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**A knowledge-perception-behaviour study on malaria in  
two island villages and two timber extraction camps  
in Tanintharyi Division**

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A malaria related knowledge-perception-behaviour study was undertaken in January 1989 in Pu-law-ton-tone (8 km from Kaw-thaung) and Pan-zin (10 km from Myeik) villages by interviewing heads of household, and in Han-ka-dine (western coast in Boke-pyin township) and Nam-ton (eastern border in Kaw-thaung township) timber camps by questioning the personnel concerned. In both the villages, more than 85% of the respondents had none or low level of knowledge concerning biting position and breeding places of *Anopheles* mosquito, cause of malaria, awareness of chemoprophylaxis and of chemotherapy against malaria. However, in comparison with the latter respondents, the former knew less significantly about the biting time of mosquito, and spread of malaria; and perceived less significantly on susceptibility, severity, and controllability of malaria, benefits of selected antimalarial control measures. The greater number of people in Pu-law-ton-tone relied malaria treatment on general medical practitioners (37%) and self-medication (29%), while those in Pan-zin on Community Health Worker (38%) and midwife (29%). Similarly, the patterns of lack and difference in knowledge and perception on malaria between Han-ka-dine and Nam-ton timber workers were comparable to those seen in the two villages. The causes of dis-similarity in knowledge and perception on malaria between the villages and the camps and the facts to be emphasized in providing health education are discussed.

**INTRODUCTION**

Malaria has been a first or second national priority health problem in Myanmar since 1978 (1, 2). In fact, it is widely distributed in various hilly, coastal and plain regions of the country (3), where the people from the plains usually contract malaria by travelling to the foothill (4). It is well known that one of the basic elements for successful malaria prevention and control requires correct human behaviour (5), which is much influenced by the knowledge,

beliefs, attitudes and values that the individuals have on malaria. As these attributes of man on malaria tend to be area-specific, epidemiologically-specific, culture-specific, and health-service-specific (6), we undertook a knowledge-perception-behaviour study in rural communities and timber extraction camps in Tanintharyi Division when an opportunity arose to visit those areas for the assessment of malaria situation.

## MATERIALS AND METHODS

### Study areas and population

The study areas consisted of two villages one on each island, and two timber extraction camps in deep forest. One village, known as Pu-law-ton-tone with 226 households is situated about 8 km in the north-west of Kaw-thaung. The other, Pan-zin with 190 households lies in the Kyun-su township, about 10 km west of Myeik. One timber extraction camp called Han-ka-dine lying in the coast (western side) of Boke-pyin township and the other, Nam-ton in the Myanmar eastern border about 60 km north of Kaw-thoung (See Figure). For the rural areas, the study population (respondents) comprised 75 (30% of) heads, spouses or adult member of household in Pu-law-ton-tone and 90 (50% of) households in Pan-zin. The respondents in the camps were 32 and 40 government personnel in Han-ka-dine and Nam-ton respectively. The respondents in the villages were chosen randomly on household basis and the respondents in the camps were those available at the time of survey.

### Interviews

A structured questionnaire was used to interview the respondents by trained interviewers on malaria related knowledge, perception and behaviour. The variables in the questions on knowledge were composed of Anopheles mosquitoes, cause and spread of malaria, preventive, diagnostic and therapeutic measures against malaria, while those on perception consisted of susceptibility, severity, controllability, eradicability of malaria, and perceived benefits and barriers against antimalarial measures. The behaviour variables were confined to malaria illness and prevention. The study was conducted in January 1989.

### Analysis

Percentage distributions of variables on malaria related knowledge, perception and behaviour were determined either singly or after making knowledge status or score and stratified by socio-demographic status if necessary. The differences in the distribution between the villages and between the timber extraction camps were compared and chi-square tests were used to show statistical differences.

## RESULTS

### Respondents

There were significantly more spouses of younger ages, and having less education in Pu-law-ton-tone than in Pan-zin. They also had shorter period of residence and less exposure to malaria in the former than in the latter village (Table 1). Similarly, the respondents in Han-ka-dine timber camp were less educated and had less past experience on malaria than in Nam-ton camp.

### Knowledge on malaria

In both the villages, more than 85% of the respondents had none or low level of knowledge concerning biting and breeding places of Anopheles mosquitoes, cause of malaria, awareness of chemoprophylaxis and of chemotherapy against malaria. However, the respondents of Pu-law-ton-tone knew less significantly than those of Pan-zin about the biting time of mosquito, spread of malaria, awareness of chemoprophylaxis and of chemotherapy except knowledge concerned with causation of malaria and effect of DDT household spraying on mosquitoes (Table 2).

Table 1. Percentage distribution of characteristics of sample household respondents in two villages in Tanintharyi Division

| Variable                          | Pu-law-ton-tone<br>(n=75) | Pan-zin<br>(n=90) |
|-----------------------------------|---------------------------|-------------------|
| <u>Family status</u>              |                           |                   |
| Head                              | 40.00                     | 61.1              |
| Spouse                            | 49.3                      | 28.9              |
| Other                             | 10.7                      | 10.0              |
|                                   | $\chi^2 = 7.112$ ;        | $P < 0.05$        |
| <u>Age in years</u>               |                           |                   |
| < 30                              | 53.4                      | 13.3              |
| 30-44                             | 33.3                      | 35.6              |
| 45                                | 13.3                      | 51.1              |
|                                   | $\chi^2 = 35.639$ ;       | $P < 0.001$       |
| <u>Sex</u>                        |                           |                   |
| Male                              | 42.7                      | 55.6              |
| Female                            | 57.3                      | 44.4              |
|                                   | $\chi^2 = 2.227$ ;        | $P > 0.05$        |
| <u>Education</u>                  |                           |                   |
| Illiterate                        | 21.3                      | 4.6               |
| Read & write                      | 22.7                      | 44.2              |
| Primary school                    | 48.0                      | 40.7              |
| Secondary school                  | 8.0                       | 10.5              |
|                                   | $\chi^2 = 12.898$ ;       | $P < 0.01$        |
| <u>Family size</u>                |                           |                   |
| 1-3                               | 14.7                      | 14.4              |
| 4-6                               | 54.7                      | 40.0              |
| 7+                                | 30.6                      | 45.6              |
|                                   | $\chi^2 = 3.583$ ;        | $P > 0.05$        |
| <u>Period of residence</u>        |                           |                   |
| < 5                               | 20.6                      | -                 |
| 6-9                               | 16.2                      | 5.8               |
| 10+                               | 63.2                      | 94.2              |
|                                   | $\chi^2 = 22.646$ ;       | $P < 0.001$       |
| <u>Past occurrence of malaria</u> |                           |                   |
| Yes                               | 65.3                      | 94.2              |
| No                                | 34.7                      | 5.8               |
|                                   | $\chi^2 = 19.636$ ;       | $P < 0.001$       |

In the case of timber extraction camps, the personnel from Han-ka-dine knew less significantly than those of Nam-ton about almost all the above items except effect of DDT household spraying on mosquitoes (Table 2).

#### Perception on malaria

In Pu-law-ton-tone, the respondents perceived less significantly than those in Pan-zin on susceptibility, severity and controllability of malaria, and benefits of selected antimalarial measures (Table 3). Also the patterns of perception difference of the above items between Han-ka-dine and Nam-ton workers was similar to those between Pu-law-ton-tone and Pan-zin except on extent of reducing working ability of malaria cases, preventive and side effects of antimalarial drugs which were favourable to Han-ka-dine personnel (Table 3).

#### Illness and preventive behaviours on malaria

At least one member of 65% of the households in Pu-law-ton-tone had malaria experience before, while 95% in Pan-zin had similar experience 37% and 29% of people in the former village relied malaria treatment on general medical practitioners and self-treatment respectively. On the other hand, 38% and 29% in Pan-zin depended their treatment respectively upon community health worker and mid-wife. In both the villages, about 40% of the household had no mosquito-nets, 10% used mosquito-nets irregularly and the rest regularly. In Pu-law-ton-tone, only 11.4% and 27.3% of the travelling household member to the forested areas respectively used anti-malarial drugs frequently and occasionally for prophylaxis, while the respective values in Pan-zin were 1.3% and 11.7%.

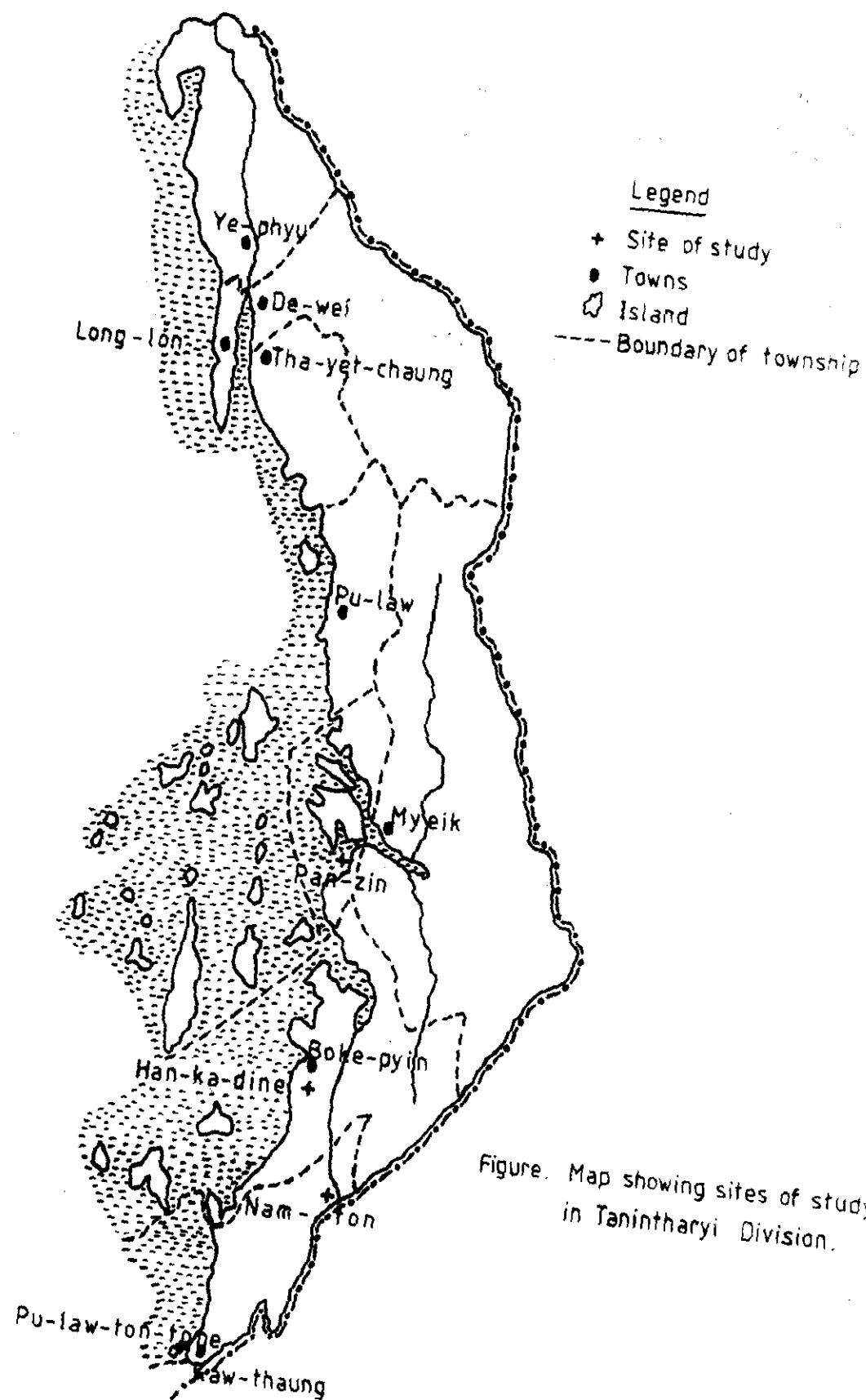


Figure. Map showing sites of study in Tanintharyi Division.

Table 2. Percentage distribution of some differential items of malaria-related knowledge between villages and between camps in Tanintharyi Division

| Items & level                                | Village                |                | Timber extraction camp |                |
|----------------------------------------------|------------------------|----------------|------------------------|----------------|
|                                              | Pu-law-ton-tone (n=75) | Pan-zin (n=90) | Han-ka-dine (n=32)     | Nam-ton (n=40) |
| <u>Biting position of Anopheles mosquito</u> |                        |                |                        |                |
| None                                         | 89.3                   | 83.3           | 93.8                   | 65.0           |
| Low                                          | 1.3                    | -              | 3.1                    | 27.5           |
| High                                         | 9.4                    | 16.7           | 3.1                    | 7.5            |
|                                              | $\chi^2 = 1.263;$      | $P > 0.05$     | $\chi^2 = 6.575;$      | $P < 0.05$     |
| <u>Biting time of anopheles mosquito</u>     |                        |                |                        |                |
| Incorrect                                    | 94.7                   | 47.8           | 93.8                   | 62.5           |
| Correct                                      | 5.3                    | 52.2           | 6.2                    | 37.5           |
|                                              | $\chi^2 = 39.949;$     | $P < 0.001$    | $\chi^2 = 7.971;$      | $P < 0.01$     |
| <u>Malaria causation</u>                     |                        |                |                        |                |
| None                                         | 78.7                   | 97.8           | 43.8                   | 10.0           |
| Low                                          | 17.3                   | -              | 53.1                   | 60.0           |
| High                                         | 4.0                    | 2.0            | 3.1                    | 30.0           |
|                                              | $\chi^2 = 14.989;$     | $P < 0.001$    | $\chi^2 = 12.559;$     | $P < 0.01$     |
| <u>Spread of malaria</u>                     |                        |                |                        |                |
| None                                         | 73.4                   | 23.3           | 37.5                   | 15.0           |
| Low                                          | 1.3                    | -              | 21.9                   | -              |
| High                                         | 25.3                   | 76.7           | 40.6                   | 85.0           |
|                                              | $\chi^2 = 40.401;$     | $P < 0.001$    | $\chi^2 = 14.105;$     | $P < 0.001$    |
| <u>Effect of DDT spray on mosquitoes</u>     |                        |                |                        |                |
| None                                         | 52.8                   | 78.0           | 62.5                   | 87.5           |
| Low                                          | 13.9                   | -              | 28.1                   | 2.5            |
| High                                         | 33.3                   | 22.0           | 9.4                    | 10.0           |
|                                              | $\chi^2 = 11.740;$     | $P < 0.01$     | $\chi^2 = 7.894;$      | $P < 0.05$     |
| <u>Awareness of chemoprophylaxis</u>         |                        |                |                        |                |
| None                                         | 54.7                   | 10.0           | 9.4                    | -              |
| Low                                          | 40.0                   | 85.6           | 43.7                   | 10.0           |
| High                                         | 5.3                    | 4.4            | 46.9                   | 90.0           |
|                                              | $\chi^2 = 37.888;$     | $P < 0.001$    | $\chi^2 = 12.722;$     | $P < 0.01$     |
| <u>Awareness of chemotherapy</u>             |                        |                |                        |                |
| None                                         | 58.7                   | 21.1           | 56.3                   | 42.5           |
| Low                                          | 36.0                   | 75.6           | 28.1                   | 27.5           |
| High                                         | 5.3                    | 3.3            | 15.6                   | 30.0           |
|                                              | $\chi^2 = 24.643;$     | $P < 0.001$    | $\chi^2 = 1.475;$      | $P > 0.05$     |

Table 3. Percentage distribution of some differential items of perception on malaria between villages and between camps in Tanintharyi Division

| Items<br>&<br>level                                              | village                   |                   | timber extraction camp |                   |
|------------------------------------------------------------------|---------------------------|-------------------|------------------------|-------------------|
|                                                                  | Pu-law-ton-tone<br>(n=75) | Pan-zin<br>(n=90) | Han-ka-dine<br>(n=32)  | Nam-ton<br>(n=40) |
| <u>Perception score of susceptibility</u>                        |                           |                   |                        |                   |
| < 0.25                                                           | 23.0                      | 1.9               | 16.7                   | 6.7               |
| 0.25-0.49                                                        | 38.5                      | 33.3              | 25.0                   | 27.3              |
| 0.50+                                                            | 38.5                      | 64.8              | 58.3                   | 66.7              |
|                                                                  | $\chi^2 = 10.126$ ;       | $P < 0.01$        | $\chi^2 = 0.707$ ;     | $P > 0.05$        |
| <u>Chance of malaria causing death</u>                           |                           |                   |                        |                   |
| Big chance                                                       | 44.2                      | 61.8              | 42.9                   | 55.3              |
| Small chance                                                     | 27.9                      | 35.3              | 47.6                   | 34.2              |
| Uncertain                                                        | 27.9                      | 2.9               | 9.5                    | 10.5              |
|                                                                  | $\chi^2 = 13.804$ ;       | $P < 0.01$        | $\chi^2 = 0.626$ ;     | $P > 0.05$        |
| <u>Thinking malaria a severe disease</u>                         |                           |                   |                        |                   |
| Severe                                                           | 68.2                      | 87.2              | 71.0                   | 80.0              |
| Not severe                                                       | 4.5                       | 3.5               | 19.3                   | 15.0              |
| Uncertain                                                        | 27.3                      | 9.3               | 9.7                    | 5.0               |
|                                                                  | $\chi^2 = 7.526$ ;        | $P < 0.05$        | $\chi^2 = 0.192$ ;     | $P > 0.05$        |
| <u>Extent of reducing working ability of malaria cases</u>       |                           |                   |                        |                   |
| Large extent                                                     | 50.8                      | 79.1              | 74.1                   | 64.1              |
| Small extent                                                     | 32.3                      | 20.9              | 25.9                   | 7.7               |
| Do not effect                                                    | 16.9                      | -                 | -                      | 28.2              |
|                                                                  | $\chi^2 = 17.784$ ;       | $P < 0.01$        | $\chi^2 = 8.527$ ;     | $P < 0.05$        |
| <u>Human is able to reduce malaria transmission</u>              |                           |                   |                        |                   |
| Yes                                                              | 27.6                      | 51.7              | 65.6                   | 90.0              |
| No                                                               | 30.4                      | 28.1              | 6.3                    | 7.5               |
| Uncertain                                                        | 42.0                      | 20.2              | 28.1                   | 2.5               |
|                                                                  | $\chi^2 = 10.454$         | $P < 0.01$        | $\chi^2 = 7.768$ ;     | $P < 0.05$        |
| <u>Antimalarial prevent malaria</u>                              |                           |                   |                        |                   |
| No                                                               | 23.9                      | 7.9               | -                      | 10.0              |
| Uncertain                                                        | 28.3                      | 7.9               | 11.1                   | 17.5              |
| Yes                                                              | 47.8                      | 84.2              | 88.9                   | 72.5              |
|                                                                  | $\chi^2 = 20.955$ ;       | $P < 0.001$       | $\chi^2 = 1.761$ ;     | $P > 0.05$        |
| <u>Antimalarial drugs have side-effects</u>                      |                           |                   |                        |                   |
| No                                                               | 28.6                      | 61.4              | 84.4                   | 62.5              |
| Uncertain                                                        | 33.9                      | 3.4               | 9.4                    | 7.5               |
| Yes                                                              | 37.5                      | 35.2              | 6.2                    | 30.0              |
|                                                                  | $\chi^2 = 25.831$ ;       | $P < 0.001$       | $\chi^2 = 4.921$ ;     | $P > 0.05$        |
| <u>Blood smear examination is reliable for detecting malaria</u> |                           |                   |                        |                   |
| No                                                               | 21.4                      | 4.6               | 3.1                    | 7.5               |
| Uncertain                                                        | 30.4                      | 10.2              | 25.0                   | -                 |
| Yes                                                              | 48.2                      | 85.2              | 71.9                   | 92.0              |
|                                                                  | $\chi^2 = 20.038$ ;       | $P < 0.001$       | $\chi^2 = 8.632$ ;     | $P < 0.05$        |

Since malaria treatment was available in the timber extraction camps, questions on illness behaviour were not asked. In both the camps, the non-mosquito net user constituted about 20%, and irregular and regular user comprised 40% each. In Han-ka-dine camp, 69% of the worker used antimalarial drugs for prophylaxis frequently, 14% used sometimes and 17% never, while the corresponding figures for Nam-ton were 96.5%, 3.5% and 0% respectively.

## DISCUSSION

The characteristics of respondents between the two island villages and the two timber extraction camps were different. The number of more female spouses as respondents and of shorter periods of residence in the Pu-law-ton-tone than in Pan-zin indicates that the former village is relatively unstable with respect to population migration and that of less educated and younger respondents might account for the observed differences in knowledge and perception on malaria as mentioned in the text. Other factors like knowledge on malaria causation, and effect of DDT house spraying on mosquitoes might be influenced by the recent experience of DDT house spraying to control an outbreak occurring in Pu-law-ton-tone in February 1988 (Malaria Inspector of Kaw-thaung, personal communication). However, at the time of survey, the parasitaemia rates in Pu-law-ton-tone ( $n=168$ ) and Pan-zin ( $n=61$ ) were 1.2% and 9.8% respectively (Drs Tun Tun Aung and Ye Htut, personal communication).

In our another study (7) in the malarious foothill and non-malarious plain villages in Kyauktaga township, a large percentage of household respondents did not have clear knowledge about malaria causation and treatment

and that the majority of people in the foothill being less educated knew less about transmission, control and severity of malaria.

Regarding respondents in the timber extraction camps, most of the personnel in Nam-ton came from Yangon and those in Han-ka-dine were recruited mainly from Dawei and Myeik townships (managers of the camps, personal communication). Moreover, the Han-ka-dine camp had already been established for 2 years and the parasitaemia rate at the time of study was about 21%, while Nam-ton had been in existence for only 3 months and the rate was about 12% (Ye Htut, personal communication). Thus, the dissimilarities in education, past exposure to malaria knowledge, duration of camp establishment and the degree of current malaria infection in the area might explain for the differences in knowledge and perception between the camps. For instance, the higher occurrence of malaria infection and longer duration of stay in Han-ka-dine would cause its occupants to perceive more on the extent of reducing working ability of malaria cases, preventive and side effects of antimalarial drugs (see Table 3).

The above findings on the lack of and differences in knowledge, preception, and behaviours towards illness and malaria prevention, and the differences in characteristic features of the villages and camps, would help to develop the contents for health education message and tactics to be employed or malaria prevention and control in the areas. For instance, the lack of knowledge and perception on malaria has been mentioned in the text and in (Tables 2 & 3). For island villages, malaria are acquired by forest travelling or as the result of man-made occupations. The latter instance had occurred in Pan-zin in 1986 from the salt-production firm,

creating salty water to retain, making An. sundiacus to breed and causing epidemic to occur (Malaria Assistant of Myeik, personal communication).

The tactics of providing health education would vary. In areas like Pu-law-ton-tone which is closed to the border area, with multiple races like Salon, Pa-su and Myanmar and has low educational status and population migration, a greater and sustained efforts with appropriate health education messages will be definitely desired. For timber extraction camps like Han-kan-dine which is located deep in the forest and where the occupants have high infection and illness rates, every worker before entry or while at the camps must be provided with the necessary information about the malaria transmission, control and prevention. Strict practices by workers on the malaria control measures need to be observed and maintained, and treatment regimens with different anti-malarial drugs have to be provided at the local camps.

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## A study of glucose and insulin profiles in acute viral hepatitis patients

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Oral glucose tolerance tests, with 75 gm of glucose load were carried out on 25 patients suffering from acute viral hepatitis including 15 pregnant women and on 25 apparently normal subjects. The mean fasting blood glucose level of hepatitis patients ( $62.3 \pm 2.6$  mg/dl) was found to be significantly ( $P < 0.01$ ) lower than that of normal subjects ( $73.6 \pm 2.0$  mg/dl). After drinking the glucose solution hepatitis patients generally had higher blood glucose levels compared to normal subjects. Blood glucose and plasma insulin concentration were measured in another 35 acute viral hepatitis patients after an overnight fast, to study the relation between these values and the parameters of Liver Function Test. The fasting blood glucose level was found to be inversely correlated to SGOT and SGPT levels in these patients.

### INTRODUCTION

The importance of the liver in blood glucose homeostasis has long been recognized. Moreover, the gradual decrease in glucose tolerance during normal pregnancy has been demonstrated<sup>1-3</sup>. When a pregnant woman suffers viral hepatitis, she is exposed to double stress regarding the glucose homeostasis. This fact became evident during 1982 viral hepatitis epidemic in Yangon, where the highest case fatality rate was found in pregnant women with non-A, non-B hepatitis<sup>4</sup>. The present study was, therefore, conducted with the aim to study the glucose and insulin profiles in acute viral hepatitis patients especially the pregnant patients.

### MATERIALS AND METHODS

Patients and control subjects were allotted into following groups of five and

were subjected to oral glucose tolerance tests (OGTTs).

- Group 1. Apparently normal male subjects.
- Group 2. Male viral hepatitis patients.
- Group 3. Apparently normal female subjects.
- Group 4. Non-pregnant female hepatitis patients.
- Group 5. Apparently normal pregnant women, five subjects in each trimester.
- Group 6. Pregnant hepatitis patients, five patients in each trimester.

Viral hepatitis patients were those admitted to Infectious Diseases Hospital and Liver Unit, Yangon General Hospital (YGH), from 18th May 1987 to 15th September 1987 for acute viral hepatitis. Patients that were admitted to the above hospitals after the required number of patients had been fulfilled in the respective groups were subjected to

overnight fast and a single fasting blood sample was taken. There were altogether 35 such patients. Although, we intended to include five patients in each trimester we had collected only two first trimester patients, eight second trimester patients and five third trimester patients. Male and female control subjects were recruited from patients' attendants. Pregnant control subjects were recruited from those attending the antenatal clinic at Central Women Hospital. Patients who were very ill and those who were receiving parenteral glucose infusion were postponed till the condition became favourable for OGTT. Patients failed to confirm on concomitant Liver Function Test (L.F.T) were excluded from the study.

Oral glucose tolerance tests were performed with 75 gm glucose load according to WHO 1985 recommendation. L.F.Ts were carried out on the same blood sample taken for glucose and insulin assays. Blood glucose concentrations were measured by glucose oxidase method as described by Marks (1959)<sup>6</sup> and plasma insulin concentrations were measured by Radio Immuno Assay Kit purchased from Amersham International plc.

Blood glucose and plasma insulin concentration curves were constructed from the blood sample obtained before the glucose drinks and half hourly for up to 2 hours after the drinks. All data are presented as mean  $\pm$  s.e.m. The area under the glucose and insulin curves above the fasting level, were calculated by triangulation. The statistical calculations were performed by means of Student's 't' test for two groups comparison and ANOVA for multiple groups comparison.

## RESULTS

Physical characteristics of control subjects and patients are shown in Table 1. Mean age, height and weight

were comparable between groups.

The glucose concentration curves of hepatitis patients following oral glucose drinks were within normal range and no one showed diabetes mellitus pattern. No differences were found between male and female groups. When the data from both sexes were combined and comparison made between hepatitis patients and control subjects, hepatitis patients showed significantly lower mean fasting blood glucose level (Figure 1). However, after the glucose drink, the mean blood glucose concentration curve of hepatitis patients demonstrated well above that of control subjects. Mean plasma insulin concentration curve of hepatitis patients showed higher levels at all points after glucose drinks although the mean fasting level was almost the same as the control (Figure 1).

Among the female subjects, the fasting blood glucose levels were found to have a lowering trend in the following order: normal female subjects, normal pregnant women, non-pregnant female hepatitis patients and pregnant hepatitis patients, and the fasting blood glucose values were significantly ( $P < 0.05$ ) different among these four groups of female subjects. The fasting plasma insulin values were not different among these groups (Figure 2). Table 2 showed mean  $\pm$  s.e.m peak and area under the curve of various groups. Regarding glucose, hepatitis patients generally achieved higher peak and greater area than their control counterpart. Regarding plasma insulin concentration there was no clear trend except that hepatitis patients (both sexes) showed significantly larger area than their control counterparts.

Correlation between SGOT, SGPT levels and fasting blood glucose levels of various groups were shown in Table 3. Apart from this there were no correlation between other parameters of L.F.T and blood glucose nor insulin levels.

Table 1. Anthropometric characteristics of control subjects and viral hepatitis patients  
(Mean  $\pm$  S.E.M)

|                  |                | Age (yr)       | Ht (cm)          | Wt (kg)        |
|------------------|----------------|----------------|------------------|----------------|
| Control Subjects | Male (5)       | 26.8 $\pm$ 3.4 | 164.7 $\pm$ 13.6 | 49.6 $\pm$ 4.1 |
|                  | Female (5)     | 29.2 $\pm$ 3.2 | 149.8 $\pm$ 10.7 | 39.7 $\pm$ 9.7 |
|                  | Both sex (10)  | 28.0 $\pm$ 2.2 | 157.3 $\pm$ 8.5  | 46.1 $\pm$ 3.8 |
|                  | pregnancy (15) | 22.0 $\pm$ 1.6 | 140.8 $\pm$ 5.6  | 44.8 $\pm$ 4.6 |
| V.H Patients     | Male (5)       | 26.0 $\pm$ 2.8 | 162.5 $\pm$ 7.5  | 46.9 $\pm$ 8.2 |
|                  | Female (5)     | 30.2 $\pm$ 3.4 | 138.7 $\pm$ 10.4 | 40.1 $\pm$ 3.7 |
|                  | Both sex (10)  | 28.1 $\pm$ 2.2 | 150.6 $\pm$ 6.7  | 43.1 $\pm$ 3.5 |
|                  | pregnancy (15) | 25.6 $\pm$ 2.0 | 144.4 $\pm$ 8.7  | 45.8 $\pm$ 5.1 |

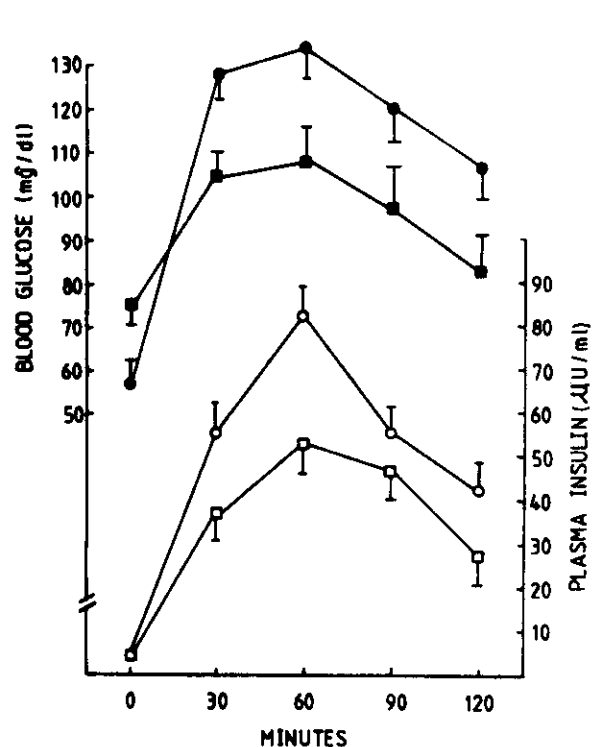


Fig. 1. Mean blood glucose levels (●, ■) and mean plasma insulin levels (○, □) following OGTTs. [control subjects (□, ■); viral hepatitis patients (○, ●)]

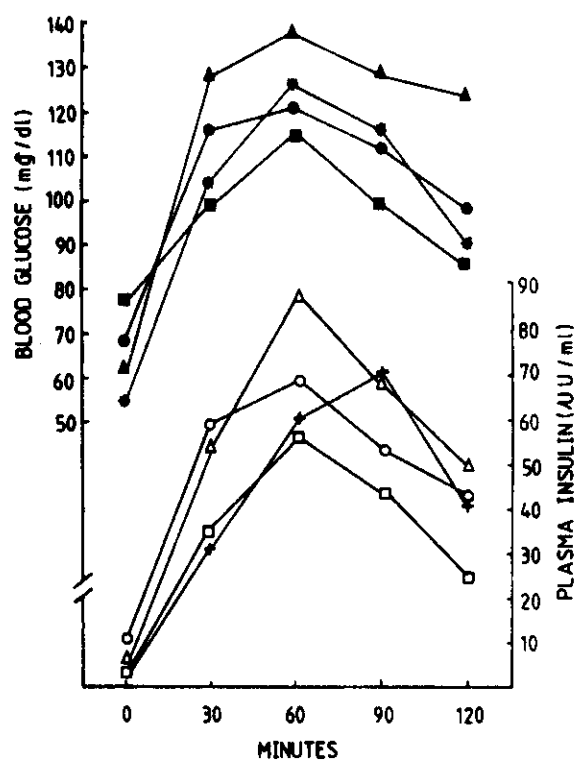


Fig. 2. Mean blood glucose levels (●, ■, ▲, ○) and mean plasma insulin levels (◆, □, △, ○) following OGTTs. [non-pregnant normal female (□, ■); normal pregnant women (○, ●); non-pregnant female viral hepatitis patients (△, ▲); and pregnant viral hepatitis patients (◆, ◆)]

Table 2. Mean  $\pm$  S.E.M peak and area under the concentration curves of control subjects and viral hepatitis patients

|                  |                | Blood Glucose    |                   | Plasma Insulin   |                 |
|------------------|----------------|------------------|-------------------|------------------|-----------------|
|                  |                | peak (mg/dl)     | Area (mg. min/dl) | peak (uU/ml)     | Area(uU.min/dl) |
| Control Subjects | Male (5)       | 119.2 $\pm$ 10.7 | 2754 $\pm$ 887    | 62.8 $\pm$ 9.4   | 4062 $\pm$ 334  |
|                  | Female (5)     | 122.4 $\pm$ 15.1 | 2859 $\pm$ 946*   | 63.0 $\pm$ 5.0** | 4047 $\pm$ 544  |
|                  | Both sex (10)  | 120.8 $\pm$ 8.8  | 2807 $\pm$ 682**  | 62.9 $\pm$ 5.1   | 4055 $\pm$ 301* |
|                  | Pregnancy (15) | 134.4 $\pm$ 5.5  | 4651 $\pm$ 405    | 77.5 $\pm$ 6.5   | 5168 $\pm$ 462  |
| V.H Patients     | Male (5)       | 134.2 $\pm$ 8.7  | 5991 $\pm$ 1201   | 86.0 $\pm$ 10.7  | 5382 $\pm$ 638  |
|                  | Female (5)     | 150.6 $\pm$ 12.6 | 7272 $\pm$ 1456   | 91.2 $\pm$ 8.2   | 6465 $\pm$ 1552 |
|                  | Both sex (10)  | 142.4 $\pm$ 7.7  | 6632 $\pm$ 915    | 88.6 $\pm$ 11.5  | 5924 $\pm$ 812  |
|                  | Pregnancy (15) | 134.2 $\pm$ 6.8  | 6100 $\pm$ 499    | 87.5 $\pm$ 15.5  | 5245 $\pm$ 829  |

\* and \*\* denotes significantly different from their viral hepatitis counterpart at  $p < 0.05$  and  $0.01$  level respectively

Table 3. Correlation between fasting glucose level and SGOT, SGPT levels

|                   | SGOT  |       | SGPT  |       |
|-------------------|-------|-------|-------|-------|
|                   | r     | p     | r     | p     |
| Male              | -0.59 | <0.02 | -0.64 | <0.01 |
| Fasting Female    | -0.58 | <0.05 | -0.33 | N.S   |
| Glucose Pregnancy | -0.57 | <0.05 | -0.35 | N.S   |
| All combined      | -0.53 | <0.01 | -0.44 | <0.01 |

## DISCUSSION

Felig et al. (1970)<sup>7</sup> observed fasting hypoglycemia (below 60 mg/dl) in eight out of fifteen consecutive patients (53.3%) with acute viral hepatitis. In the present study fasting hypoglycemia was found in 60% of the hepatitis patients (both sexes combined). Even higher percentage (86.7%) was found in pregnant patients, whereas, only three out of fifteen apparently normal

subjects (20%) showed fasting hypoglycemia and no one in the control subjects both male and female showed fasting hypoglycemia. Moreover, the mean fasting blood glucose level of hepatitis patients (62.3 $\pm$ 2.6 mg/dl) was found to be significantly ( $P < 0.01$ ) lower than that of normal subjects (73.6 $\pm$ 2.0 mg/dl). The reasonable explanation for this low fasting glucose level would be due to decreased liver glycogen content resulted from parenchymal cells damage.

Although, the individual glucose concentration curves of the hepatitis patients following OGTTs were within normal limits regarding the diabetes criteria, they generally attained higher peak and larger area under the curve, both of which indicated decreased tolerance to glucose load. Infact, unequivocal diabetes type of glucose curves had been identified in cases of chronic liver diseases<sup>8</sup>.

The mechanism responsible for disordered blood glucose regulation have not

established. Failure of glycogen synthesis and hyperinsulinism resulting from inadequate hepatic insulin degradation have been postulated. In the present study the fasting insulin level of hepatitis patients were not elevated, though their fasting glucose level were significantly lower. Felig et al. (1970) observed that liver glycogen content, determined histologically, was markedly reduced in the patients with hepatitis. However, the mean insulin concentration curve following OGTT was higher and the mean peak and area under the curve were definitely higher in these patients (Table 2). Therefore, the role of inadequate hepatic insulin degradation could not be ignored.

Megyesi et al. (1967)<sup>8</sup> found no correlation between impairment of glucose tolerance and presence or absence of jaundice. In the present study we found significant inverse correlation between fasting blood glucose level and SGOT, SGPT levels. Therefore we concluded that the more severe the hepatitis as indicated by high SGOT, SGPT levels, the more likely is the patient to develop hypoglycemia, and being pregnant is the another factor that can increase the susceptibility of the patient to develop hypoglycemia.

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## Effect of enterotoxigenic Escherichia coli heat labile toxin on intestinal enzymes in rat

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Differential study of the marker enzyme levels for intracellular organelles in intestinal mucosa and muscularis layer following E. coli labile toxin (LT) administration to rat revealed that there were no changes in the enzyme levels in mucosa but some changes (a decrease of acid phosphatase and an increase of proteinase) in muscularis layer. The results suggest that the LT, being a high molecular weight toxin, may not be easily invaginated into intestinal epithelial cells and interact with the subcellular particles. The changes in the enzyme activities observed in the muscularis layer could be the abnormal metabolic consequences of prolonged hypersecretion produced by LT.

### INTRODUCTION

One of the major enteric pathogens isolated from stools of patients with acute diarrhoea in a suburban community in Myanmar is enterotoxigenic Escherichia coli (1). Enterotoxigenic E. coli has two types of toxins, namely heat stable (ST) and heat labile (LT) (2). Although there have been many studies on the mechanism of fluid secretion due to the toxins (3), relatively little is known about the effects of the toxins on subcellular organelles of intestinal epithelial cells. Recently, Aye-Kyaw et al. (4) have indicated that the ST mainly affects lysosome by increasing the lysosomal enzyme activities and lysosomal membrane stability. The present study is, therefore, intended to look at the effects of another toxin of enterotoxigenic E. coli namely LT, on the subcellular marker enzymes.

### MATERIALS AND METHODS

Experimental. About sixty days old Wistar rats, weighing 60 to 80 g, were used throughout the experiment. Six rats, which had been fasted overnight and served as the test (infected) group, were anaesthetized and for each rat, the abdomen was opened, the intestine was tied with sterile thread at two points about 20 cm apart and injected with the enterotoxigenic E. coli LT (obtained from human R 8-2 strain and supplied by Bacteriology Research Division of the Department of Medical Research) prepared in normal saline, at a dilution level of 1/50 which is optimum for producing maximum fluid accumulation. The control group, which also consisted of 6 rats, was sham-operated, that is each animal received the same amount of saline (without toxin) and the same treatment as the infected group, simultaneously. After

the incubation period, the animals were sacrificed. The tied portion of the intestine was removed, and the volume of fluid accumulated in it was measured. Its weight and length were then recorded. The intestine was then washed with chilled 0.25 M sucrose containing 0.5% of 0.2 M Tris-HCl (pH 7.4), blotted and cut into short lengths, which were then cut longitudinally to expose the mucosa. The mucosa was removed by scraping with a scalped blade.

The mucosal scrapings obtained from each rat were pooled, weighed and homogenized in (20 x weight of mucosa) ml of 0.25 M sucrose with a Potter-Elvehjem homogenizer. The remaining muscularis layer (left after mucosa layer had been scraped off) was also weighed, cut into small pieces and homogenized in (20 x weight of tissue) ml of 0.25 M sucrose. The homogenates were frozen and thawed five times to lyse the cells and were then used for enzyme assays.

Enzyme assays. Acid phosphatase (EC 3.1.3.2) was assayed at pH 5.0 according to the method described by Aye - Kyaw (5) using  $\beta$ -glycerophosphate as

substrate. Alkaline phosphatase (EC 3.1.3.1) and glucose 6-phosphatase (EC 3.1.3.9) were assayed according to Parkinson et al. (6). The reactions in the above enzyme assays were stopped by 10% (w/v) perchloric acid and the released inorganic phosphate was measured by the method of Aye-Kyaw et al. (7). Succinic dehydrogenase (EC 1.3.99.1) was measured according to the method described by Bonner (8). Proteinase (EC 3.4.21.14) activity was determined at pH 8.5 using casein as substrate by the method of Takahashi and Ohsaka (9). Protein was determined by the method of Miller (10).

Enzyme activities were expressed as milliunits (mU) or units (U) per milligram protein in the homogenate where 1 U is equal to 1  $\mu$ mole of substrate transformed or product produced per minute. Statistical analyses were performed using Student's unpaired  $t$  test.

## RESULTS

Table 1 shows the values of body weight and secretory response as measured by fluid accumulation and gut

Table 1. Body weight, volume of fluid accumulated in the intestine and gut weight/length of the control and infected rats

| Group                                | Body weight<br>(g) | Fluid<br>accumulated<br>(ml) | Gut<br>weight/length<br>(mg/cm) |
|--------------------------------------|--------------------|------------------------------|---------------------------------|
| Control 1                            | 76.60              | 0                            | 67.94                           |
| 2                                    | 86.10              | 0                            | 73.09                           |
| 3                                    | 73.45              | 0                            | 67.61                           |
| 4                                    | 60.50              | 0                            | 70.11                           |
| 5                                    | 67.90              | 0                            | 69.58                           |
| 6                                    | 77.50              | 0                            | 71.85                           |
| (Mean $\pm$ S.E)                     | (70.84 $\pm$ 3.44) |                              | (70.03 $\pm$ 0.75)              |
| Infected 1                           | 72.70              | 3.0                          | 64.63                           |
| 2                                    | 69.10              | 2.0                          | 67.09                           |
| 3                                    | 77.70              | 3.6                          | 83.79                           |
| 4                                    | 70.40              | 4.5                          | 78.78                           |
| 5                                    | 82.30              | 4.5                          | 76.28                           |
| 6                                    | 57.00              | 3.0                          | 84.44                           |
| (Mean $\pm$ S.E)                     | (71.53 $\pm$ 3.22) |                              | (79.17 $\pm$ 3.04)              |
| Significance<br>(Control - Infected) | N.S.               |                              | $P < 0.05$                      |

Probability (P) was calculated by Student's  $t$  test.

N.S., not significant at the 5% probability level

weight/length ratio of each and individual rat. It is observed that the body weights of the infected rats did not differ from the control rats probably indicating the validity of experimental design. There was a significant accumulation of fluid in the intestines of infected rats whereas the control rats did not show any fluid accumulation. The gut weight/length ratio values were also significantly increased in the infected group probably confirming the positive secretory response to the toxin.

As shown in Table 2, the protein levels (expressed as milligrams per gram tissue) of intestinal mucosa and muscularis layer of the infected group did not show any statistical significant difference from the control group suggesting that there may be no significant atrophic changes in intestinal cell as a result of toxin infection.

Table 3 shows differentially the specific activities of different marker enzymes, acid phosphatase, alkaline phosphatase, glucose 6-phosphatase, succinic dehydrogenase and proteinase in intestinal mucosa and muscularis layer of controls

and infected animals. It was found that the enzymes showed no changes in the mucosa between the control and the infected group. However, there were some changes in muscularis layer. Acid phosphatase, the marker enzyme for lysosome, was significantly decreased whereas proteinase, the soluble enzyme, was significantly increased in the infected group when compared with the control.

Table 2. Protein levels of intestinal mucosa and muscularis layer in the control and infected groups

| Group                   | Protein concentration<br>(mg per g tissue) |                         |
|-------------------------|--------------------------------------------|-------------------------|
|                         | Mucosa                                     | Muscularis layer        |
| Control (C)             | 120.5 <sup>±</sup> 18.4                    | 119.9 <sup>±</sup> 6.4  |
| Infected (I)            | 95.3 <sup>±</sup> 4.6                      | 146.3 <sup>±</sup> 14.6 |
| Significance<br>(C - I) | N.S.                                       | N.S.                    |

Each value represents the mean <sup>±</sup> standard error for six rats.

N.S., not significant at the 5% probability level.

Table 3. Enzyme levels of intestinal mucosa and muscularis layer in the control and the infected groups

| Enzyme                 | Mucosa                    |                           | Muscularis layer        |                           |
|------------------------|---------------------------|---------------------------|-------------------------|---------------------------|
|                        | Control                   | Infected                  | Control                 | Infected                  |
| Acid phosphatase       | 9.49 <sup>±</sup> 2.32    | 7.07 <sup>±</sup> 0.56    | 7.32 <sup>±</sup> 0.59  | 4.63 <sup>±</sup> 0.50*   |
| Alkaline phosphatase   | 285.06 <sup>±</sup> 76.11 | 236.69 <sup>±</sup> 47.54 | 15.17 <sup>±</sup> 8.97 | 5.56 <sup>±</sup> 1.40    |
| Glucose 6-phosphatase  | 2.54 <sup>±</sup> 0.91    | 3.61 <sup>±</sup> 0.69    | 4.25 <sup>±</sup> 1.79  | 2.74 <sup>±</sup> 0.40    |
| Succinic dehydrogenase | 33.87 <sup>±</sup> 8.09   | 20.91 <sup>±</sup> 3.46   | 21.60 <sup>±</sup> 2.97 | 16.34 <sup>±</sup> 1.85   |
| Proteinase             | 1.46 <sup>±</sup> 0.13    | 1.44 <sup>±</sup> 0.28    | 0.57 <sup>±</sup> 0.10  | 1.11 <sup>±</sup> 0.19 ** |

Each value represents the mean <sup>±</sup> standard error for six rats. Acid phosphatase, alkaline phosphatase and glucose 6-phosphatase activities are expressed as milliunits per milligram protein. Succinic dehydrogenase, and proteinase activities are expressed as units per milligram protein.

\*P < 0.01, \*\*P < 0.05 (on comparison with the respective controls).

## DISCUSSION

Although there have been many studies on the clinical aspect and the mechanism of secretion in acute diarrhea caused by the toxins of *E. coli*, relatively few studies have been carried out on the effects of the toxins on subcellular organelles. Recently, a study by Aye-Kyaw et al. (4) have indicated that among subcellular organelles *E. coli* ST mainly affects lysosomes, which is consistent with the concept proposed by Walker and Isselbacher (11) for the uptake and transport of macromolecules by the intestine. However, in the present study, *E. coli* LT apparently does not affect intestinal epithelial cell particles, since no changes in the marker enzymes were found in the mucosa. The difference between the effects of ST (4) and LT (present study) may lie in the difference in molecular weights of the toxins. LT, having a molecular weight of about 73,000, is relatively larger than ST, the molecular weight of which is reported to be about 5,000 (12), which may be the reason that unlike ST, LT cannot be easily invaginated into the cell and does not interact with the cell particles resulting in no changes in the marker enzymes. Consequently, LT could escape intracellular digestion by lysosome and thus, unlike ST, it has the physiologic action of prolonged hypersecretion (12). It is of interest to note the pattern of distribution of alkaline phosphatase between mucosa and muscularis layer (Table 3). Alkaline phosphatase, the brush border enzyme, was found to be mainly associated with the mucosa, which probably indicates the efficiency of the method of mucosal scraping used in the present study, making the differential study of the enzyme levels between mucosa and muscularis layer more valid.

The alterations in the enzyme levels observed in muscularis layer, like a decrease of acid phosphatase and an

increase of proteinase, could be the abnormal metabolic consequences of prolonged hypersecretion produced by LT infection. It is also possible that the increased proteinase levels in the muscularis layer could be the result of infiltration of blood granulocyte proteolytic enzymes which have been reported to increase substantially following bacterial toxin administration (13, 14).

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## Intestinal helminthiases in twenty one villages in Taikkyi township

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A field research project was undertaken under the title "The relationship between the control of Ascaris infection and child nutritional status" in 21 villages in Taikkyi township. At the beginning of study in August 1984, a mass stool examination was done on 3 whole villages (n=1052) and on 2-12 year old children (n=1070) in the remaining 18 villages. In addition, deworming was carried out in randomly selected 8 villages, involving different age groups. The results were confined to the baseline study. Ascaris lumbricoides was the principal intestinal helminth and its infection rate was 77.38% in the population as a whole. The prevalences of other infections were 5.63% for Trichuris trichiura, 2.53% for hook worm and 2.07% for Strongyloides stercoralis. By age, the prevalence of Ascaris infection was conspicuously high in the 5-9, 10-14 and 15-19 years old. The mean worm burden per person in all the ages was 6.6 and the age-specific worm burden was highest in the 5 to 9 year age group, about 10 worms per child. The findings were compared with those of other studies. It is high time to develop a community-based mass chemotherapy programme to control ascariasis in Myanmar based on national experiences in previous control and research projects.

### INTRODUCTION

The wet and humid rural areas of Myanmar are endemic to intestinal helminthiasis(1,2) particularly to ascariasis(3). In order to study the effect of ascariasis on nutrition, a two-year field research project was undertaken in Taikkyi township, under the title "The relationship between the control of Ascaris infection and child nutritional status". At the beginning of the study in August 1984, a baseline mass stool examination was carried out in the whole study area. The aim of this communication was to describe the pattern and occurrence of intestinal helminthiases especially on ascariasis in

a large number of villages at the project site.

### Study area and Villages

The project site lies in the west of Taikkyi town, about 80 km north of Yangon. It covers an area of 64 km<sup>2</sup> in which 21 villages are scattered within a distance of 1.5 km between any two villages and separated by paddy fields. There is a dirt road passing through the study area from Taikkyi. Transportation between villages is mainly by trishaws and on foot (see Figure).

There were 1005 households and a total

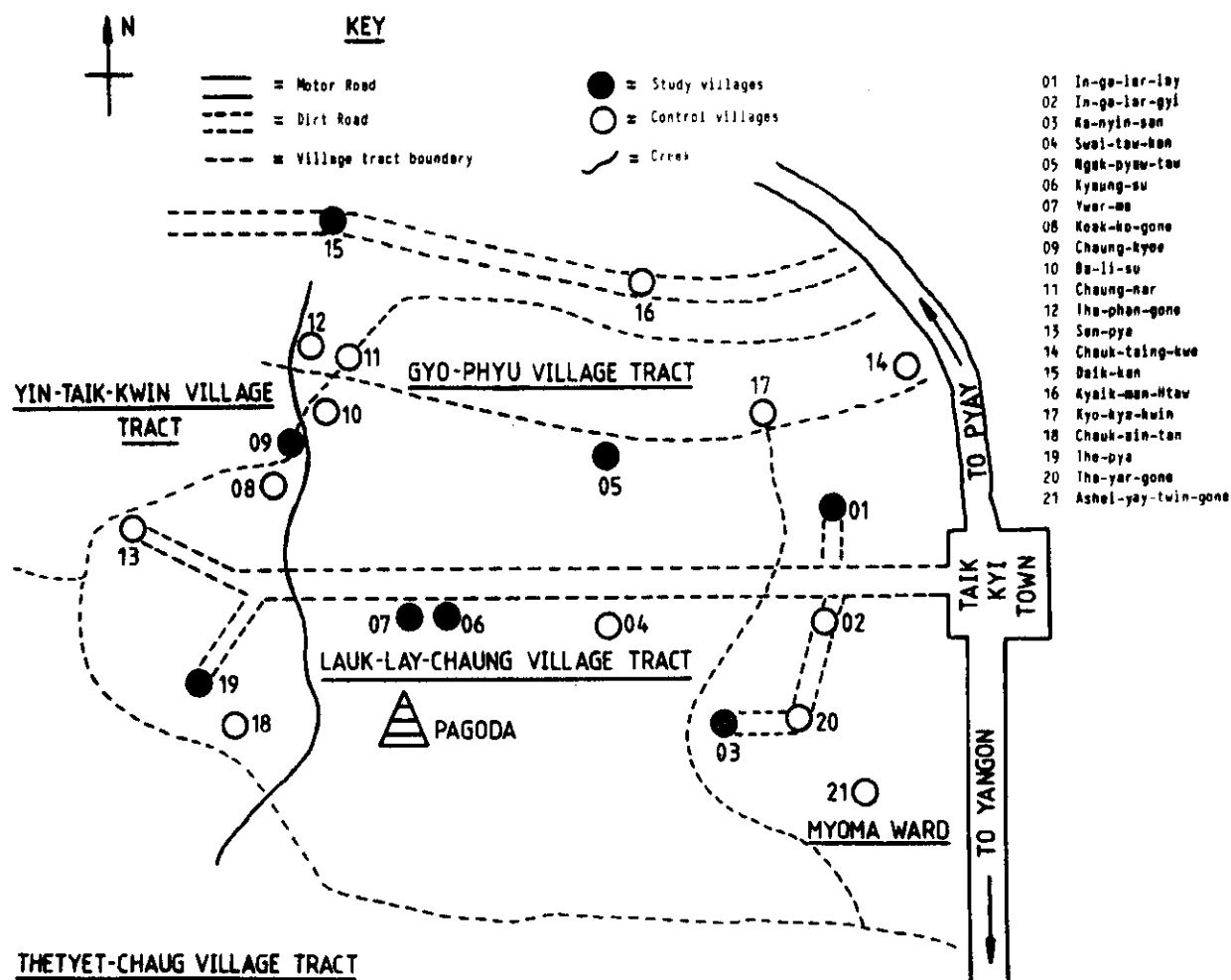


Figure. Map showing the location of villages in the study area in Taikkyi township

population of 4790, having 1356 children of 2-to-12 years old (for nutrition impact study) in the 21 villages. About 86.0% of the heads of household were paddy-cultivators and only 4.9% of them were illiterate. Moreover, 48.1% and 41.0% of the households were provided with pit and surface latrines respectively.

#### Study population and methods

For this report, the study populations comprised all the inhabitants of the three villages, namely Kanyinsan (n=493), Thepya (n=266) and Daikkan (n=293) and a total of 1070 children

aged 2-12 years in the remaining 18 villages. The survey consisted first of a stool examination of the above whole and children populations to detect eggs of intestinal helminths by direct microscopical examinations at the field laboratory set up in Taikkyi hospital. However, three duplicates of each stool sample will be examined for confirmation of absence of Ascaris eggs. Secondly, worm counts were made on each infected individual with Ascaris lumbricoides by examination of stools for a period of 72 hours after giving levamisole hydrochloride (Ketrax tablets, ICI) were used and doses were given according to manufacturer's

instructions). Computation was made of prevalences for intestinal helminthiasis and, age and sex prevalence for ascariasis and mean Ascaris worm burden per host.

## RESULTS

### Prevalence of intestinal helminthiasis

In the three villages where the whole population were selected for stool examination, 871 of 1052 individuals participated in the study. The most predominant intestinal helminth was A. lumbricoides constituting 77.38%. T. trichiura infection formed 5.63%, and hook worm and S. stercoralis infections were the least frequent at about 2% each (Table 1). Similar patterns of intestinal helminth infections were also seen when the children of 2-12 year age group in the other 18 villages were examined. However, the trichiuriasis prevalence in this age-group ranged from zero in 5 villages, viz chaung-kyoe, Swai-taw-kan, San-pya, Tha-yar-gon and chaung-nar to 16% in one village, Chauk-ain-tan.

Table 1. Prevalence of parasitoses by intestinal helminth in Kanyinsan, Thepya and Daikkan villages in Jaikkyi township, August 1984

| Helminth               | Kanyin-<br>san<br>(n=382) | Thepya<br>(n=233) | Daik-<br>kan<br>(n=256) | Total<br>(n=871) |
|------------------------|---------------------------|-------------------|-------------------------|------------------|
| <u>A. lumbricoides</u> | 77.23                     | 70.82             | 83.59                   | 77.38            |
| <u>A. trichiura</u>    | 4.45                      | 6.44              | 6.64                    | 5.63             |
| Hook worm              | 1.83                      | 1.29              | 4.69                    | 2.53             |
| <u>S. stercoralis</u>  | 0.79                      | 3.00              | 3.13                    | 2.07             |

n = No. of persons examined

### Prevalence of ascariasis by age and sex

There was an overall slight preponderance of females to males of getting Ascaris infections in the three villages (Table 2). However, no consistent pattern of infections occurred between age groups by sex. Before attaining the age of 1 year, about 40% acquired the infection and the prevalence was conspicuously high, over 80% in each of

Table 2. Prevalence of ascariasis by age and sex in Kanyinsan, Thepya and Daikkan villages in Jaikkyi township, August 1984

| Age<br>group | K a n y i n s a n |       |        |       | T h e p y a |        |        |       | D a i k k a n |        |        |       | T o t a l |       |        |       | Both male<br>& female |       |
|--------------|-------------------|-------|--------|-------|-------------|--------|--------|-------|---------------|--------|--------|-------|-----------|-------|--------|-------|-----------------------|-------|
|              | Male              |       | female |       | Male        |        | female |       | Male          |        | female |       | Male      |       | female |       | n                     | %     |
|              | n                 | %     | n      | %     | n           | %      | n      | %     | n             | %      | n      | %     | n         | %     |        |       |                       |       |
| 1            | 9                 | 44.44 | 2      | 50.00 | 1           | 100.00 | 2      | 0.00  | 3             | 33.33  | 3      | 33.33 | 13        | 46.15 | 7      | 28.57 | 20                    | 40.00 |
| 1- 4         | 20                | 85.00 | 28     | 71.43 | 13          | 92.31  | 6      | 66.67 | 12            | 83.33  | 17     | 94.12 | 45        | 86.67 | 51     | 78.43 | 96                    | 82.29 |
| 5- 9         | 32                | 87.50 | 20     | 95.00 | 16          | 87.50  | 17     | 82.35 | 22            | 86.36  | 18     | 94.44 | 70        | 87.14 | 55     | 90.91 | 125                   | 88.80 |
| 10-14        | 26                | 92.31 | 18     | 88.89 | 15          | 80.00  | 14     | 92.86 | 15            | 80.00  | 19     | 94.74 | 56        | 85.71 | 51     | 92.16 | 107                   | 88.79 |
| 15-19        | 17                | 76.47 | 17     | 82.35 | 8           | 75.00  | 14     | 92.86 | 7             | 71.43  | 14     | 92.86 | 32        | 75.00 | 45     | 88.89 | 77                    | 83.12 |
| 20-24        | 16                | 50.00 | 21     | 71.43 | 13          | 46.15  | 10     | 50.00 | 8             | 62.50  | 10     | 90.00 | 37        | 51.35 | 41     | 70.73 | 78                    | 61.54 |
| 25-34        | 21                | 85.71 | 25     | 88.00 | 12          | 58.33  | 15     | 66.67 | 16            | 87.50  | 22     | 68.18 | 49        | 79.59 | 62     | 75.81 | 111                   | 77.48 |
| 35-44        | 11                | 63.64 | 17     | 76.47 | 9           | 55.56  | 12     | 83.33 | 11            | 81.82  | 15     | 86.67 | 31        | 67.74 | 44     | 81.82 | 75                    | 76.00 |
| 45-54        | 20                | 75.00 | 21     | 80.95 | 13          | 76.92  | 15     | 60.00 | 9             | 100.00 | 11     | 90.91 | 42        | 80.95 | 47     | 76.60 | 89                    | 78.65 |
| 55-64        | 7                 | 57.14 | 10     | 60.00 | 4           | 75.00  | 7      | 71.43 | 5             | 80.00  | 8      | 87.50 | 16        | 68.75 | 25     | 72.00 | 41                    | 70.73 |
| 65+          | 11                | 72.73 | 13     | 46.15 | 7           | 28.57  | 10     | 40.00 | 6             | 50.00  | 5      | 80.00 | 24        | 54.17 | 28     | 50.00 | 52                    | 51.92 |
| All ages 190 | 76.84             | 192   | 77.60  | 111   | 70.27       | 122    | 71.31  | 114   | 79.82         | 142    | 86.62  | 415   | 75.90     | 456   | 78.73  | 871   | 77.38                 |       |

the 5-9, 10-14 and 15-19 year age groups. The rate then declined gradually to a level of about 50% in the oldest (Table 2).

On examining the prevalence of ascariasis in the 2-12 year old children as a whole in the 18 villages, the average value was 80.78% and ranged from 63.41% in Kyaungsu to 94.12% in Balisu.

Table 3. Mean worm burden per person in Daikkan and Thepya villages by age in Taikkyi township, August 1984

| Age group (years) | Daikkan |                        | Thepya |                        |
|-------------------|---------|------------------------|--------|------------------------|
|                   | n       | $\bar{x} \pm s^2$      | n      | $\bar{x} \pm s^2$      |
| < 5               | 19      | 7.1 <sup>±</sup> 60.9  | 20     | 3.9 <sup>±</sup> 39.1  |
| 5 - 9             | 35      | 10.3 <sup>±</sup> 99.5 | 32     | 9.9 <sup>±</sup> 148.6 |
| 10 -14            | 27      | 8.2 <sup>±</sup> 66.4  | 27     | 7.0 <sup>±</sup> 51.7  |
| < 15              | 81      | 8.8 <sup>±</sup> 79.3  | 79     | 7.4 <sup>±</sup> 91.5  |
| 15 -24            | 23      | 4.9 <sup>±</sup> 35.4  |        |                        |
| 25 -44            | 26      | 4.7 <sup>±</sup> 46.6  |        |                        |
| 45 +              | 21      | 2.0 <sup>±</sup> 8.0   |        |                        |
| All ages          | 151     | 6.5 <sup>±</sup> 63.2  |        |                        |

n = No. of persons dewormed  $\bar{x} \pm s^2$  = mean  
± variance

#### Mean worm burden per host

Of the 21 villages, 8 were randomly selected for providing chemotherapy. Different age groups were allocated by village. In one village (Daikkan), all age groups were involved, while in the other village (Thepya), only those under 15 years of age were given anthelmintic treatment, and only 2-12 year old children were assigned for the treatment in the remaining villages.

In Daikkan, the overall mean worm burden per person was 6.6. The age-

specific mean worm burden was highest in the 5-9 year age group in both Daikkan and Thepya (Table 3). On analysing the worm burden in the 2-12 year old children in the 8 villages, the average number of worms per child was 11.9, the range being from 8.1 in Thepya to 20.2 in Ywarma village.

## DISCUSSION

The predominant intestinal helminth in this study is also *A. lumbricoides* as observed in previous surveys (1,2) and in the recent study in Yangon (4). Generally, the prevalence of *T. trichiura* in Taikkyi has similar magnitude as reported in other rural areas (1,2,5,6) but the prevalence was very much lower than those found in some urban areas (7,8,9). In localities where trichiuriasis prevalence is high or over 70% like in Yangon(8), study on transmission dynamics should be undertaken to elucidate control measures against trichiuriasis in communities. With regard to hook worm infection rate, it is much lower than those reported in Insein township (5,6,10). This might be due to difference in nature of occupation. For instance, in Taikkyi, very few persons or none worked in high risk areas like banana plantations for acquiring hookworm infection. In our study, we did not find other intestinal helminths, other than a very low percentage of *S. stercoralis* as observed in previous surveys (1,2,4,7,9). It should be borne in mind that this study was mainly directed to detect ascariasis and the reported prevalences for other helminths are regarded as minimal estimates and they are based on direct stool microscopic examination.

Thus, the picture of intestinal or soil-transmitted helminthiasis in rural Myanmar remain the same as occurred in the 1960s, owing to lack of improvement in socio-economic conditions, environmental sanitation and personal

hygiene and lack of deployment of effective control measures. The problem of helminthiasis, particularly ascariasis will be expected to remain the same for a considerable time in the future unless a short-term project like a community based periodic mass chemotherapy control programme is advocated. In this respect, the national experiences of ascariasis control measures and programmes have been reviewed by one of the authors (11,12) and the health policy-makers and health managers should give serious consideration over the proposed ascariasis control programme contained therein.

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## Emergency laparotomy in Yangon General Hospital

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The information recorded in the Operating Theatre Registers kept at the Accident and Emergency Service (AES) of the Yangon General Hospital in 1985 and 1986 were collected to determine the magnitude, distributions and durations of operation of laparotomy conditions. Annually, about 1394 cases of major surgery were performed at the AES. Of these, laparotomy constituted about 91.3%, and appendicitis topped the list. By sex, male preponderance was observed in all diseases or conditions. By age, 20 to 29 years formed the commonest age group. The day-to-day performance of laparotomy cases was randomly and independently distributed (Poisson distribution) while those cases performed per week, month or similar days of the week in a year were constant (uniform distribution). Least numbers of cases were admitted between midnight and 8:00 am and operated on between 4:00 am to 12 noon. Within 4 hours of arrival, 50% of cases of common diseases had their operations started. The overall duration of operation per case lasted about 1 hour. It is recommended to study on patients and management in a wider context of the AES to improve the emergency services.

### INTRODUCTION

The published work on emergency surgical operations in Myanmar is sparse. The reports on emergency operations are usually submerged under the general heading, 'surgical operations' which included all other operations. These operations are described in total numbers by type of anaesthesia occurring in various hospitals by medical speciality or by hospital-bed (1). In a city like Yangon, it is important to bring out the magnitude of emergency surgery as a separate entity to help develop planning of emergency surgery services. As a first step, we studied the emergency operations involving laparotomy in the Accident and Emergency Service (AES) of the Yangon General Hospital

(YGH). The aims of the study were: (i) to determine the extent and patterns of emergency laparotomy by disease, (ii) to describe the age and sex distribution, (iii) to describe the distribution by time (daily, weekly, monthly and days-of-week variation, times at admission and operation, and time lag between admission and operation) and (iv) to determine the durations of operation.

### MATERIALS AND METHODS

For the materials, the emergency operating theatre registers of the AES in YGH were utilized for the study. The variables extracted from each laparotomy case include patient's hospital registration number, age, sex, diagnosis, date and time at admission,

times at beginning and end of operation. Data were collected for 1985 and 1986. Simple analysis was done in order to have total numbers, percentage distribution, mean and standard deviation of the variables of interest of the laparotomized cases. Chi-square goodness-of-fit tests for Poisson and uniform distributions and statistical significant test for difference between two means (Student's t-tests) were used as and when required (2).

## RESULTS

### Extent and patterns of laparotomy

Over the years 1985 and 1986, a total of 278 cases of major and 4815 minor

surgical operations were performed at the AES of YGH. Of the major operations, laparotomy constituted about 91.3%. Within the laparotomized cases, appendicitis topped the list (39.7%), followed by peptic ulcer perforation (18.4%), abdominal injury (10.4%), hernia (7.6%) and intestinal obstruction (5.2%).

### Sex and age distribution

During the years under study, 1732 male patients came to the AES for abdominal operations while only 810 females came for the same purpose. Male preponderance was seen in all diseases or conditions (Table 1). By sex and age, the overall percentage

Table 1. Percentage distribution of laparotomy cases by age and sex at the Accident and Emergency Service, YGH during 1985 and 1986

| Disease                  | Sex | Age in years |       |       |       |       |      | Total |        |
|--------------------------|-----|--------------|-------|-------|-------|-------|------|-------|--------|
|                          |     | <20          | 20-29 | 30-39 | 40-49 | 50-59 | 60+  |       |        |
| Appendicitis             | M   | 18.6         | 41.4  | 20.0  | 10.3  | 6.7   | 3.0  | 100.0 | (554)* |
|                          | F   | 18.5         | 40.2  | 21.5  | 9.9   | 7.3   | 3.5  | 100.0 | (455)  |
| Peptic ulcer perforation | M   | 3.5          | 27.5  | 24.2  | 14.4  | 17.2  | 13.1 | 100.0 | (396)  |
|                          | F   | 5.5          | 6.9   | 6.9   | 21.9  | 20.5  | 38.4 | 100.0 | (73)   |
| Hernia                   | M   | 7.9          | 22.0  | 14.7  | 17.5  | 15.3  | 22.6 | 100.0 | (177)  |
|                          | F   | -            | 6.7   | 13.3  | 6.7   | 13.3  | 60.0 | 100.0 | (15)   |
| Haematemesis & melena    | M   | 1.3          | 20.5  | 9.0   | 12.8  | 21.8  | 34.6 | 100.0 | (78)   |
|                          | F   | 3.3          | 3.3   | 13.3  | 20.0  | 16.7  | 43.3 | 100.0 | (30)   |
| Intestinal obstruction   | M   | 5.1          | 23.0  | 9.0   | 21.8  | 12.8  | 28.2 | 100.0 | (78)   |
|                          | F   | 10.9         | -     | 25.5  | 14.6  | 10.9  | 38.2 | 100.0 | (55)   |
| Enteric perforation      | M   | 25.0         | 36.8  | 17.1  | 13.1  | 4.0   | 4.0  | 100.0 | (76)   |
|                          | F   | 47.1         | 17.7  | 29.4  | 5.9   | -     | -    | 100.0 | (17)   |
| Abdominal injury         | M   | 21.4         | 43.2  | 20.5  | 10.2  | 3.4   | 1.3  | 100.0 | (234)  |
|                          | F   | 29.0         | 32.3  | 32.3  | 6.4   | -     | -    | 100.0 | (31)   |
| Others                   | M   | 11.5         | 20.9  | 18.7  | 21.6  | 15.1  | 12.2 | 100.0 | (139)  |
|                          | F   | 6.7          | 26.9  | 24.6  | 11.9  | 17.9  | 11.9 | 100.0 | (134)  |
| Sub-total                | M   | 12.8         | 32.9  | 19.3  | 13.6  | 11.0  | 10.5 | 100.0 | (1732) |
|                          | F   | 14.9         | 29.5  | 21.1  | 11.2  | 10.5  | 12.7 | 100.0 | (810)  |
| Total                    |     | 13.5         | 31.8  | 19.9  | 12.9  | 10.9  | 11.2 | 100.0 | (2542) |

\* Number of cases in parentheses

distributions for all diseases conditions were similar. However, in the group under peptic ulcer perforation, more males were in the 20-29-year-age group and more females in the 60 years and above. With regard to hernia, haematemesis and malena, and intestinal obstruction cases, males predominated in the 20-29 and 60 year-and above age groups while females predominated only in the 60 years and above. By age, more than 80% of adults under 40 years were operated on appendicitis, entric perforations, and abdominal injuries (Table 1).

#### Time distribution

The performance of laparotomy by day ranged from zero to 10 cases and the daily distribution of cases is in agreement with the Poisson probability distribution model (Table 2), indicating that daily laparotomy operations done are randomly independent to each other or unpredictable. Similarly, the occurrences of cases by week, month and similar days of the week in a year correspond to uniform distribution model (Table 2), showing that cases are constant in distribution.

On examination the time at admission of cases of common laparotomy conditions, most of them more or less occurred during the day or night except from mid-night to 8:00 am for appendicitis, haematemesis and malena, and abdominal injury; from mid-night to 4:00 am for peptic ulcer perforation and intestinal obstruction; from 4:00 to 8:00 am for hernia (Table 3). However, by time at operation, about 8% or less were operated on between 4 to 8 am or 8 am to 12 noon.

The average values ( $\pm$  SD) for time lag for operation i.e. time difference between time at admission and time at operation are shown in Table 4. By frequency distribution, less than 5% of the emergencies were operated on in

the first hour of arrival to the AES. Within 4 hours, about 50% and within 6 hours, about 75-80% of the common surgical condition cases except intestinal obstruction had their operations started (Table 4). As regard to intestinal obstruction cases, only 30% and 60% began to have surgery within 4 and 6 hours of arrival, and 30% or so were operated on only after 8 hours of admission (Table 4).

#### Duration of operation

The overall average duration taken for a single case of emergency laparotomy was 62 minutes. The time taken for the operation on cancer of stomach or rectum took the longest (118 minutes), followed by haematemesis and malena (107 minutes), abdominal injury (83 minutes), etc. (Table 5).

### DISCUSSION

In general, in the Accident and Emergency Service of YGH, about 3.5 cases per day, 25 cases per week, 110 cases per month or 1270 cases per year or over 90% of all the surgical operations performed were laparotomy cases and the operations on appendicitis were the commonest. In addition, men came to the Service more than twice than women; 20-29 years of age were mostly operated; Patient arrival and performance of surgery were more frequent between 8:00 pm to 12 mid-night; the majority of cases had to wait 4-6 hours for surgical operation; and the surgery took about 1 hour for each case.

It seems that durations of operations take longer in children than in adults for the same disease. For instance, in this study, the mean durations  $\pm$  SD in minutes taken on intestinal obstruction, appendicitis, intestinal perforation and intussusception cases were  $82 \pm 37.2$ ,  $48.5$

Table 2. Distribution by laparotomy by time in 1985

## Frequency by days

| No. of Patients | No. days Observed | No. days* expected |
|-----------------|-------------------|--------------------|
| 0               | 11                | 10.42              |
| 1               | 36                | 37.06              |
| 2               | 71                | 65.89              |
| 3               | 79                | 78.10              |
| 4               | 62                | 69.44              |
| 5               | 50                | 49.38              |
| 6               | 23                | 29.27              |
| 7               | 20                | 14.87              |
| 8               | 11                | 6.61               |
| 9               | 1                 | 2.61               |
| 10              | 1                 | 1.35               |
| Total           | 365               | 365.00             |

\* estimated by Poisson distribution model

 $\bar{x} = 3.56, \chi^2 = 8.39, P > 0.05$ 

## Frequency by month

| Month     | No. observed |
|-----------|--------------|
| January   | 106          |
| February  | 106          |
| March     | 108          |
| April     | 99           |
| May       | 111          |
| June      | 89           |
| July      | 124          |
| August    | 128          |
| September | 111          |
| October   | 113          |
| November  | 89           |
| December  | 114          |
| Total     | 1298         |

 $\bar{x} = 108.17, \chi^2 = 14.29, P > 0.05$ 

## Frequency by weeks

| No. of Patients | No. of weeks Observed |
|-----------------|-----------------------|
| 9               | 1                     |
| 12              | 1                     |
| 19              | 4                     |
| 21              | 2                     |
| 22              | 5                     |
| 23              | 13                    |
| 24              | 1                     |
| 25              | 5                     |
| 26              | 2                     |
| 27              | 1                     |
| 28              | 2                     |
| 29              | 3                     |
| 30              | 4                     |
| 31              | 4                     |
| 32              | 1                     |
| 33              | 2                     |
| 37              | 1                     |
| Total           | 52                    |

 $\bar{x} = 24.96, \chi^2 = 53.52, P > 0.05$ 

## Frequency by days of week

| Day        | No. observed |
|------------|--------------|
| Sundays    | 206          |
| Mondays    | 179          |
| Tuesdays   | 186          |
| Wednesdays | 205          |
| Thursdays  | 164          |
| Fridays    | 181          |
| Saturdays  | 177          |
| Total      | 1298         |

 $\bar{x} = 185.43, \chi^2 = 7.54, P > 0.05$

Table 3. Percentage distribution of common laparotomies by time at admission at the Accident and Emergency Service, YGH during 1985

| Time        | Appendicitis    | Peptic ulcer perforation | Hernia        | Intestinal obstruction | Haematemesis & melena | Abdominal injury |
|-------------|-----------------|--------------------------|---------------|------------------------|-----------------------|------------------|
| 20:00-23:59 | 31.3            | 20.9                     | 26.3          | 16.1                   | 21.3                  | 34.1             |
| 00:00-03:59 | 6.4             | 9.4                      | 15.4          | 5.4                    | 8.2                   | 7.8              |
| 04:00-07:59 | 6.0             | 12.6                     | 4.4           | 14.3                   | 8.2                   | 3.9              |
| 08:00-11:59 | 21.0            | 22.5                     | 20.9          | 28.5                   | 26.2                  | 14.7             |
| 12:00-15:59 | 14.1            | 16.8                     | 15.4          | 14.3                   | 14.8                  | 17.8             |
| 16:00-19:59 | 21.2            | 17.8                     | 17.6          | 21.4                   | 21.3                  | 21.7             |
| Total       | 100.0<br>(467)* | 100.0<br>(191)           | 100.0<br>(91) | 100.0<br>(56)          | 100.0<br>(61)         | 100.0<br>(129)   |

\*Number of cases in parentheses

Table 4. Cumulative frequency distribution of common emergency laparotomies by time lag for operation at the Accident and Emergency Service, YGH during 1985

| Class interval in hours | Appendicitis    | Peptic ulcer perforation | Hernia        | Intestinal obstruction | Abdominal injury |
|-------------------------|-----------------|--------------------------|---------------|------------------------|------------------|
| < 1                     | 4.1             | 2.5                      | 4.5           | 2.0                    | 0.9              |
| 1-2                     | 17.1            | 15.3                     | 20.3          | 8.1                    | 22.4             |
| 2-3                     | 35.9            | 29.1                     | 34.9          | 16.3                   | 41.4             |
| 3-4                     | 52.1            | 48.0                     | 56.2          | 28.5                   | 54.3             |
| 4-5                     | 70.1            | 66.4                     | 69.7          | 44.9                   | 68.1             |
| 5-6                     | 80.9            | 77.1                     | 75.3          | 57.1                   | 74.1             |
| 6-7                     | 89.1            | 83.7                     | 83.2          | 61.2                   | 79.3             |
| 7-8                     | 92.6            | 88.8                     | 88.8          | 69.4                   | 85.3             |
| 8+                      | 100.0<br>(462)* | 100.0<br>(196)           | 100.0<br>(89) | 100.0<br>(49)          | 100.0<br>(116)   |
| $\bar{x}$ (minutes)     | 255             | 285                      | 275           | 366                    | 263              |
| $\pm$ S.D               | $\pm$ 152       | $\pm$ 172                | $\pm$ 185     | $\pm$ 182              | $\pm$ 165        |

\* Number of cases in parentheses

Table 5. Duration of operation means  $\pm$  S.D in minutes of surgical abdominal emergency condition at the Accident and Emergency Service in YGH

| Disease/<br>condition           | 1985 |                   | 1986 |                   | Total |                   |
|---------------------------------|------|-------------------|------|-------------------|-------|-------------------|
|                                 | n    | $\bar{x} \pm S.D$ | n    | $\bar{x} \pm S.D$ | n     | $\bar{x} \pm S.D$ |
| Appendicitis                    | 502  | 48.3 $\pm$ 20.4   | 507  | 48.8 $\pm$ 22.3   | 1009  | 48.5 $\pm$ 21.4   |
| Peptic ulcer<br>perforation     | 222  | 57.5 $\pm$ 27.0   | 245  | 59.0 $\pm$ 27.0   | 467   | 58.3 $\pm$ 27.0   |
| Hernia                          | 96   | 67.5 $\pm$ 33.4   | 96   | 65.5 $\pm$ 36.2   | 192   | 66.5 $\pm$ 34.7   |
| Haematemesis<br>& melena        | 61   | 105.8 $\pm$ 40.3  | 47   | 108.5 $\pm$ 40.9  | 108   | 107.0 $\pm$ 40.4  |
| Intestinal<br>obstruction       | 58   | 85.3 $\pm$ 41.3   | 75   | 79.4 $\pm$ 33.7   | 133   | 82.0 $\pm$ 37.2   |
| Enteric<br>perforation          | 57   | 60.4 $\pm$ 24.5   | 36   | 60.8 $\pm$ 22.1   | 93    | 60.6 $\pm$ 23.5   |
| Intussusception                 | 17   | 77.1 $\pm$ 61.5   | 5    | 55.0 $\pm$ 24.0   | 22    | 72.0 $\pm$ 55.5   |
| Cancer<br>stomach/rectum        | 4    | 128.8 $\pm$ 61.7  | 5    | 111.0 $\pm$ 70.1  | 9     | 118.9 $\pm$ 63.0  |
| Abdominal injury                | 147  | 84.6 $\pm$ 40.7   | 118  | 81.6 $\pm$ 43.4   | 265   | 83.3 $\pm$ 41.9   |
| Peritonitis/<br>Haemoperitonium | 37   | 70.8 $\pm$ 27.4   | 25   | 65.2 $\pm$ 32.5   | 62    | 68.5 $\pm$ 29.4   |
| Others                          | 95   | 65.0 $\pm$ 35.2   | 85   | 65.6 $\pm$ 27.5   | 180   | 65.3 $\pm$ 31.7   |
| Overall average                 | 1296 | 62.8 $\pm$ 34.2   | 1244 | 61.4 $\pm$ 32.8   | 2540  | 62.1 $\pm$ 33.5   |

$\pm$  21.4, 60.6 $\pm$ 23.5 and 72 $\pm$ 55.5 respectively (see Table 5), while those for children were 109 $\pm$ 37 (n=131), 60 $\pm$ 25 (n=111), 95 $\pm$ 32 (n=29), and 85 $\pm$ 33 (n=81) (3), and the differences in durations except in intussusception are found to be highly significantly different ( $p < 0.01$ ). Duration of operation for laparotomy depends upon a number of factors such as, diagnosis, age of patients, history of taking food (fasting required before operation), need of expert consultation, number of investigations required and operation procedure. The influence of these factors on laparotomy should be explored to help plan the operation services. In this connection, the influence of

operation procedure on operation duration of diseases in children has been shown in our another study (3).

By the same token, it is recommended to investigate or study on patients and management particularly with respect to efficient use of resources in terms of beds, operating theatre time, medical and nursing staff and other facilities, in a wider context of the Accident and Emergency Service to improve the services in general. It needs to remember that although the arrival of patients to the Service may be constant or predictable by week or month, how many emergency patients that will be treated, operated or

admitted in a day will not be known or able to control.

#### ACKNOWLEDGEMENTS

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cularly to Dr Soe Myint Aung and Dr Nwe Ni Tun in collecting and analysing the data.

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### Anopheline mosquitoes of Myanmar-III.

## Anopheles (Cellia) philippinensis Ludlow, 1902 & Anopheles (Cellia) nivipes (Theobald), 1903 in Myanmar and their differentiating characters

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Malaria workers in Myanmar have only recorded the prevalence of Anopheles philippinensis in the country and anopheles nivipes have not yet been identified in Myanmar. The Department of Medical Research (DMR) survey teams to Tha-bye-wa, Oktwin Township collected many specimens provisionally identified in the field as An. philippinensis. Iso-female lines were raised from wild caught female to prepare larval skin, pupal skin and adult specimens for detail morphological examination. The specimens collected from the forested foot-hill area of Tha-bye-wa was found to be An. nivipes, which was recorded for the first time in Myanmar. The distribution, seasonal prevalence, adult bionomics and larval habitats for both species, as described by previous workers were compared with the observations of the DMR survey teams.

### INTRODUCTION

Anopheles (Cellia) philippinensis Ludlow has a very wide distribution in Myanmar. Khin Maung Kyi(1) recorded this species from all parts of the country and in all types of terrain. However, nobody has ever examined the morphological characters of this species, in Myanmar, in detail to differentiate it from Anopheles (Cellia) nivipes (Theobald). Apiwathnasorn(2) and Knight and Stone(3) did not include Myanmar in the distribution of An. nivipes although Reid(4) stated that An. nivipes distribution extended eastwards from Assam through Myanmar, Thailand, Malaya and possibly Indo-China. One of the authors (MP), while on a visit to Armed Forces Research Institute for Medical Sciences, Bangkok, was

encouraged by Dr. Ralph Harbach (now at Smithsonian Institution) to carry out studies on the prevalence of An. nivipes and its possible relationship to malaria transmission in Myanmar. The distribution, seasonal prevalence, breeding habitats and adult bionomics for both species were studied by DMR survey teams and the results are presented. The differentiating characters between the two species of Myanmar were also recorded.

### Distribution

The distribution of An. philippinensis in Myanmar was described in detail by Khin Maung Kyi(1) and presented in Figure (1). The locations where An. philippinensis and An. nivipes have been recorded by DMR teams were presented in Table (1) and Figure (2).

Table 1. Locations where An. philippinensis and An. nivipes were collected by DMR teams

| locality                  | Township     | Season   | Topography and remarks on locations                                                                                     |
|---------------------------|--------------|----------|-------------------------------------------------------------------------------------------------------------------------|
| <u>An. philippinensis</u> |              |          |                                                                                                                         |
| <u>YANGON DIVISION</u>    |              |          |                                                                                                                         |
| Ye-sit-kan                | Taik-kyi     | June-Oct | Reservoir and catchment area with thick forest. Yangon Water Supply Centre. Low hills with some cultivated areas nearby |
| Kyauk-ain                 | Hlegu        | Oct-Nov  | Paddy cultivating plain area                                                                                            |
| <u>BAGO DIVISION</u>      |              |          |                                                                                                                         |
| Bwe-chin, Phado           | Kyauk-ta-gar | Aug-Oct  | Paddy cultivation plain area                                                                                            |
| Hle-lan-gu                | Thayarwady   | Aug-Sept | Foot-hills village with paddy cultivation area nearby                                                                   |
| A-le-kone                 | Gyo-bin-nauk | October  | Paddy cultivation plain area about 5 miles from foot-hills                                                              |
| <u>MANDALAY DIVISION</u>  |              |          |                                                                                                                         |
| Wetwun                    | Maymyo       | Aug-Nov  | Cultivated area on plateau about 3000 feet altitude                                                                     |
| Se-daw-gyi                | Madaya       | Aug-Nov  | Cultivated area by irrigation system. Close to foot-hills                                                               |
| <u>MON STATE</u>          |              |          |                                                                                                                         |
| Kwan-ka-thaung Ward       | Mudon        | July-Oct | Mudon Town area with edge of Kwan-ka-thaung Ward near rubber plantation                                                 |
| <u>An. nivipes</u>        |              |          |                                                                                                                         |
| <u>BAGO DIVISION</u>      |              |          |                                                                                                                         |
| Tha-bye-wa                | Oktwin       | July     | Foothill village surrounded by teak forest at an altitude of 700 feet                                                   |
| <u>MON STATE</u>          |              |          |                                                                                                                         |
| Kwan-ka-thaung            | Mudon        | July     | Mudon Town area with edge of Kwan-ka-thaung Ward near rubber plantation                                                 |

Adult bionomic

Prevalence in relation to forest

Entomological survey teams from DMR recorded only An. nivipes during July at Tha-bye-wa village, which is situated deep inside a teak forest reserve areas. Anopheles philippinensis was not

collected at all in this area. At Kwan-ka-thaung (Myoma 4) ward, Mudon Town, which is close to light jungle and rubber plantations, An. nivipes was collected in the monsoon season but only at a ratio of 1:4 to An. philippinensis. In the paddy-cultivating plains of Kyauk-ain, Hle-gu Township, no An. nivipes was recorded

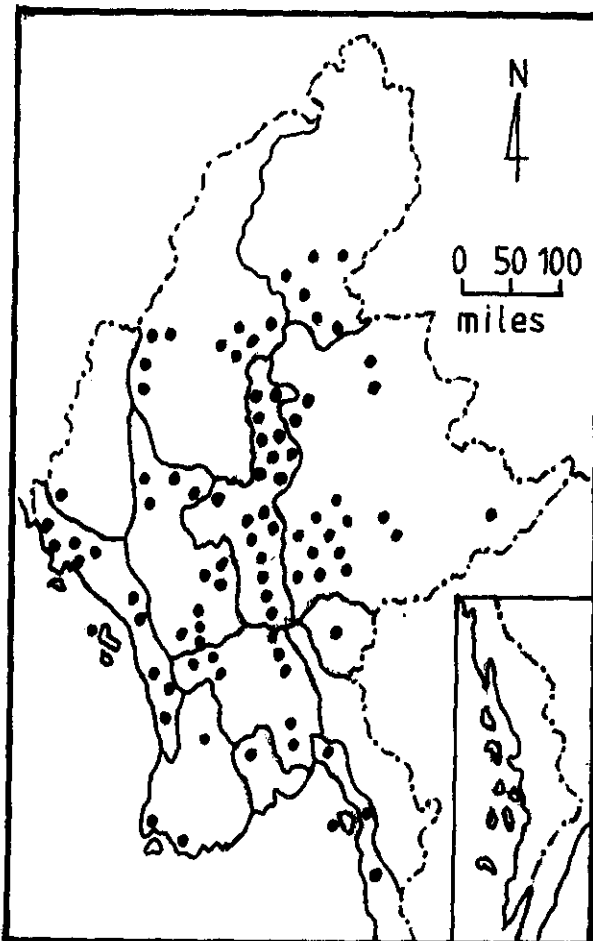


Figure 1. Recorded distribution of An. philippinensis by Khin Maung Kyi

at all in collections carried out in the monsoon and immediate post-monsoon months.

Anopheles nivipes was thus found to be associated with deep forest areas where it can be collected in significant numbers. An. philippinensis on the other hand, is more associated to paddy cultivation and breeds closer to human habitations. At Kwan-ka-thaung ward, DMR teams found An. philippinensis larvae breeding in earthen pots kept at the foot of stairs in houses washing feet.

#### Seasonal prevalence

Khin Maung Kyi(1) stated that An. philippinensis is prevalent from August

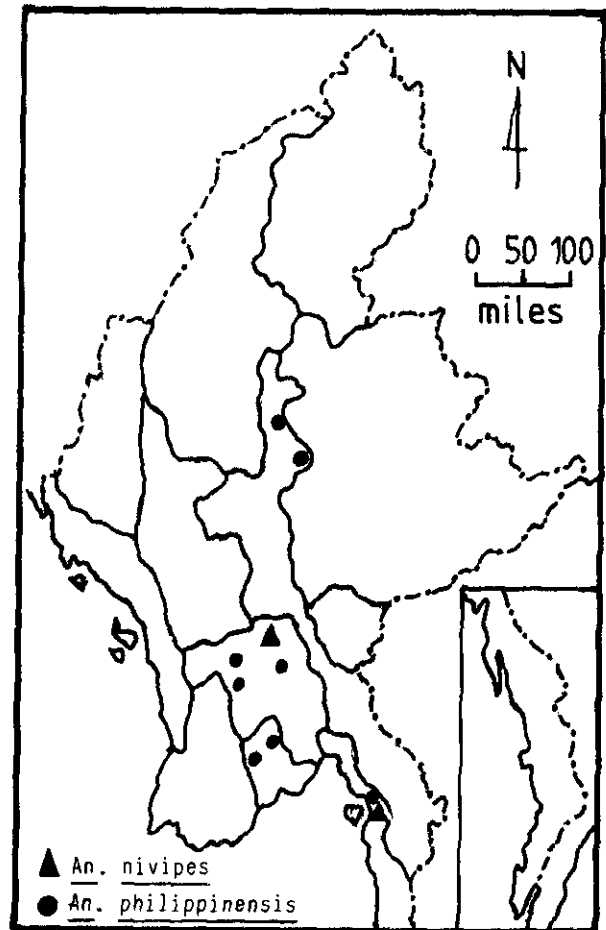


Figure 2. Recorded distribution of An. philippinensis and An. nivipes (DMR surveys)

to November with peak density in October. DMR survey teams also found this species in the latter part of monsoon with peak densities towards the end of September. However, by the end of November An. philippinensis could be collected only in small number in Kyauk-ain, Hle-gu Township.

Anopheles nivipes, was however, collected mainly during the rainy season in both Tha-bye-wa and Mudon. During subsequent surveys in the cool/dry and hot/dry seasons no An. nivipes could be collected. Its density was found to be highest in September at the end of Monsoon season in Tha-bye-wa.

### Breeding sources

Khin Maung Kyi(1), quoting previous workers in Myanmar such as Feegrade and Macan, stated that An. philippinensis was found to breed in ponds, cultivated or derelict paddy fields, tanks and swamps. DMR survey teams found An. philippinensis breeding in small ponds near paddy fields and in paddy fields in association with An. annularis when the paddy plants are tall enough to provide good shade.

However, DMR teams could not detect any breeding source of An. nivipes at both Tha-bye-wa or Mudon although all potential breeding sources were surveyed thoroughly.

### Biting habits

#### Host preference

Both An. philippinensis and An. nivipes were observed to be highly zoophilic by DMR teams. Myo Paing et al.(5) found that in Tha-bye-wa village, one night catch with human-baited bed net yield only 2 An. nivipes as compared to 11 specimens collected by cattle baited bed net placed nearby. Similarly, An. philippinensis was mainly collected from cattle-baited big bed nets.

However, Khin Maung Kyi(1) stated that at Innwaing village, Mawlamyine and at Ye-ta-khun-taung camp, Patheingyi, An. philippinensis was collected biting human-baits in large numbers during October 1970.

#### Time and place of biting

Both An. philippinensis and An. nivipes were found to bite mostly early in the night about 1900 to 2300 hours. Both the species are also almost always collected by outdoor biting catches and by cattle-baited big bed nets outdoors. DMR survey teams found very few An. philippinensis biting indoors. As for An. nivipes it was exclusively collected

from outdoor catches.

### Daytime resting

No An. philippinensis or An. nivipes were found resting indoors during the daytime in all the areas surveyed although numbers of the DMR survey teams made special effort to collect daytime resting catches.

### Relationship to malaria transmission

Khin Maung Kyi(1) stated that, in the absence of cattle, it is quite possible that An. philippinensis could act as a secondary vector during transmission season. In Bangladesh, Elias(6) assumed An. philippinensis to be a vector of major importance in the vast plain area of the country. From the results of DMR survey teams, taking into consideration its predominantly zoophilic biting habits, An. philippinensis could not be involved in malaria transmission in Myanmar.

In the forested mountainous border area between Myanmar and Thailand, An. nivipes was observed to be a secondary vector of malaria (Ralph Harbach, personal communication). In the DMR studies, at both Tha-bye-wa and Mudon, An. nivipes was predominantly zoophilic and it is assumed that in the presence of cattle, this species could not play a role in malaria transmission in Myanmar.

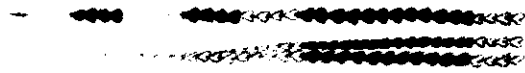
### Toxonomic Discussion

The differentiation of An. philippinensis and An. nivipes was carried out according to Reid(4 & 7). The results of our studies showed some difference with the findings of Reid. The main difference was that we could easily identify An. nivipes accordingly from the larval skin slides, whereas Reid(7) stated that pupal skin characters were more diagnostic. In the Myanmar specimens, we found that the pupal characters such as the refractile border

Figure 3.

A. philippinensis

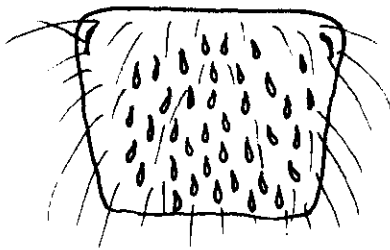
An. nivipes



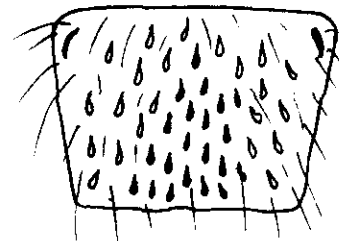
(a)



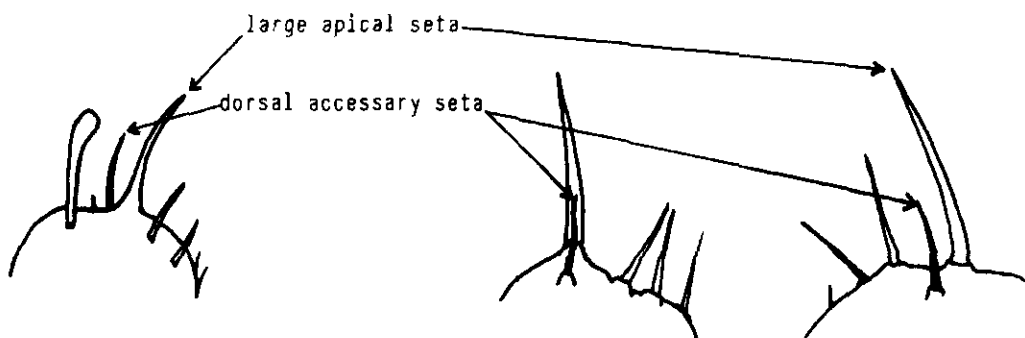
(b)



(c)



(d)



(External)

(in line)

(internal)

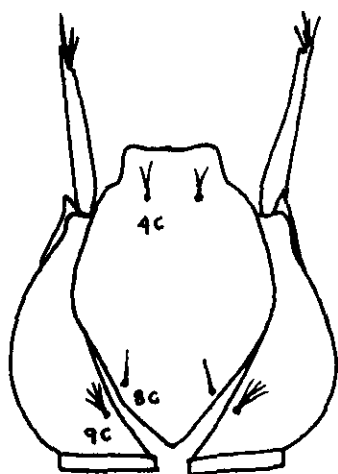
(e)

(f)

ADULT MORPHOLOGY

Figure 4.

An. philippinensis

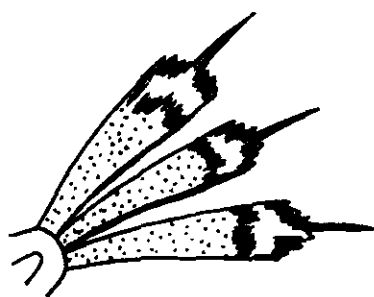


(a)

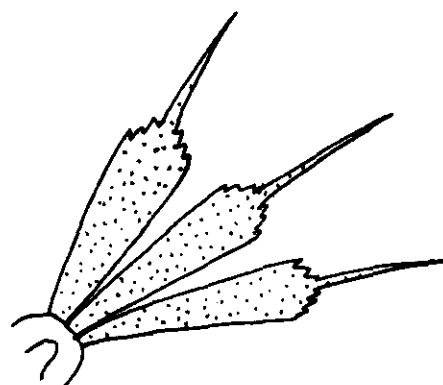
An. nivipes



(b)



(c)



(d)

length to total paddle length index and fringe teeth number were inconsistent. We could also differentiate the larval skin characters from freshly shed skins placed in a drop of water under cover slip, thus, developing a rapid method of differentiation. The adult male characters were found to be consistent and reliable.

#### Adult male characters

Figure (3a) shows the wing of An. philippinensis male with basal extension of dark mark on Vein R ending at the level of Presector pale (Psp) on costa.

Figure (3b) shows the wing of An. nivipes male with the basal extension of dark mark on Vein R extending basally to the level of Humeral dark (Hd) on costa.

Figure (3c) shows the An. philippinensis male sternite VIII (dorsal after rotation) with scales in the center of the segment all white, whereas in Figure (3d) An. nivipes sternite VIII the scales in the center of segment were dark.

Differences in the male genitalia between the two species was also noted and conformed to the findings of Reid(4). As described by Reid, the accessory seta of harpago was either in line or internal to the large apical seta in An. nivipes Figure (3f) and in An. philippinensis external to the large apical seta (Figure 3e).

#### Larval skin characters

In the Myanmar specimens that we examined, larval characters were found to be diagnostic and very consistent. Figure (4b) shows the cephalic hairs of the two species where nivipes was found to have more branches in 4,8,9c as compared to An. philippinensis Figure (4a). According to Figure (4c,d) the palmate hair in Abdominal

segment III-IV show marked difference between the two species. We could observe fresh skin in a drop of water on a slide and differentiate between nivipes and philippinensis by this character alone. From the colonies of nivipes and philippinensis, blind trials were tried on fresh larval skin identification with very accurate results.

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## Anopheline mosquitoes of Myanmar. IV.

### Anopheles (Cellia) annularis Van der Wulp, 1884

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Anopheles annularis, a very common anopheline mosquito in Myanmar, was studied in detail by survey teams of the Department of Medical Research. Field survey results were presented and compared with the findings of previous workers. Laboratory studies on DDT resistances, genetics of DDT resistance, comparison of two strains and laboratory colonization were described and the results presented.

#### INTRODUCTION

Although not important as malaria vector of malaria, like Anopheles dirus and Anopheles minimus, Anopheles annularis could be assumed to be a secondary vector of local importance. The Department of Medical Research (DMR) studied this species in detail due to its very significant difference in DDT susceptibility levels in different parts of Myanmar. This paper presents the results of DMR survey teams on the field biology of An. annularis as well as on the detail laboratory studies carried out in the Medical Entomology Research Division laboratories.

#### DISTRIBUTION

Khin Maung Kyi (1) stated that Anopheles annularis has a very wide distribution in Myanmar being recorded from all four corners of the country. Citing previous workers in Myanmar, Khin Maung Kyi(1) presented the distribution in Figure (1) which showed that it is recorded from all 14 states and divisions of the country. The sites where An. annularis was collected by DMR survey teams were presented in Table (1) and Figure (2).

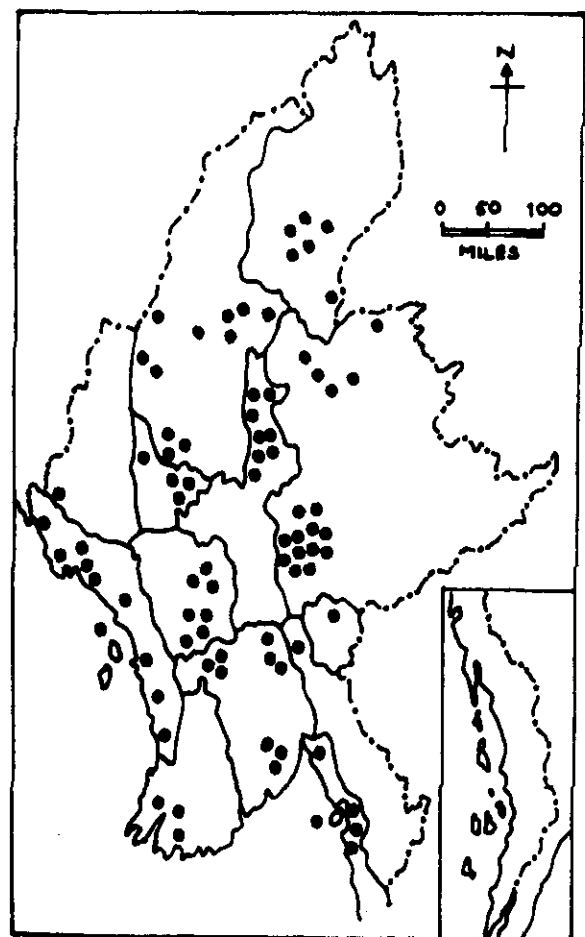


Figure 1. An. annularis distribution by Khin Maung Kyi

Table 1. Distribution of An annularis as recorded by DMR teams

| Locality                                            | Township          | Season/Time                                      | Remarks about locality                                                                                            |
|-----------------------------------------------------|-------------------|--------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <b><u>YANGON DIVISION</u></b>                       |                   |                                                  |                                                                                                                   |
| Thiri Avenue, 7 mile<br>Prime Road                  | Mayangon          | June                                             | High-class residential area, $\frac{1}{2}$ mile from Inya lake.                                                   |
| Nga-dat-kyi Pagoda<br>Road                          | Bahan             | January                                          | Many small ponds and streams blocked to store water for gardening. Residential area.                              |
| Aye-yar 15th street                                 | North<br>Okkalapa | January                                          | Near paddy field & small ponds. Caught while Prof: Izo Kanda demonstrated the use of big-bed nets by cattle bait. |
| Insein Strand Road                                  | Insein            | September                                        | Collection site in cattle shed near a pond. Semi-urban, lower middle-class residential area.                      |
| Ye-sit-kan                                          | Taik-kyi          | June                                             | Reservoir for Yangon City water supply.                                                                           |
| Ye-net-gyi<br>Road Construction<br>Camp, Phaung-gyi | Mlegu             | June &<br>January                                | Near forest and with ponds in the vicinity where many <u>annularis</u> larvae were collected.                     |
| <b><u>BAGO DIVISION</u></b>                         |                   |                                                  |                                                                                                                   |
| Hle-lan-gu village                                  | Thayarwady        | July                                             | Near scrub jungle with paddy fields nearby.                                                                       |
| Thayarwady Town                                     | Thayarwady        | August                                           | Caught at edge of town, about 200 yds from paddy fields. Adults collected from cattle sheds.                      |
| Waing & Kaing-daw-su<br>villages                    | Ok-po             | November                                         | Villages situated between paddy fields and streams near foot-hills.                                               |
| Ye-bya-timber<br>Extraction camp                    | Ok-po             | November                                         | Deep forest timber extraction camp on Western slope of Bago Yoma. Many streams in the area.                       |
| Myo-chaung village                                  | Kyauk-ta-ga       | Monsoon &<br>immediate<br>post monsoon<br>months | Foot-hill village with paddy field on one side.                                                                   |
| Ngok-to & Gwe-gone<br>villages                      | Kyauk-ta-ga       | Monsoon &<br>immediate<br>post monsoon<br>months | Foot-hill villages with paddy field on one side                                                                   |
| Bwe-chin village                                    | Kyauk-ta-ga       | Monsoon &<br>immediate<br>post monsoon<br>months | In paddy cultivating plain area                                                                                   |
| Tha-bye-wa village                                  | Oktwin            | July                                             | Deep forest area of Bago Yoma foot-hills                                                                          |
| Hsa-chaung village                                  | Letpadan          | July                                             | Near foot-hill and with light scrub jungle                                                                        |
| Khin-tan village                                    | Pyu               | August                                           | Foot-hill area with paddy fields nearby                                                                           |
| <b><u>MANDALAY DIVISION</u></b>                     |                   |                                                  |                                                                                                                   |
| Moe-swe village                                     | Pyinmana          | November                                         | Timber extraction area                                                                                            |
| Se-daw-gyi                                          | Madaya            | September                                        | Irrigation scheme near foot-hills.                                                                                |
| Wet-wun & Nyaung-tha-<br>yar & Myaing-gyi villages  | Maymyo            | July-September                                   | Plateau area over 3000 feet altitude. Farming area.                                                               |
| <b><u>RAKHINE STATE</u></b>                         |                   |                                                  |                                                                                                                   |
| Ka-thè-su Ors. Sittwe                               | Sittwe            | February                                         | Coastal urban area with many small ponds for growing water cress.                                                 |
| <b><u>MON STATE</u></b>                             |                   |                                                  |                                                                                                                   |
| Mudon Town                                          | Mudon             | July                                             | Urban ward of Mudon town.                                                                                         |

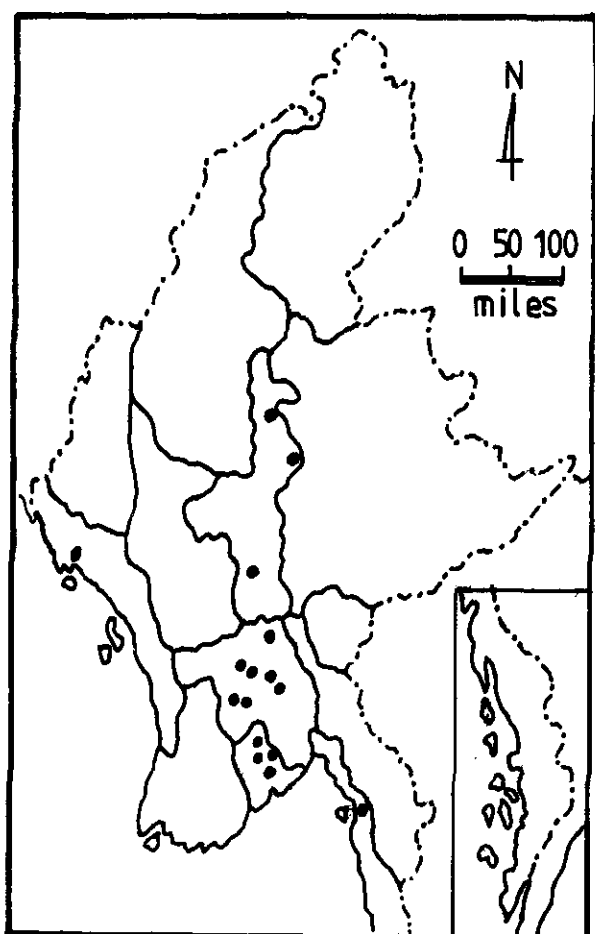


Figure 2. An. annularis distribution DMR

The distribution of An. annularis, as recorded by DMR teams, was significant in that this species was collected in Yangon City itself. One female was collected in Bahan Township at the author's compound on cattle bait. For confirmation of its identification, a colony was raised and identified from many  $F_1$  adult specimens. An. annularis was also collected from Thiri Avenue, 7th mile Prome Road where 2 persons were diagnosed by blood films to be having P. falciparum infection. If detail surveys were carried out in Yangon, especially in the suburban and satellite towns, An. annularis will definitely be collected in significant numbers. The only place where DMR survey teams did not record An. annularis was in the timber extraction

camps of Kawthaung Township. It can be assumed that An. annularis is very widely distributed over the country and that it is found in towns, especially near ponds, swamps etc.

Anopheles annularis is also collected from different ecological areas of the country such as (a) paddy cultivating plain areas (b) forested foot-hill areas of Bago Division (c) plateau areas like Maymyo township which is about 3000 feet above sea-level and also (d) in suburban areas of Yangon City and other towns.

### BREEDING SOURCES

Breeding sources of An. annularis was described by Khin Maung Kyi(1) as mainly in collections of water with thick grassy edges or containing abundant stagnant aquatic vegetation in ponds, borrow-pits, tanks, swamps, stagnant drains and in rice fields. Khin Maung Kyi(1) also stated that in his experience, An. annularis is most abundant in weed-choked ponds and less intensively in growing rice fields.

Survey teams from DMR found that An. annularis breeding was closely associated to paddy cultivation in the plain areas as observed in Bwe-chin village, Kyauk-ta-gar Township where the density of this species is directly related to paddy cultivation. In many areas, An. annularis was found breeding in small ponds near paddy fields and also in small ponds and swamps near villages. In Yangon City limits, it was assumed to breed in small ponds and in small streams blocked by gardeners for watering.

In Sittwe Town, the main breeding source of An. annularis was found to be small ponds used for growing water cress for human consumption.

In the paddy fields and small ponds nearby, it was found breeding mainly in association with An. philippinensis and An. vagus.

## SEASONAL PREVALENCE

In the rural paddy cultivating plain areas, Anopheles annularis breeding starts to build up about end of July or August when paddy transplantation was completed. Peak densities were observed about the middle of September to October when the growing paddy plants offer good shade for the larvae. In Bwe-chin, An. annularis larvae could be collected easily from paddy fields in September. Adult density starts to rise at Bwe-chin by July and peak density was obtained in September. During September 1984, a 20 minute biting catch in a cattle shed at Bwe-chin yielded more than 30 fully blood fed An. annularis. Similar high densities were also recorded in Myaing-gyi, Maymyo Township and Hsa-chaung, Letpadan Township by cattle baits. In the paddy cultivating areas, An. annularis density decreased when the fields dry up and after harvesting time An. annularis became scarce.

In foot-hill villages and in sub-urban areas where ponds and blocked streams are the breeding sources, An. annularis could still be collected up to January though in smaller numbers than in the rainy season. In general, An. annularis could be recorded in large numbers mainly during the late monsoon months of September and October.

### Host preference

Host preference of Anopheles annularis was clearly observed during surveys by DMR teams in various parts of the country. At Bwe-chin village, Kyauk-ta-gar Township, during September 1984, all night human bait catches yielded very small number of An. annularis whereas in cattle sheds, this species could be collected large in numbers biting cattle. At Thayarwady and Sittwe Towns, An. annularis was collected mainly resting in the cattle

sheds. During our surveys, it was observed that An. annularis is predominantly a cattle feeder but will also feed on man if its preferred host is not available as evident by its collection from human baited big bed nets. Khin Maung Kyi(1) stated that An. annularis is a zoophilic mosquito under normal circumstances and that if cattles are not available will readily divert to man. Khin Maung Kyi also described that at Kwan-ka-thaung, Mudon where, due to scarcity of cattle, it was deviated to man.

## BITING TIME

Anopheles annularis was observed to bite mainly in the first half of the night. At Myo-chaung, Kyauk-ta-gar Township, one An. annularis was caught biting the DMR survey team leader at about 1830 hours indoors. With cattle baits, An. annularis was collected in large numbers before 2200 hrs..

### DDT resistance

Anopheles annularis of Sittwe Town was found to be highly resistant to DDT by Saw Marcus Winn (personal communications). Susceptibility tests carried out at DMR on An. annularis from Thayarwady showed this strain to be very susceptible to DDT. The two strains of An. annularis, possessing different susceptibility status to DDT, were hybridized and the polytene chromosomes studied in detail by Myat Myat Thu et al.(2). The results of hybridization showed that the two strains are genetically identical. The polytene chromosome results however showed rich polymorphism in both the strains. The DDT resistance was found to be of incomplete dominant type and the gene for resistance was found to be monofactorial and not sex-linked.

### Susceptibility to *Bacillus sphaericus*

Susceptibility of *Anopheles annularis* larvae to *Bacillus sphaericus* was studied at DMR and reported by Tin Tin Hlaing(3). The LC<sub>50</sub> & LC<sub>90</sub> of *An. annularis* to strain 2362 was found to be 1.8 ppm & 9.8 ppm respectively for 24 hours exposure. For 48 hours exposure, the LC<sub>50</sub> & LC<sub>90</sub> were 0.44 ppm & 4.4 ppm respectively. With *B. sphaericus* strain 2297 the LC<sub>50</sub> & LC<sub>90</sub> for 24 hours exposure was 4.0 ppm & 20.2 ppm and for 48 hours exposure 3.2 ppm & 11.8 ppm respectively.

### Colonisation & biology under laboratory conditions

Laboratory colonisation of *An. annularis* was achieved easily at DMR by induced mating. Two strains of *An. annularis*, DDT-resistant strain from Sittwe and DDT susceptible strain from Thayarwady, were maintained in the laboratory. The biology of *An. annularis* under laboratory conditions was studied in detail and reported by Su Nyunt Co(4).

### Relationship to malaria transmission

Khin Maung Kyi (1) stated that according to dissection records malaria sporozoite in *An. annularis* was recorded in Myanmar only prior to 1930. Subsequent dissection by Khin Maung Kyi and his staff showed no positive results, although epidemiologically *An. annularis* could be assumed to be a vector in Sittwe. In the other areas surveyed by DMR teams all dissection results on *An. annularis* proved negative. Khin Maung Kyi(1)

described that during the cyclone disaster in Rakhine State in August-September 1968, severe malaria epidemic occurred and during this time *An. annularis* was found to breed in profusion around villages and also found resting in human habitations. From epidemiological and parasitological evidences, Khin Maung Kyi(1) assumed this species to be responsible for malaria epidemics in Myohaung and Myinbya areas during that time.

From our observations *An. annularis* could not be considered an important vector due to its strong zoophilic habits. However, in special situations it can cause transmission in localised areas under favourable conditions.

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**Morphological characters for quick identification of  
Anopheles (Cellia) culicifacies Giles, 1901 and  
its seasonal prevalence observed some areas**

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Anopheles (Cellia) culicifacies Giles, 1901 has a very wide distribution in the Union of Myanmar. Khin Maung Kyi(1) stated that although not a major vector of Malaria in the Union of Myanmar, this species has a considerable secondary importance, whenever present, in hyperendemic foot-hill areas and also presented dissection records on An. culicifacies carried out in this country. Although Harrison(2) described An. culicifacies as the easiest to identify amongst the Myzomyia series of Anopheles (Cellia) in Thailand, we felt that important characters necessary for quick and reliable identification of this species should be studied in the country. From wild caught female An. culicifacies collected from Tha-bye-wa, Oktwin, Myaing-gyi village, Maymyo and Se-daw-gyi, Madaya, iso-female lines were raised and  $F_1$  adults examined. All the iso-female lines were confirmed as An. culicifacies by larval and pupal skin morphology. A total of over 200 An. culicifacies females were examined.

Characters for identification of female adults

1. Harrison and Scanlon(3) in their identification key, stated that in An. culicifacies, the preapical dark band of palpi was much longer than the apical pale band. In our observations, the preapical dark band was found to be between 2 to 4 times that of the apical pale band

(but mostly averaging about, 3 times). This character is necessary to differentiate from minimus group where some morphological variants have preapical dark bands slightly broader than the apical pale bands. We also noticed that in the specimens that we examined, the preapical pale band was narrower than the preapical pale bands of An. minimus (Illustrated in Figure (1A)).

2. The wing characters of An. culicifacies was quite constant except for the pale interruptions in the basal 1/3 of costa. The important points to note in examining the wings are as follows:
- (a) a pale spot at the fork of Vein  $R_2$  &  $R_3$
  - (b)  $R_{4+5}$  mostly dark except for small pale spots at apex and base
  - (c) a pale spot at fork of Vein  $M_{1+2}$  &  $M_{3+4}$
  - (d)  $Cu_1$  mostly dark except for a small pale spot near m-cu cross vein and at the apex.
  - (e) basal pale spots on Cu & 1A large and lie on the same plane.

In our specimens we found that there is either one large pale interruption or one large and one small pale interruption in the basal 1/3 of costa.

Wing vein numbering was according to Harrison and Scanlon(3) and the wings characters are illustrated in Figure (1B).

A

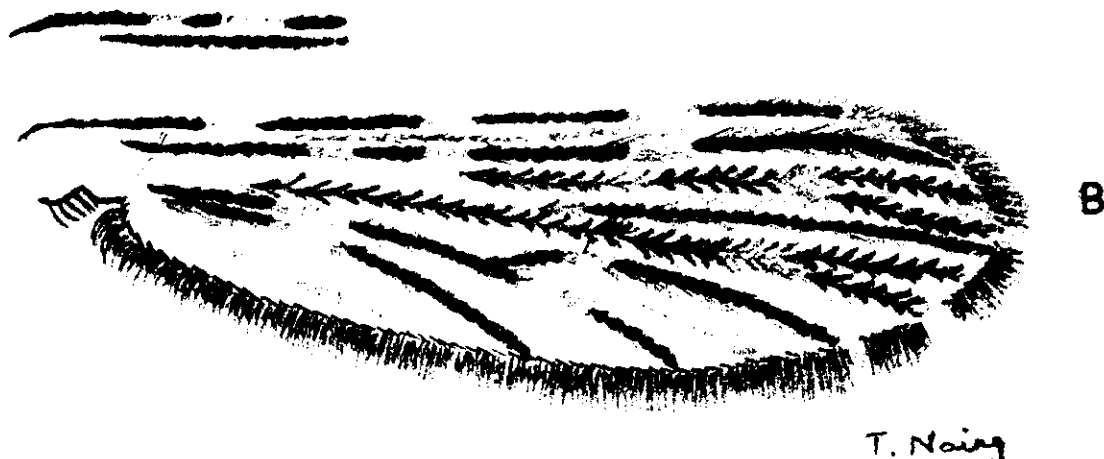


Figure 1. An. (Cellia) culicifacies (A) ♀ palpi (B) wing

#### Seasonal prevalence and breeding sources

Unlike the findings of Khin Maung Kyi(1), we observed that at Tha-bye-wa, Oktwin Township, Bago Division, An. culicifacies was collected in high numbers during January and February. From larval surveys, many culicifacies larvae were collected from pools along the sand banks of Tha-bye-chaung and Kha-baung-chaung.

In Se-daw-gyi, Madaya, An. culicifacies was collected mostly during the non-monsoon month from October to December. Larvae were still collected in large numbers from a stream in June 1989.

In Myaing-gyi, Maymyo, An. culicifacies was collected in large numbers in the immediate post-monsoon months but appreciable numbers were still collected during June 1989. Breeding sources in this area is presumed to be small streams.

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## Intramuscular quinine kinetics in Myanmar children with Falciparum Malaria

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### INTRODUCTION

With the global upsurge of malaria, children are the most vulnerable group for the malady, especially the severe form. Quinine is still effective for severe falciparum malaria, resistant to chloroquine. The most appropriate form employed currently is an intravenous infusion, due to its reliable bioavailability, being painless and hardly any danger of tissue damage. Nevertheless, it is expensive and requires skill, factors less favourable in many countries, including Myanmar. Even though cheaper and easier to use, the intramuscular form has been reported to fall short of the desired characteristics of its intravenous counterpart(1). The present study, therefore, aimed at seeing to the suitable form of intramuscular quinine in Myanmar children and noting the drug pharmacokinetics.

### METHODS

Among falciparum malaria patients attending the Thayarwady Civil Hospital during 1988 malaria season, 10 children age of 6-12 years weight of 16-44 kg were studied. Half of the patients were highly parasitised (more than 5% of their erythrocytes parasitised). Intramuscular quinine dihydrochloride in 1.5% benzyl alcohol (prepared specially by the Myanmar Pharmaceutical Industry) was used. Quinine-free urine

of the subject was a prerequisite inclusion criterion of selection, and the urinary content was determined using the Mayer Tunret reagent test.

Regimen employed was an initial loading intramuscular dose of 20mg/kg body weight, followed at 8-hourly intervals by 10 mg/kg body weight for total of 3 doses. Then oral quinine sulphate 10 mg/kg t.d.s. was maintained till day 7.

Clinical data were recorded 6-hourly throughout the 7-day hospital stay for blood film for parasites, blood pressure, pulse rate, temperature and signs and symptoms of toxicity. ECG recordings were done immediately and at the day 3, day 7 and day 14. To those having unsatisfactory responses were then switched over to a regimen of oral mefloquine with secured effectiveness.

Blood samples for plasma quinine were collected at various time intervals: 0, 1, 2, 4, 6, 8, 12, 24 hours on day 1, and at day 2, 3, 4 and 7. Plasma was separated immediately and stored at minus 20 degree centigrade until assayed by the Benzene extraction fluorescence method(2), using the Turner Filter Fluorometer Model III (Sequoia-Turner Corporation). Plasma quinine concentration until 6 hours after the initial loading dose were analysed for single dose pharmacokinetics.

### RESULTS

Among the 10 children, 8 responded well to the treatment. In 9 out of 10

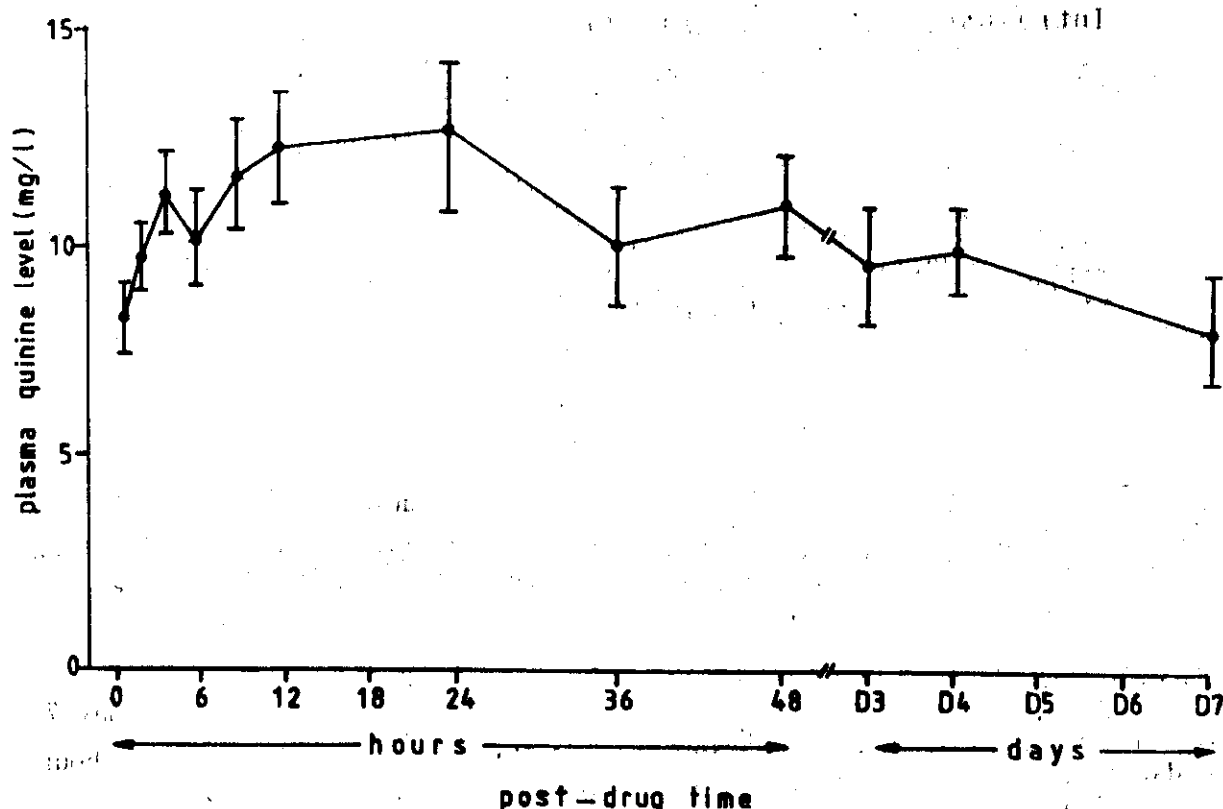


Figure 1. Plasma quinine level in children patients treated with intramuscular quinine. Each point represents the mean of 10 subjects. Vertical bars indicate respective standard errors of the means.

| Parameters                                        | Data                        |
|---------------------------------------------------|-----------------------------|
| Plasma half-life ( $t_{1/2}$ )                    | $5.7 \pm 0.4$ hr            |
| Concentration at zero hour ( $C_0$ )              | $18.7 \pm 1.4$ mg/l         |
| Maximum concentration ( $C_{max}$ )               | $12.5 \pm 0.8$ mg/l         |
| Time to reach maximum concentration ( $T_{max}$ ) | $3.2 \pm 0.3$ hr            |
| Elimination rate constant ( $K_{el}$ )            | $0.13 \pm 0.04$ hr $^{-1}$  |
| Apparent volume of distribution (aVd)             | $1.1 \pm 0.7$ l/hr          |
| Systemic clearance (Cl)                           | $2.3 \pm 0.2$ ml/min/kg     |
| Area under the curve ( $AUC_{0-6}$ )              | $9.2 \pm 0.7$ mg/ml x hours |

Table 1. The pharmacokinetic parameters of single intramuscular quinine 20mg/kg in Myanmar children

children quinine level reached above the minimum inhibitory concentration (MIC) level of 5.7 mg/l (Myint-Lwin: Personal communication) at the first hour.

The mean quinine concentrations of the subjects tested is shown in Figure 1, and major pharmacokinetic data are depicted in Table 1.

## DISCUSSION

The modified intramuscular form of quinine dihydrochloride (i.e. in 1.5% benzyl alcohol) was well tolerated by the patients and did not produce any

tissue necrosis. From the pharmacokinetic findings, it possessed therapeutic characteristics, namely, the MIC in children, comparable to that of the intravenous(3). Even though the drug had a shorter half-life in Myanmar children than those from Papua New Guinea being 8.3 hours(4), the maximum drug level achieved could have exceeded the latter, being 12.5 mg/l. These factors indicated that the present drug schedule, where quinine is given at 8 hour interval is sufficient in terms of blood level to get the desired therapeutic effect. Assurance of the quinine bioavailability with a failure rate of 20% found in the study is comparable to its intravenous counterpart(3).

Therefore, the drug form developed is most desirable to be employed, with an added advantage of easier application.

#### ACKNOWLEDGEMENTS

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Pharmaceutical Industry for supplying the study with the modified preparation of quinine dihydrochloride injection.

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## Immune mechanisms in mouse malaria (2):

### A correlation of survival periods and cell mediated immune responses

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The survival period of BALB/c nu/nu mice after *Plasmodium berghei* NK65 infection was significantly longer than the BALB/c nu/+ mice after an infection with the same parasite. No such difference was found after infection with *Plasmodium yoelii* 17X. The difference in the survival periods was not due to the difference in parasitaemia level. Studies of several immunological parameters revealed that the mice that survived longer showed an increased ability to generate interleukin-2 (IL2) and cytotoxic T lymphocytes (CTL) from their spleen cells. We had reported that the survival period had no relationship with the amount of antimalarial antibody production nor the formation of immune complexes(1). T cell functions were found to be generally decreased and natural killer (NK) cell activity was raised in all the mice regardless of their different survival periods. Our experiments suggested the contribution of IL2 in the clinical outcome of a malarial infection.

## INTRODUCTION

Different species of rodent malaria parasites induce different immunological reactions in the host. The resulting pathological effects range from mild self-limiting diseases to rapidly fatal complicated infections. There are ample evidences to show that after infection with some species of malaria, hosts with T cell dysfunction can survive longer than hosts with normal T cell functions (2,3,4,5,6). Despite the postulations based on the permeability changes(7), rheologic alterations(8) and cytoadherence phenomenon(9) immunological processes seem to determine the final outcome of the disease process

(10). Three most serious complications of human malaria are thought to have an immunological basis: anaemia, glomerulonephritis and cerebral malaria (11). We have been trying to test various immunological parameters of mouse malaria in correlation with their survival period which is used as an indication of the severity of the disease. We have reported that the specific antibodies, both IgG and IgM, and circulating immune complexes have no relationship with the severity of malaria(1). This report is to present our additional findings on the cell mediated immunological parameters and their influence on the severity.

## MATERIALS AND METHODS

BALB/c nu/nu mice were inoculated intravenously through the tail vein with  $10^7$  red blood cells infected with Plasmodium yoelii 17X(lethal) or Plasmodium berghei NK65. Five mice from each group were observed for their survival period after inoculation. Tail blood smears were prepared everyday and stained with Giemsa stain to count the percentage of infected red cells.

### Collection of spleen cells

The remaining mice were used for cellular immunological tests. They were sacrificed by cervical dislocation to collect the spleen cells. The spleen was removed aseptically, minced and passed gently through a fine mesh stainless steel sieve into PBS. It was centrifuged at 1500 rpm for 5 min and the cell pellet was resuspended in RPMI. Cell viability was assessed by the trypan blue dye exclusion method.

### Mitogen stimulation and mixed lymphocyte reaction

For mitogen stimulation and mixed lymphocyte reaction (MLR), 3 mice from each group were sacrificed on the day-4. Blastogenic response was measured by the incorporation of  $^3\text{H}$ -thymidine into nuclear DNA of the cultured cells using 96 well microtitre plates. The mitogen reactivity was determined by culturing the spleen cells in the presence of 25 ug/ml of phytohaemagglutinin (PHA) or 5 ug/ml of concanavalin A (Con A) or 25 ug/ml of lipopolysaccharide (LPS). MLR was performed in the same way using mitomycin C-treated stimulator cells of (C57BL/6J(H-2<sup>b</sup>) or C3H(H-2<sup>k</sup>) mice. BALB/c spleen cells were used as a self stimulating control. The same stimulator cells were used for cytotoxic T lymphocyte(CTL) induction.

### Cytotoxic T lymphocyte

For CTL induction, 3 mice from each group were sacrificed on the day-7 to get the responder cells. After 5 days culture with the mitomycin C-treated cells(see above), the stimulated cells were cocultured with the target cells EL4(H-2<sup>b</sup>), X5563(H-2<sup>k</sup>) and P815(H-2<sup>d</sup>) respectively at different ratios of concentrations. Percentage cytotoxicity was determined using the  $^{51}\text{Cr}$  chromium release assay method.

### Interleukin-2(IL2) assay

To induce IL2 production, 6 mice on each group were sacrificed on the day-4 and the spleen cells were stimulated with Con A for 24 hours. The culture supernatant was assayed for the presence of IL2 activity by measuring its ability in a gradient of concentrations to support the growth of an IL2 dependent cell line, CTLL-2(12). Tritiated thymidine uptake by CTLL-2 cell is determined as an index of IL2 activity.

### Natural killer cell activity

Four mice from each group were sacrificed on the day-3 and the spleen cells from each group were pooled. For an enrichment of natural killer(NK) activity, the pooled spleen cells were passed through a nylon wool column to remove B cells. The resulting enriched effector cells were cocultured with different proportions of target YAC-1 cell. Percentage  $^{51}\text{Cr}$  cytotoxicity was measured using the  $^{51}\text{Cr}$  chromium release assay method.

### Calculations

All the tests were done in triplicate wells and the mean of three results were used. The mean of all the results from each group was shown in

the illustrations. For mitogen stimulation and MLR, net count per minute (cpm) was calculated by subtracting the background count, which is from the triplicate culture without any stimulation. For CTL induction and NK activities, percentage cytotoxicity is calculated by using the detergent-lysed wells as the maximum and cultures without effector cells as the minimum values of cell lysis. Student's t test for unpaired samples is used to determine a statistically significant difference.

## RESULTS

Nude mice survived longer than nu/+ BALB/c mice after *Plasmodium berghei* infection ( $p < 0.001$ ), but no such difference was noted after *Plasmodium yoelii* infection. The early death of nu/+ mice after *P. berghei* infection was not attributable to the rapid increase in the parasitaemia level (Figure 1). Mitogenic and allogeneic stimulations generally elicited diminished responses which showed no correlation with the survival pattern (Figures 2 and 3).

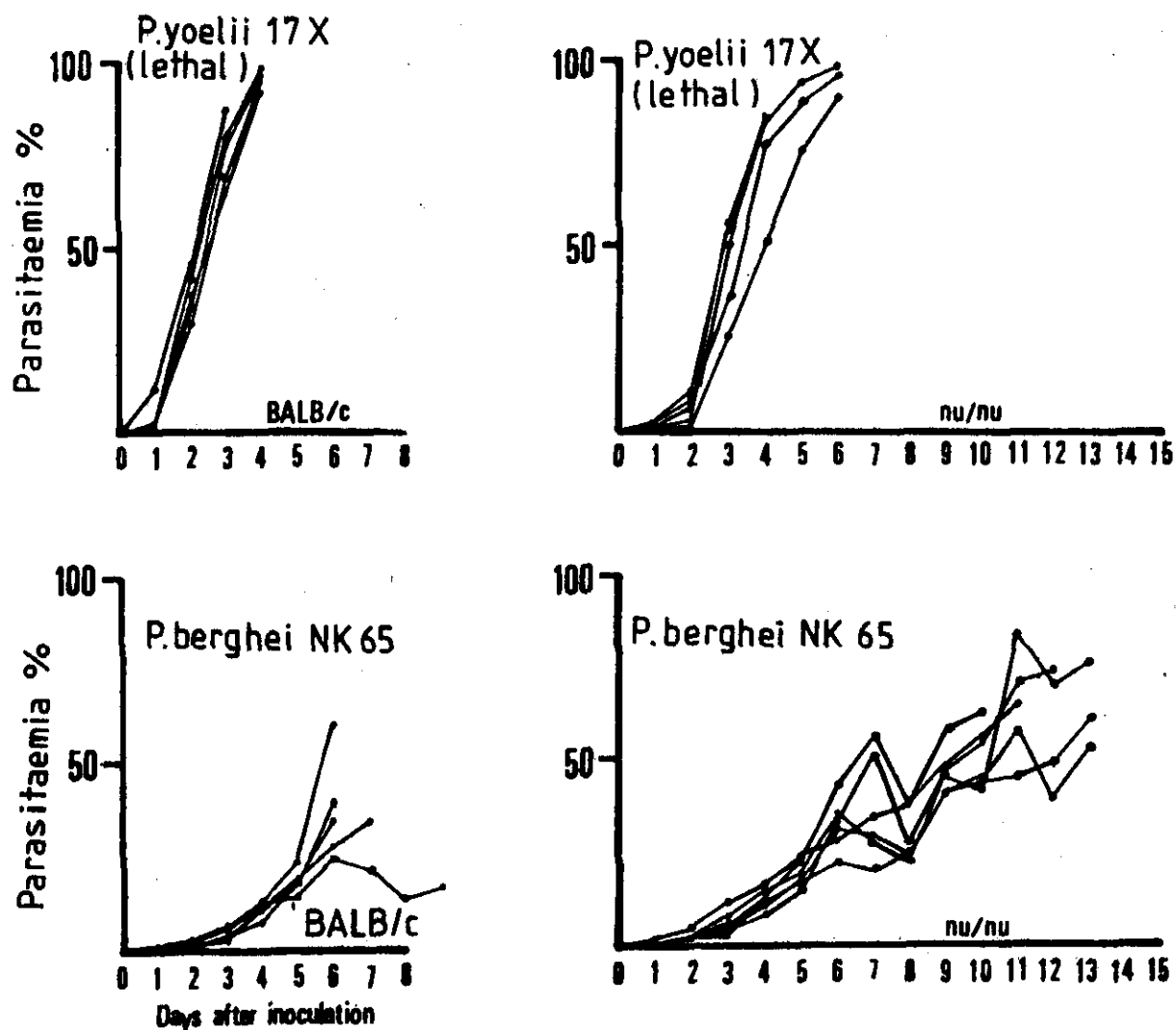
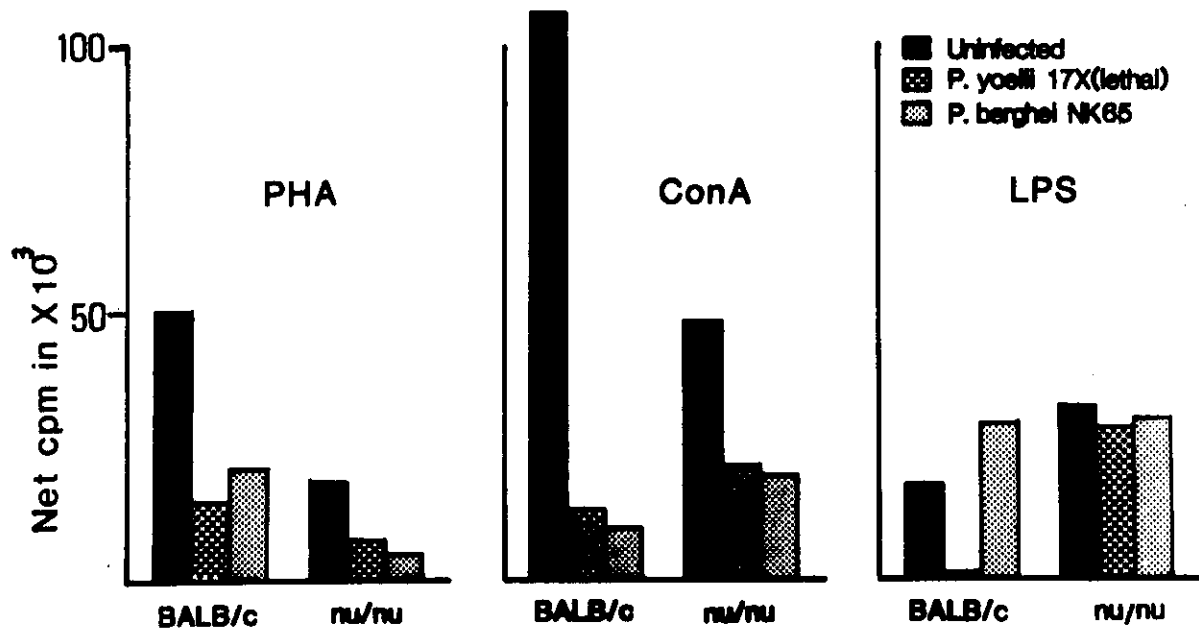


Figure 1. Parasitaemia levels and survival days of BALB/c nu/+ and nu/nu mice infected with *Plasmodium yoelii* or *Plasmodium berghei*

## MITOGEN STIMULATION



## MIXED LYMPHOCYTE CULTURE

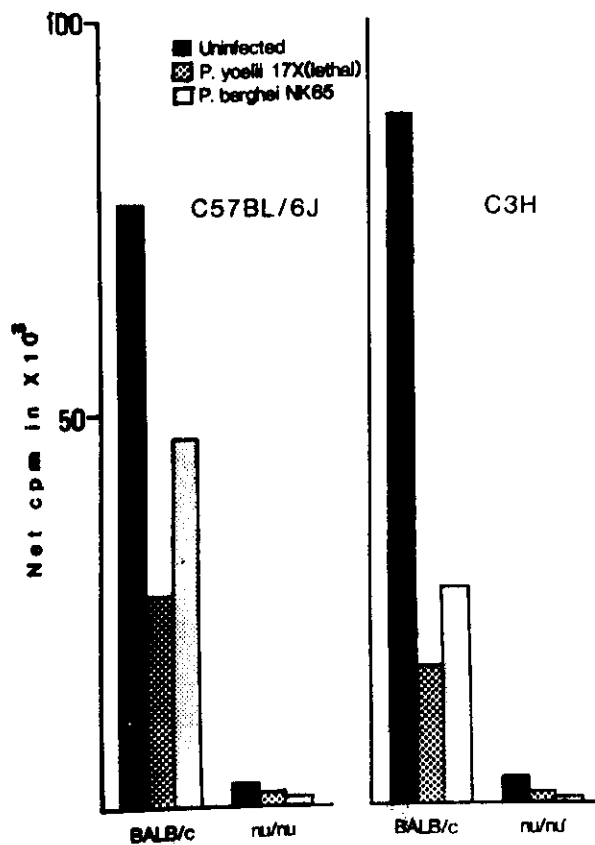


Fig. 2. Response to mitogens expressed as net count per minute (cpm) in the four groups of mice and uninfected controls. (Phytohaemagglutinin, PHA; concanavalin A, Con A; Lipopolysaccharide, LPS).

Fig. 3. Results of mixed lymphocyte reaction after C57BL/6J and C3H stimulations.

A mild reduction in the emergence of CTLs was observed in all the mice except Plasmodium berghei-infected nu/+ group where two mice against X5563 cells and one mice against EL4 cells showed increased levels of cytotoxicity, however the difference was not statistically significant (Figure 4). This specific group is the one with a markedly longer period of survival.

Fig. 4. Development of cytotoxic T lymphocytes (CTL) against H-2<sup>D</sup> (C57BL/6J) and H-2<sup>K</sup> (C3H) were shown as cytotoxicity to the target cells EL4(H-2<sup>D</sup>) [closed circles] and X5563 (H-2<sup>K</sup>) [open circles] respectively. Non-responsiveness to the control target cell P815 (H-2<sup>D</sup>) is not shown.

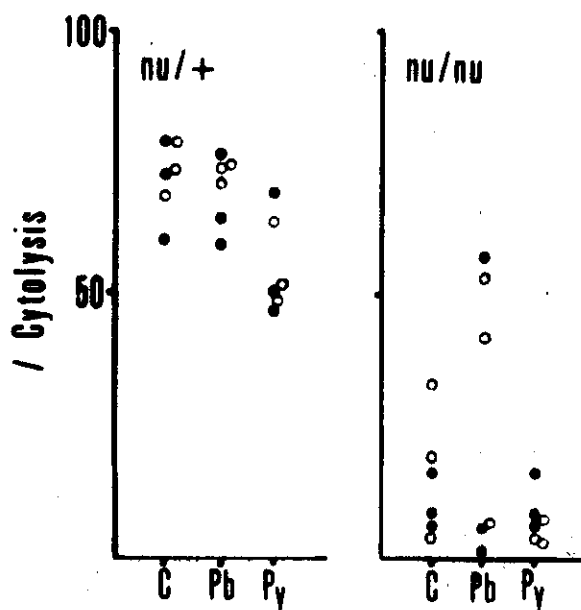
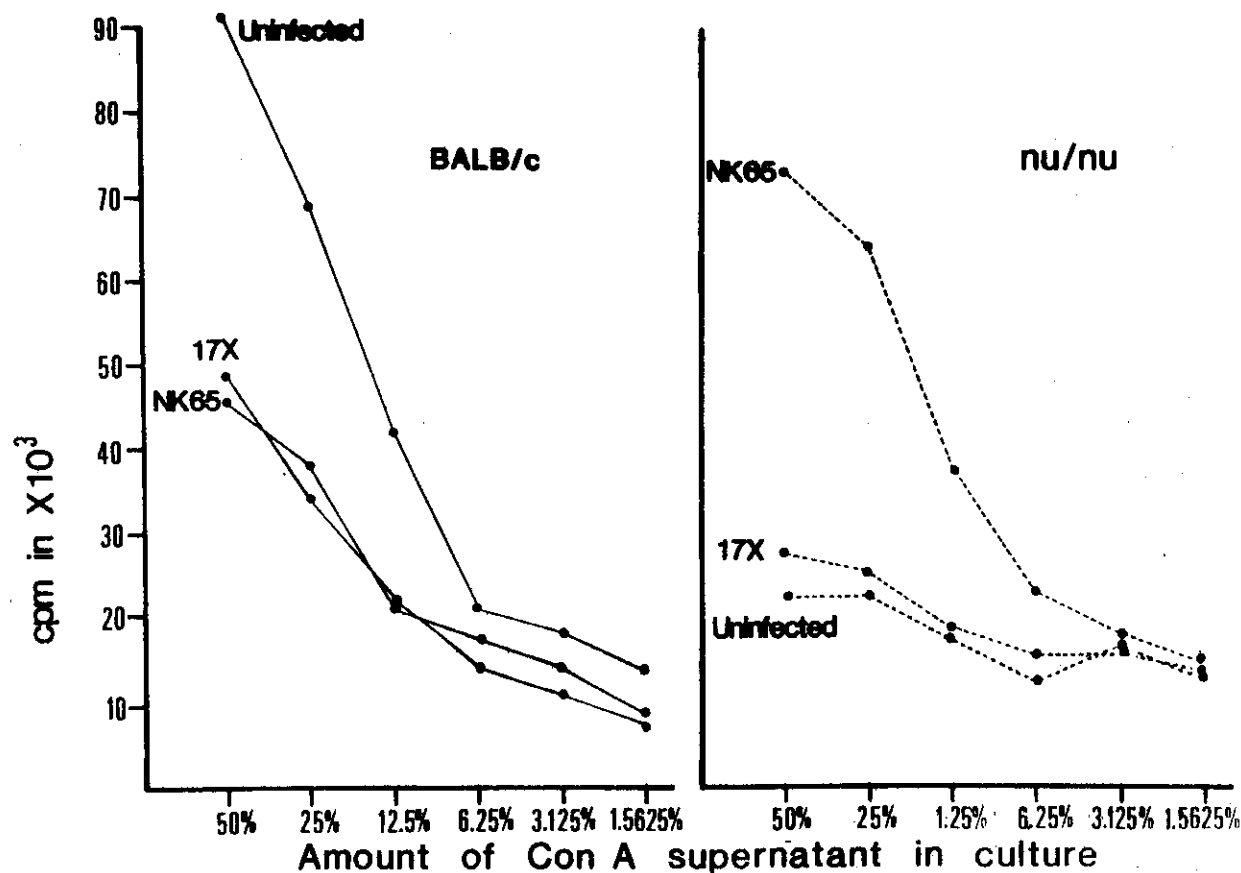


Figure 5. Concanavalin A induced IL2 production is demonstrated as the efficiency to support the growth of CTLL-2 cell line.

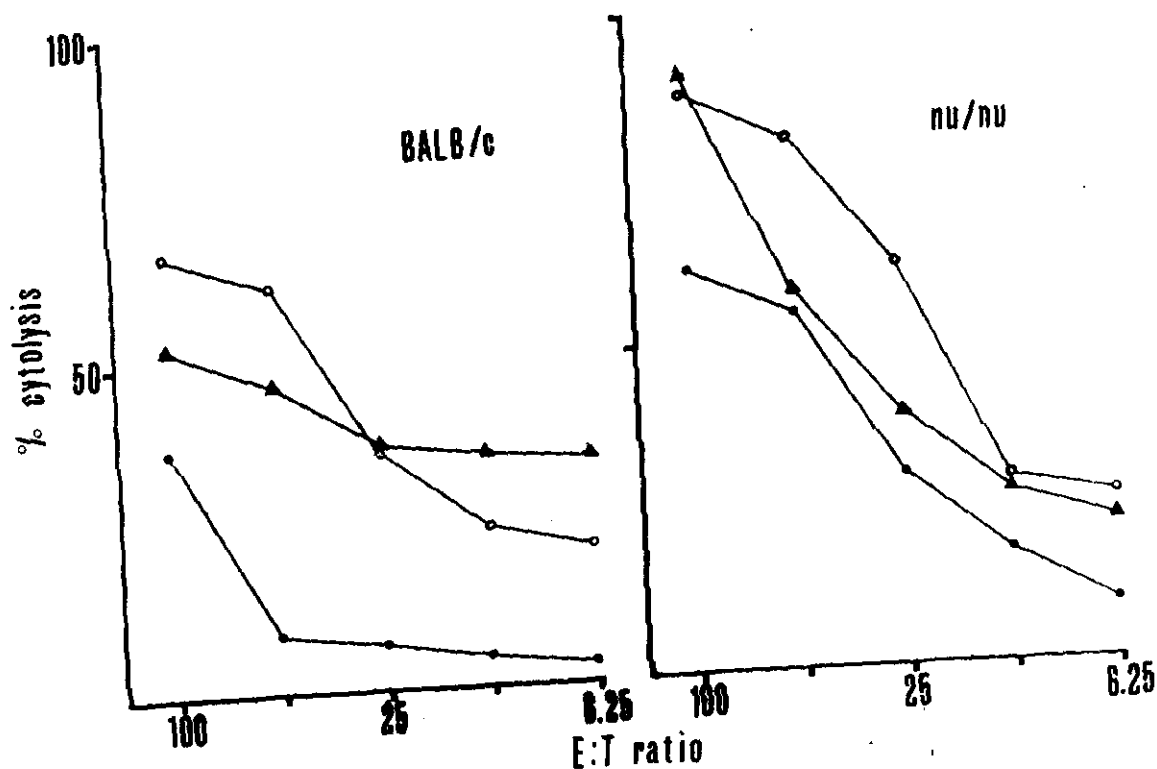


Figure 6. Natural killer cell activity is shown as the degree of cytotoxicity to the target cell line YAC-1 at various effector: target (E:T) ratios. Closed circles, uninfected group; open circles, *P. berghei* infected group; triangles, *P. yoelii* infected group.

In addition, Con A-induced IL2 production was also markedly raised in the same group whereas reduced levels were found in all other groups (Figure 5). NK cell activity was raised in all the groups without any difference between long-lived and short-lived groups (Figure 6).

Among the cell mediated immune parameters tested, IL2 productivity and CTL response seemed to have an association with the longer survival of the host (Figure 7).

### DISCUSSION

Many explanations and speculations had been put forward regarding the prolonged survival of T cell-deprived hosts after some types of malarial infections, but many of the hypotheses did not fit with our findings. The

results from our previous experiments (1) concerning circulating immune complexes and antimalarial antibodies were in contrast with the previous thinkings of immune complex-mediated injury(13,14,15) and formation of intra-vascular infected cell agglutinates due to T-dependent agglutinins in the T cell deprived hosts(4,5). The finding of reduced mitogenic and allogeneic responses agreed with the previous findings of immunosuppression(16,17).

Nude mice, having a higher NK activity(18), were presumed to have a resistance to malaria infection. A correlation had been demonstrated between the susceptibility to malaria and NK activity(19). However, in our experiment NK activity seemed to play no role in the resistance to malarial infection. The raised NK activity due to the drastic immunological stimulation by malaria had previously been

| SUMMARY                      |                      |       |                |       |
|------------------------------|----------------------|-------|----------------|-------|
|                              | P.yoelii 17X(lethal) |       | P.berghei NK65 |       |
|                              | BALB/c               | nu/nu | BALB/c         | nu/nu |
| Survival days                | -                    | -     | -              | ↑     |
| Parasitaemia                 | -                    | -     | -              | -     |
| Mixed lymphocyte culture     | ↓                    | -     | ↓              | -     |
| T cell mitogens              | ↓                    | ↓     | ↓              | ↓     |
| B cell mitogen               | ↓                    | -     | ↑              | -     |
| CTL induction                | ↓                    | ↓     | -              | ↑     |
| ConA induced IL2 production  | ↓                    | ↓     | -              | ↑     |
| Natural killer cell activity | ↑                    | ↑     | ↑              | ↑     |

Figure 7. Summary of the results on the cell mediated immune responses.

noted(20,21).

By contrast, the ability to produce IL2 after Con A stimulation was markedly increased in the group that survived longest. This group also gave more CTLs in response to both H-2<sup>b</sup> and H-2<sup>k</sup> antigens. Increased IL2 productivity may in some way confer a protection against the malignant pathological effects of *Plasmodium berghei* in nude mice. Raised CTL activity may be the result of high IL2 secretion because IL2 had been known to induce specific CTLs particularly in nude mice(22,23). The exact mechanism of how the high level of IL2 confers protection needs to be further investigated.

Macrophage overactivity in mouse malaria is well documented(24).

Increased interleukin-1 production by the activated macrophages resulting in an IL2 production and then subsequent increase in CTL formation is a possible sequence. It needs to be confirmed by measuring interleukin-1 production of the adherent cells.

Our results paved a way to further observations on the varying pathological effects of the different plasmodia on human patients. Falciparum malaria can cause cerebral complication and rapidly fatal outcome whereas vivax infection is benign and uncomplicated. It will be interesting to observe whether such a difference is associated with an inequality in IL2 productivity and/or CTL activity. The synergistic effect of different lymphokines particularly IL2 and tumour necrosis factor(TNF) on the

clinical outcome of malaria has recently been under extensive investigations(25).

Our finding of raised IL2 productivity is a further evidence of the presence of functional T cells in nude mice. Despite the well known fact of a lack of thymus, nude mice were found to exhibit some T cell functions if a sensitive assay method was used or after an intense immunological stimulus(26, 27,28).

In summary, our experiment excluded the possibility of an association between the severity of malaria and the the specific antibodies(IgG & IgM), immune complexes(1), NK activity and mitogenic as well as allogeneic T cell responses whereas an association with IL2 productivity and CTL response was demonstrated highlighting the importance of lymphokines in the immunopathogenesis of malaria (Figure 7).

#### ACKNOWLEDGEMENT

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**"NOTICE"**

Please read: "Myanmar" for "Burma" "Yangon" for  
"Rangoon" "Ziwaka road" for "Zafar Shah road"