Bacillus sphaericus* and *Bacillus thuringiensis* could be used safely in field control projects, as these microbial agents are non-pathogenic to man and to non-target organisms as reported by Ali(4), Molloy and Jamnback(5), Purcell(6) and Shadduck *et al* (7).

The results of laboratory and field trials of *Bacillus sphaericus* -1593 on *Culex quinquefasciatus* was reported by Myo-Paing *et al* (8). However, no trials has been carried out against *Anopheles* in Myanmar. The present study was conducted to compare the larvicidal

activity of *B. sphaericus*-2362 and 2297 against *Anopheles dirus* and *Anopheles annularis* in the laboratory and also to determine the persistency and recycling potential of *B. sphaericus* -2362, the more potent of the 2 strains, in *Anopheles* larvae.

MATERIALS AND METHODS

- (A) *Anopheles* species and strains used
Two species of *Anopheles* were used:
i) *Anopheles dirus* (Mudon strain) and
ii) *Anopheles annularis* (DDT-resistant strain from Sittwe)
- (B) *Bacillus sphaericus* strains used
i) *Bacillus sphaericus*-2362 ABG-6184 primary powder from Abbott laboratory lot 86/958/BD containing 7.6×10^{10} spores per gram.
ii) *Bacillus sphaericus*-2297 (SPH-84 standard) from Pasteur Institute as lyophilised powder.
- (C) Bioassay tests of *Bacillus sphaericus*-2362 and 2297 against *An. dirus* and *An. annularis* larvae.

Laboratory bioassay tests were carried out according to the method described by Dubitskij(1) with some modifications. Enamelled bowls of 160 mm diameter, containing 100 ml pond water were used and twenty-five second instar larvae were added in each bowl. The bowls were labelled with serial dilutions and the required dilution was prepared in each bowl. Dilutions were made from 0.01% stock solution and the required strength dilutions were prepared by using the dilution formula described by WHO(9). Each concentration was tested in triplicate on 3-4 occasions, yielding a total of 9-12 replicates per concentration. In every bioassay tests, 3 bowls with just 100 ml pond water containing twenty-five second instar larvae per bowl were used as controls. After 24-, 48-, and 72 hour exposure periods, mortality readings were taken. In all the controls, the mortality rates did not exceed 5%. LC_{50} and LC_{90} in mg/litre were obtained from dosage response lines established on probit long paper, and after testing the goodness of fit, Swaroop (10), plotting per cent mortality versus *B. Sphaericus* concentrations. All the bioassay tests were carried out at room temperature $28.08 \pm 1.71^\circ C$, and the larvae were fed on powdered mouse chow during the tests.

(D) Determination of the persistency and recycling potential of *Bacillus sphaericus* in *Anopheles dirus* larvae.

Since *B. sphaericus* -2362 was found to be more potent and effective than *B. sphaericus* -2297 from bioassay trials, persistency and recycling potential was determined with *B. sphaericus* -2362 against *Anopheles dirus* larvae only.

10 bowls each containing twenty-five second instar larvae in pond water, were initially inoculated with a dosage of 1 mg/l of *B. sphaericus* concentration which produced 100% mortality within 24 hours (result,

obtained from the bioassay trials). After 24 hours, the bowls were divided into the following groups.

Group I: 5 bowls with cadavers and pond water.

Group II: 5 bowls where all cadavers were removed and only pond water was left.

Group III: 5 fresh bowls, each containing 100 ml of pond water were prepared. Cadavers removed from each bowl in Group II were ground up with a glass rod in a beaker and added to each bowl in this group to give 5 bowls of ground cadavers in 100 ml pond water.

Weekly bioassay tests were carried out in all 3 groups with twenty-five, second instar *An. dirus* larvae. Larval mortalities were recorded after 24-, 48-, and 72-hour exposure periods. All cadavers were always left in the bowls in Group I, but all the dead larvae from bowls of Group II and III were removed after reading the results of weekly bioassay. 5 bowls with just 100 ml pond water with twenty-five, second instar larvae per bowl were used as controls for every bioassay test. Larvae were fed on powdered mouse chow during the tests. In the controls, the mortality rate did not exceed 0.42% throughout the experiment.

RESULTS

(A) Bioassay tests of *Bacillus Sphaericus*-2362 and *Bacillus sphaericus*-2297

It can be observed from Tables 1 and 2 that, *Anopheles dirus* was more susceptible than *Anopheles annularis* to both *Bacillus sphaericus* -2362 and 2297. The LC_{50} and LC_{90} values were much higher in *Anopheles annularis* than that of *Anopheles dirus* after 24- and

48-hour exposure periods with both *B. sphaericus*-2362 and 2297.

Table 1. Susceptibility of 2 species of *Anopheles* mosquito larvae to *Bacillus sphaericus*-2362, after exposed continuously for 24 & 48 hours

Mosquito species	LC ₅₀ (mg/l)		LC ₉₀ (mg/l)	
	24 hrs	48 hrs	24 hrs	48 hrs
<i>Anopheles dirus</i>	0.086	0.060	0.960	0.120
<i>Anopheles annularis</i>	1.800	0.440	9.800	4.400

Table 2. Susceptibility of 2 species of *Anopheles* mosquito larvae to *Bacillus sphaericus*-2297, after exposed continuously for 24 & 48 hours

Mosquito species	LC ₅₀ (mg/l)		LC ₉₀ (mg/l)	
	24 hrs	48 hrs	24 hrs	48 hrs
<i>Anopheles dirus</i>	0.520	0.030	14.000	3.100
<i>Anopheles annularis</i>	4.000	3.200	20.200	11.800

When the effectiveness of *B. sphaericus* strains 2362 and 2297 were compared against *Anopheles dirus* and *Anopheles annularis* larvae, it was found that *B. sphaericus*-2362 was found to be more effective than *B. sphaericus* -2297 against both *Anopheles* species. The results are shown in Tables 3 and 4.

(B) Persistency and recycling potential of *B. sphaericus* -2362 in *Anopheles dirus* larvae

When the larval cadavers were present, nearly 90% mortality rates were

Table 3. Comparison between 2 strains of *Bacillus sphaericus* against *Anopheles dirus* larvae

<i>B. sphaericus</i> strains	LC ₅₀ (mg/l)		LC ₉₀ (mg/l)	
	24 hrs	48 hrs	24 hrs	48 hrs
<i>B. sphaericus</i> -2362	0.086	0.060	0.960	0.120
<i>B. sphaericus</i> -2297	0.520	0.030	14.000	3.100

Table 4. Comparison between 2 strains of *Bacillus sphaericus* against *Anopheles annularis* larvae

<i>B. sphaericus</i> strains	LC ₅₀ (mg/l)		LC ₉₀ (mg/l)	
	24 hrs	48 hrs	24 hrs	48 hrs
<i>B. sphaericus</i> -2362	1.800	0.440	9.800	4.400
<i>B. sphaericus</i> -2297	4.000	3.200	20.200	11.800

obtained for about 3 months and the larvicidal action gradually declined and mortality rate maintained at 42% up to 192 days (about 6½ months). In contrast, when the larval cadavers were removed, only about 46% - 50% mortality rates were obtained up to day 17, and the larvicidal activity gradually declined. These results clearly indicated that cadavers of *Anopheles* larvae play an important role in the maintenance and growth of *Bacillus sphaericus*-2362. On the other hand, the results from Group III with ground cadavers in pond water showed that, 80% - 100% mortalities were obtained up till day 31, which we could assume that the *Bacillus* spores

suspended in the water and effective larvicidal activity persisted up to a period of one month.

DISCUSSION

Results obtained from laboratory trials were observed to be very favourable for *Bacillus sphaericus* as a biological control agent against *Anopheles*, especially as an alternative larvicide. For practical use in a control programme, the larval breeding habitats of the target *Anopheles* species must be taken into consideration. *Anopheles dirus*, the major vector of malaria in Myanmar was found to breed in small rock pools in deep forest and in domestic wells in Mudon. Department of Medical Research survey teams also found *An. dirus* breeding in bamboo stumps at Tha-bye-wa village, Oktwin township. In such widely distributed small breeding sources, practical use of *Bacillus sphaericus*, no matter however effective is almost impossible.

The larvicidal activity of *Bacillus sphaericus* is best utilised against *Culex quinquefasciatus*, as reported by Myo-Paing et al.(8). Laboratory trials as well as small scale field trials in septic tanks showed that *Bacillus sphaericus* is very effective against *Culex quinquefasciatus* and that it can withstand high organic pollution in the septic tank waters. Another major source of *Culex quinquefasciatus* breeding is the road-side concrete drains with high organic pollution. If *B. sphaericus* can withstand the high organic pollution of septic tanks, it could be assumed to be also effective in concrete drains. However, trials carried out in a blocked earthen drain with muddy bottom, *B. sphaericus* was found to be ineffective against *Culex quinquefasciatus*, even at very high dosages (Myo-Paing, personal communication).

Mulla et al.(11) evaluated *B. sphaericus*-

2362 in organically enriched field ponds and found that the spores settled quickly and accumulated in the mud zone, out of reach of feeding mosquito larvae. However, he obtained 90% initial control in ponds with or without low level of enrichment, and concluded that good control of larvae was obtained for about 3 weeks. On the other hand, Nicolas et al.(12) reported that the persistence of larvicidal activity of the *B. sphaericus* -2362 isolate in a highly polluted habitat, demonstrated prolonged residual activity. The authors attributed this activity to very slow settling of the spores.

Majori et al.(13) found that, in small artificial ponds with clean water, both *B. sphaericus* isolates 1593 and 2362 killed all III and IV instar *Anopheles gambiae* larvae at 0.24 kg/ha by 48 hours after treatment, but reduced the numbers of I and II instar larvae by only 81% (strain 1593) and 95% (strain 2362).

An. annularis breeds predominantly in ponds, used to grow water cress at Sittwe. Here, *An. annularis* is an important vector of malaria and is highly resistant to DDT(3). In such situations, it could be possible to use *Bacillus sphaericus* as an alternative larvicide. Moreover, Department of Medical Research survey teams found *Anopheles* breeding in large numbers in pools of stagnant water on sand banks near the confluence of Tha-bye-chaung and Ka-baung-chaung near Tha-bye-wa village. The water is not deep and the bottom is not muddy. In such situations, application of *Bacillus sphaericus* will have a very favourable impact as part of integrated vector control programme.

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Accepted for publication 22 January 1990

Preliminary report on the morphological variations
of Anopheles (Cellia) minimus adult females

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Anopheles minimus is a common anopheline species in Myanmar, especially in the forested foot-hill areas. During routine surveys many variations in adult morphology were noted. Iso-female lines were raised from wild-caught females and the morphological variations in F_1 adults were studied. At the same time the identification of specimens was confirmed by detailed examination of larval skin slides. A guide for field workers on identification of *An. minimus* was also presented.

INTRODUCTION

Anopheles minimus is considered as a primary vector of malaria in Myanmar, especially in the forested foot-hill areas by Myo-Paing et al.(1). Harrison (2) also considered *An. minimus* as a confirmed vector of malaria in Thailand. As such, the Medical Entomology Research Division, Department of Medical Research (DMR) carried out detailed studies on *An. minimus* in Myanmar. In carrying out mosquito collections in various parts of the country, we encountered differences in morphology of *An. minimus* specimens caught. We also observed that the morphological variations were numerous and identification in the field during surveys could prove to be very difficult. In fact, many misidentifications must have occurred in Myanmar, as Harrison (2) has pointed out. Harrison considered the "*fluviatilis*" of Myanmar, identified by Khin-Maung-Kyi(3), as probably hypermelenic *minimus* variants. It was also felt that field workers in Myanmar using simple keys need detail information and facts to identify *An.*

minimus from some of the species of the *Myzomyia* series. Accordingly, a detail study was carried out by DMR to determine the morphological variations of *An. minimus*.

This paper is intended as a preliminary report to alert entomological field workers on the possibility of misidentifications if too much importance is attached to only one or two morphological characters for identification. A comprehensive paper will be prepared after examining more specimens from more States and Divisions of the country.

MATERIALS & METHODS

Wild caught *An. minimus* females were given blood meals and separated into individual beakers for oviposition and to raise separate iso-female lines. Iso-female lines were raised in different trays and larval skin and pupal skin slides were prepared. The F_1 adult females that emerge from each iso-female line were pinned and given specific numbers. Morphological

variations of F_1 adult females were observed in detail according to Harrison (2). The larval skin and pupal skin morphology was used in confirming the identification of adult specimens. Wing venation numbers used were according to Harbach and Knight(4).

Altogether 206 adult female specimens were examined, 11 from Wet-wun, Maymyo Township which is on a plateau area over 3000 feet above sea-level, 9 from Nam-Tun Timber Extraction Camp, a deep forest area in Kawthoung Township at the extreme Southern tip of Myanmar, while the remaining 186 specimens were from Tha-bye-wa village, Oktwin Township at the foothills of Bago Yoma.

RESULTS

Out of 206 adult female specimens examined, 198 specimens had similar morphological characters on both wings. In the remaining 8 specimens the morphology of the 2 wings were not similar in the same specimen.

The morphological variations observed in the palpi were presented in Table 1.

Table 1. Morphological characters of *An. minimus* palpi

Sr.	Morphological character of palpi	Number observed	Percentage
1.	Preapical dark band on palpi narrow, about $\frac{1}{2}$ of apical pale band	98	(98/206) 47.5
2.	Preapical dark band equal or almost equal to apical pale band	82	(82/206) 39.8
3.	Preapical dark band wider than apical pale band	25	(25/206) 12.1
4.	Apical $\frac{1}{2}$ of antenna pale	1	(1/206) 0.5

DISCUSSION

As a preliminary report this paper was written after detailed examination of 206 specimens of *An. minimus* from 3 ecologically different areas of Myanmar. The sample size is indeed small but the results are being published because the authors felt that morphological variations of this primary vector should be made available to all field entomological workers in Myanmar. Typical morphological features and identification points of *An. minimus* were described by Harrison(2) and by Illustrated key published by US Army component, SEATO(5).

Our observations showed many variations in morphology even among F_1 adults emerging from the same iso-female lines. The variation recorded were as follows.

PALPI

Palpi morphological variations are as illustrated in Figure 1 and Table 1. In our sample, 98 (47.5%) specimens had preapical dark band narrow and 82 (39.8%) specimens had preapical dark bands equal or almost equal to the apical pale band. Thus in 180 (87.4%) of the specimens examined, the palpi was observed to be of typical *An. minimus* morphology. However, 25 (12.1%) were observed with preapical dark bands much wider than the apical pale bands. On examination of such specimens if emphasis is given to this palpi morphology alone, the observer can misidentify the specimen to be of *culicifacies* group. When further observing the Wing Vein R_{4+5} if a wide pale median area is seen, the observer will identify the specimen as *fluviatilis*. Such misidentifications must have occurred in Myanmar. According to Harrison(2) *minimus* specimens exhibiting *fluviatilis* type palpi also occur in Hong Kong.

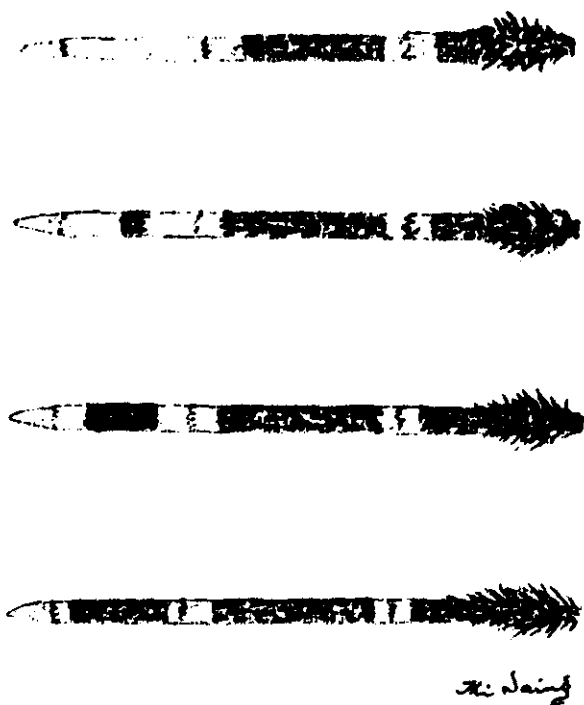


Figure 1. *Anopheles minimus* palpi variations

WING MORPHOLOGY

Wing morphological variations are as presented in Figure 2 and Table 2.

(a) Basal 1/3 of COSTA

The presence of one pale area in the basal 1/3 of costa was observed in 173 (87.4%) of specimens examined. There were however variations in that 155 (78.3%) had large pale areas whereas 18 (9.1%) had small pale areas. *Anopheles varuna* was the most common species to be mistaken for *An. minimus*. If the basal 1/3 of costa morphology of the wing is the only morphological character considered, 17 (8.6%) of specimen will be misidentified, as *minimus* specimens in our study were observed to have basal 1/3 costa entirely dark too.

Table 2. Morphological variations observed in both wings of each *An. minimus* specimens examined

Morphological characters of the WINGS observed (both wings similar)	Number Observed	Percentage
(A) COSTA		
1. Basal 1/3 of costa with one pale interruption:	173	(173/198)87.4
(a) pale area large	155	(155/198)78.3
(b) pale area small	18	(18/198) 9.1
2. Basal 1/3 of costa entirely dark	17	(17/198) 8.6
3. Basal 1/3 of costa with 2 pale interruptions:	8	(8/198) 4.0
(a) both pale areas very small	1	(1/198) 0.5
(b) one pale area very small, one area larger	7	(7/198) 3.5
(B) VEIN R ₂		
(a) without median pale area	187	(187/198)94.4
(b) with median pale area	11	(11/198) 5.6
(C) VEIN R ₄₊₅		
(a) with wide median pale area	196	(196/198)99.0
(b) with dark median area	2	(2/198) 1.0
(D) VEIN M ₁		
(a) without median pale area	156	(156/198)78.8
(b) with median pale area	42	(42/198)21.2

(E) VEIN M_{3+4}

(a) median pale area wide	190	(190/198)96.0
(b) median area of vein dark scaled	8	(8/198) 4.0

(F) FRINGE SPOT AT 1A

(a) absent	196	(196/198)99.0
(b) present	2	(2/198) 1.0

A small percentage, 4.0% (8/198), of *minimus* specimens were seen to have *pampanai* characters in having 2 pale

areas in the basal 1/3 of costa. In one specimens, both the pale areas were very small, while in the remaining 7 specimens, one pale area was very small but the other area was observed to be larger.

WING VEIN R_2

Typical *minimus* wing vein R_2 as described by Harrison(2) have no median pale area. 187 (94.4%) of our specimens were also found to have R_2 entirely dark. Only 11 (5.6%) had median pale area on R_2 similar to *aconitus*.

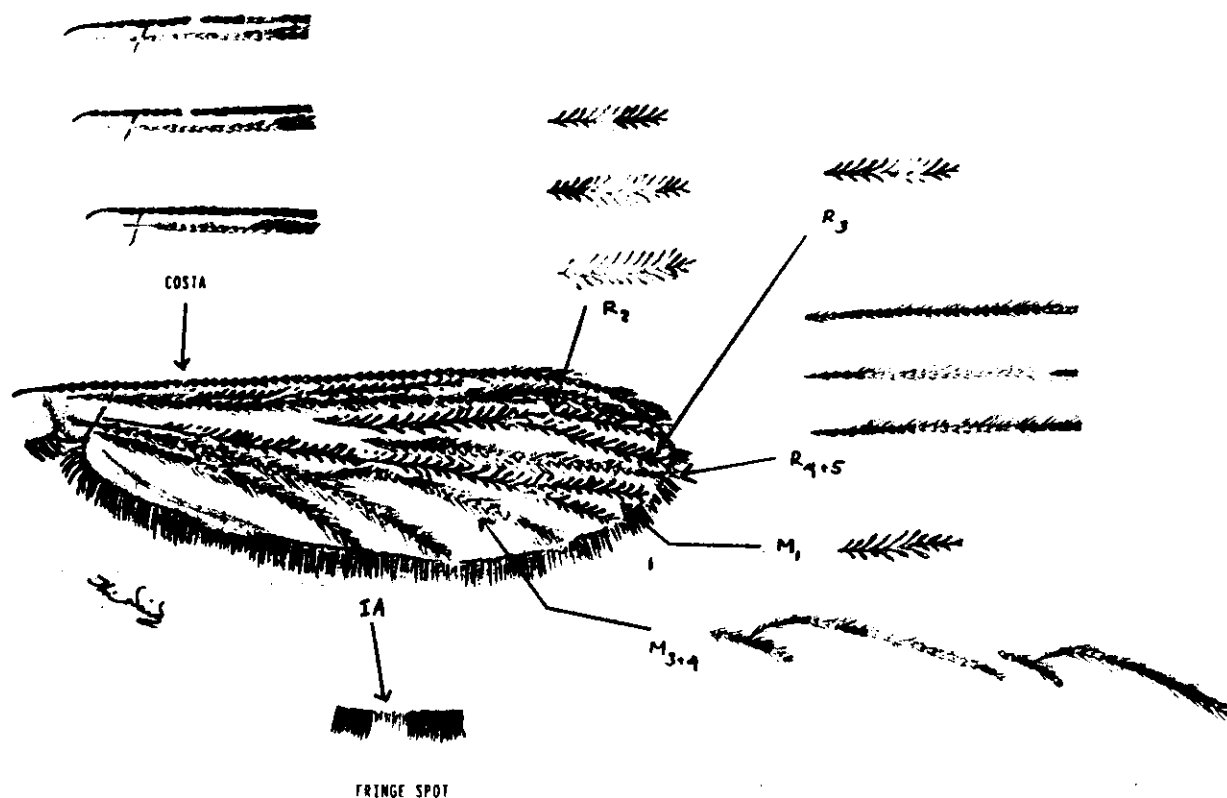


Figure 2. *Anopheles minimus* Wing morphological variations

WING VEIN R₄₊₅

This morphological character was found the most characteristic and constant. 196 (99%) of *An. minimus* specimens examined had a wide median pale area with the dark area in the apical part of vein very narrow or in some specimens somewhat wider. However, in 2 (1%) specimens Vein R₄₊₅ was found to have long dark median area.

Vein M₁

In 156 (78.8%) specimens of *An. minimus* Vein M₁ was observed to be without a median pale area. This character was not considered important as 42 (21.2%) of the specimens have a median pale area.

Vein M₃₊₄

This wing vein M₃₊₄ or Cu 1 as described by Harrison(2), is a very important morphological character for identification of *minimus* in which, the area beyond m-cu cross vein is predominantly pale. Only in 8(4%) specimens, the median area of M₃₊₄ was dark, resembling *An. varuna*.

FRINGE SPOT ON VEIN 1A

This character is mainly used to differentiate *minimus* from *aconitus*. 190 (96.0%) of specimens were without a fringe spot at 1A while the fringe spot was found only in 2 (1.0%) of specimens.

In specimens where different variations were observed in different wings in the same mosquito, much confusion could result in differentiating between *minimus* and *varuna*. Detailed variations are presented in Table 3.

There is possibility of errors in differentiating *minimus* species group from *culicifacies* by palpi banding pattern. From our observations, in *An. culicifacies*, the preapical dark band was found to be very broad and between two distinctly smaller pale

bands. From specimens of *An. culicifacies*, confirmed by larval skin slides by one of the authors (T.T.N), the preapical dark band was found to be distinctly broader than *minimus*. This character will help in correct identification of *minimus* species group.

From the wing characters, the main difficulty is differentiating between *minimus* and *varuna*. If the characters in basal 1/3 of costa are considered together with the morphology of Vein R₄₊₅ and Vein M₃₊₄, the probability of making a wrong identification could be very much reduced.

For our preliminary observations, we came to the conclusion that in identifying *An. minimus*, the following points must be examined in detail and considered.

(A) Palpi

1. The preapical dark band narrow or equal to apical pale band. Sometimes the preapical dark band can be a little wider than the apical pale band but never about 1.5 to 2 times as observed in *culicifacies*.

(B) Wing

The following characters have to be examined in detail and considered together.

- (i) Based 1/3 of costa dark or with pale areas
- (ii) Vein R₂ with or without median pale spot
- (iii) Vein R₄₊₅ having wide median pale area and with very narrow apical dark area or median area mainly dark
- (iv) Vein M₃₊₄ with wide median pale area or median area predominantly dark
- (v) Fringe spot at apex of Vein 1A usually absent

Table 3. Specimens showing variations between left and right wings in the same mosquito

Specimen Number	Site of variations in wing	Left wing	Right wing
1	Basal 1/3 of costa	1 large pale area	2 pale areas; 1 small, one large
2	Basal 1/3 of costa	1 small pale area	1 large pale area
3	Basal 1/3 of costa	1 small pale area	entirely dark
4	Basal 1/3 of costa	1 small pale area	entirely dark
5	Basal 1/3 of costa Fringe spot at 1A	1 large pale area present	entirely dark absent
6	Wing vein M ₁	only few scales pale in median area	median area of vein pale
7	Wing vein R ₃	dark	with median pale area
8	Wing vein R ₃	dark	with median pale area

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Accepted for publication 22 January, 1990

The anopheline mosquitoes of Myanmar.V.

Anopheles (Cellia) maculatus Theobald, 1901

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Anopheles maculatus was found to be distributed in association with forested foot-hills. In some areas of Myanmar this species could be collected in very high densities. This species could be regarded as a possible vector of malaria in Myanmar. Epidemiological evidences and laboratory infection study results were presented. The presence of different sibling species of *An. maculatus* based on morphological characters was also presented.

INTRODUCTION

Malaria is one of the most important public health problems prevalent in the Union of Myanmar. Although regular systematic control operations are being carried out, malaria remains a serious problem. All medical entomologists and malariologists in the country will readily agree that *Anopheles dirus* Peyton and Harrison and *Anopheles minimus* Theobald are the two primary vectors of malaria in Myanmar. To update information available on the vectorial status of other anophelines and also to fill gaps in our knowledge about the ecology of anophelines, the Department of Medical Research (DMR) conducted longitudinal, multi-disciplinary studies on the dynamics of Malaria Transmission in Bago Yoma area and also initiated studies on vectorial status of anophelines in different ecological areas of the country.

Furthermore, a multi-disciplinary survey team visited Thai-Myanmar Border Timber Trading points in Kawthaung and other timber extraction areas in Bok-pyin Townships. This survey provided very useful information on *Anopheles maculatus* in the extreme southern area of the country.

Distribution

From the results of surveys by DMR teams, *An. maculatus* was primarily recorded from forested foot-hills, deep forest camps and in rocky mountainous area about 4000 feet above sea level. It was not found in plain areas far away from the foot-hills. The distribution of *An. maculatus* as recorded by DMR teams is presented in Table 1 and Figure 1.

Seasonal prevalence

As *An. maculatus* is mainly a stream breeder, its prevalence is always low in the monsoon season when the streams are periodically flushed by heavy rains. With the end of monsoon, by early October, *An. maculatus* density starts to increase. In Tha-bye-wa village itself, *An. maculatus* was collected in very large number during January 1988 by a single cattle-baited big bed-net. In the same catching station, *An. maculatus* was very scarce during July. In Ka-baing village in Mogoke Township, *An. maculatus* was collected in large number during September, about the end of rainy season. In the southern extreme areas of Tanintharyi

Table 1. The distribution of An. maculatus as recorded by DMR teams

LOCALITY	TOWNSHIP	SEASON	REMARKS ABOUT LOCALITY
<u>BAGO DIVISION</u>			
Iha-bye-wa village	Oktwin	Jan. - Feb.	Foot-hill village about 700 feet above sea level. Deep forest on all sides. Very high density of <u>An. maculatus</u>
Hti-phado Camp	Kyauk-ta-gar	Nov. - Jan.	Deep-forest wood cutting camp in Bago Yoma hills. Very high density of <u>An. maculatus</u>
Ngok-to village Gwe-gon village	Kyauk-ta-gar	Sep. - Jan.	Villages near foot-hills of Bago Yoma
Ye-bya Camp	Ok-po	Nov. - Jan.	Forest extraction camp at Bago Yoma.
Waing & Kaing-taw-su villages	Ok-po	Nov. - Jan.	Villages close to foot-hills of Bago Yoma
<u>MANDALAY DIVISION</u>			
Kabainq village	Mogok	Sept.-Oct.	Rocky mountainous area 4000 feet above sea level. Forest all around. Very high density of <u>An. maculatus</u>
Wet-wun village tract	Maymyo	Sept.-Nov.	Plateau area about 3000 feet above sea level
Se-daw-gyi	Madaya	Sept.-Oct.	Irrigation scheme area. Near foot-hills & forest
<u>TANINTHARYI DIVISION</u>			
Shwe-du Hydroelectric Station	Myeik	Jan.	Hydroelectric station in forested hill area. <u>An. maculatus</u> found breeding in running stream with stones & rocky bed in direct sunlit area.
Mon-su village	Myeik	Jan.	Small village near foot-hills & rubber plantations. <u>An. maculatus</u> found breeding in stream as well as in small well
Han-ga-daing Camp	Bok-pyin	Jan.	Deep forest timber extraction camp near sea. Found breeding in Jungle streams under dense shade
Nam-lun Camp	Kaw-thaung	Jan.	Deep forest timber extraction camp. found breeding under shade in Jungle stream

Division, An. maculatus was collected by DMR team in high densities in January. From our observations, An. maculatus could be recorded in significant number from the end of monsoon i.e. about end of September to February or March.

Breeding sites

From our DMR survey team results, An. maculatus breeds primarily in jungle streams, either in eddying pools or in clear running streams. Unlike the findings reported by Khin-Maung-Kyi(1),

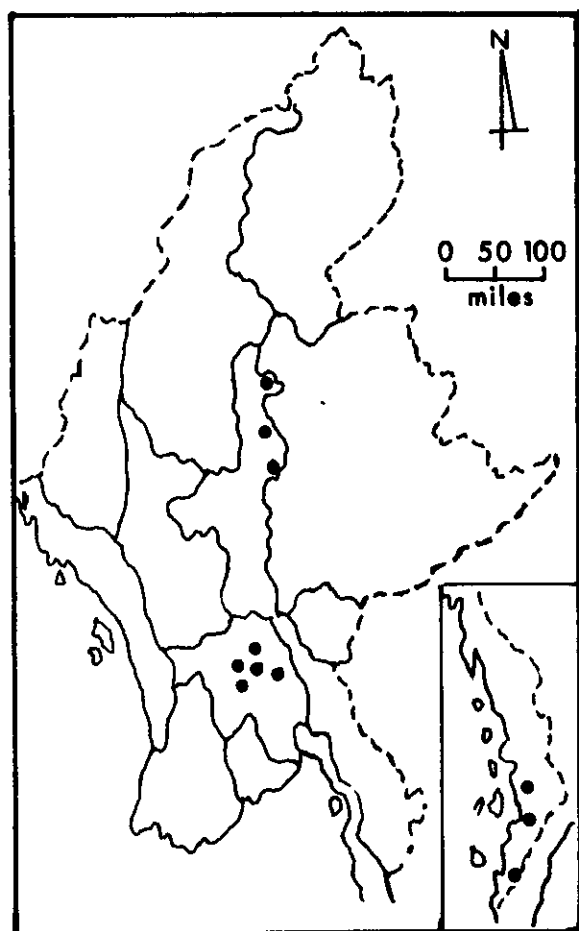


Fig. 1. *An. maculatus* collected by DMR teams

we found *An. maculatus* breeding in jungle streams under dense shade in the teak forests of Tha-bye-wa. In the timber extraction camps of Kaw-thaung, *An. maculatus* was also found breeding in rocky streams under dense as well as partial shade together with *An. minimus*. In Nam-noi Stream, Kaw-thaung, *An. maculatus* larvae were collected under rocks and pebbles near the edges of the stream. In Shwedu Hydroelectric station, Myeik Township, this species was collected in a water overflow stream between rocks under direct sunlight although the locality is situated in a forested hilly area. At Mon-su village, *An. maculatus* was found breeding in very sluggishly flowing stream with plenty of vegeta-

tion and shade and also in a small well (2 feet square) about 2-3 feet deep, and which also harboured a fish (*Ophicephalus* spp).

At the foot-hill villages of Ngok-to and Gwe-gon, Kyauk-ta-gar township, *An. maculatus* was found breeding in paddy fields in association with *An. minimus*

Host preferences

DMR survey teams collected *An. maculatus* from both human and animal baits. In the deep forest timber extraction camps such as Nam Tun and Htiphado, *An. maculatus* readily attacks man and was caught from human baits. At Htiphado, during November 1985, *An. maculatus* was caught at a density of about 90 per man night. At Kabaing, *An. maculatus* was collected equally biting both human and cattle baits. However, at Tha-bye-wa, cattle baits yielded the highest number of *An. maculatus*. The biting preference of this species could be presumed to be zoophilic in many areas but in certain situations it shows almost equal preference for human and cattle blood meals. In the absence of cattle and other animals in deep forest timber extraction camps, *An. maculatus* will readily feed on man.

Day time resting

Throughout our surveys DMR teams have never found *An. maculatus* resting indoors by daytime, even though many houses in the foot-hill areas are enclosed only on 3 sides. However, at times of peak densities, few *An. maculatus* could be collected in cattle sheds. Basing on the biting density and the number collected in cattle shed, this species could be assumed as an outdoor resters.

Relationship to malaria transmission

Chin-Maung-Kyi(1) reported that no dissections were done on this species

due to its zoophilic habits and went to state that *An. maculatus* cannot be regarded as being important for malaria transmission in Myanmar. Our DMR survey teams dissected *An. maculatus* throughout its 2 years longitudinal study at Phado area, Kyauk-ta-gar Township but found no gut and salivary gland infection. However, at Kabaing, Mogok Township, during the time of our survey, *An. maculatus* was collected in large numbers with the other anopheline species caught only in very small numbers. At the same time, many people in the village were found to be having malaria attacks. From this epidemiological evidence, *An. maculatus* could be considered as a potential vector in Kabaing. also at Mon-Su village, Myeik Township during our DMR survey carried out in January 1989, *An. maculatus* was the only anopheline collected in large numbers breeding near the village. At the same time the malaria infection rate in the villagers, as detected by the DMR parasitology team, was 11.64% (Ye-Htut, personal communications). From these epidemiological evidences, we consider *An. maculatus* to be a vector of malaria, at least in some areas of the Union of Myanmar.

Hybridization and cytogenetic studies between two strains of *An. maculatus* from two ecologically different areas

Two strains of *An. maculatus*, one from Ka-baing which was considered a vector on epidemiological grounds and the other from Phado which was found not infected with malaria by gut and gland dissection were hybridized and cytogenetically studied in detail at DMR to observe any differences.

Yu-Nyunt-Oo(2) reported that although playing different roles in malaria transmission, the two strains were genetically identical by hybridization and F_1 backcross results.

Cytogenetic studies of F_1 hybrids also showed that, from polytene chromosome

results, the two strains are genetically identical.

Morphological studies on sibling species of *An. maculatus*

Rattanaarithikul and Green(3) described six forms of *An. maculatus* and provided formal names to the sibling species. Specimens of *An. maculatus* were taken to AFRIMS, Bangkok by the author in 1986 and were identified morphologically as *An. sawadwongporni*. Rattanaarithikul and Green, a new species. Further morphological studies carried out from other areas also showed the specimens to be *sawadwongporni*. Specimens collected by baited traps and collected as larvae at Mon-su village, Myeik Township was identified to be *An. draxadicus* Christophers, which was resurrected from synonymy by Rattanaarithikul and Green(3).

Laboratory malaria infection studies on *An. maculatus*

Considering its prevalence and density in the forested foot-hills, it was felt that *An. maculatus* could play a role in malaria transmission at least in some areas of the country. Laboratory infection studies were carried out to find out its transmission potential. Infection studies were carried out in Mington village, Hlegu Township. Two hundred *An. maculatus* were fed on several *P. falciparum* gametocyte carriers, brought back to the laboratory and maintained at 28°C in an environmental chamber with sugar solution as food. Twenty five *An. maculatus* survived on the 12th day. On dissecting the salivary glands, sporozoites were detected in 6 mosquitoes giving an infection rate of 24% among mosquitoes surviving the extrinsic incubation period of *P. falciparum*.

From the laboratory studies, *An. maculatus* could be considered as a potential vector of malaria in Myanmar. This finding is in agreement with Reid(4) who also stated that *An. maculatus* is a major vector of malaria

in Malaya in nearly all hilly areas not covered by forests.

From epidemiological evidences observed from DMR surveys in Tanintharyi peninsular area, *An. maculatus* could assumed to be a vector in that area. In Peninsular Malaysia, which is continuous with the Tanintharyi peninsular area, *An. maculatus* was confirmed as a major vector of malaria by Reid(4). In addition, our laboratory infection studies showed that *An. maculatus* could be infected to produce sporozoites.

After also taking into consideration the fact that this species was collected by DMR terms on human baits and human-baited big bed nets, *An. maculatus* could be regarded as a vector in some localities of the country.

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Accepted for publication 22 January 1990.

