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Hepatitis B markers in a study group with  
human immunodeficiency virus  
infection in Myanmar, 1989

*Than-Aung, Khin-Pyone-Kyi, Khin-Yi-Oo,  
Khin-Mya-Lwin & Soe-Lwin*

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During 1989, 200 sera, positive for Human Immunodeficiency Virus (HIV) antibody by both Enzyme Linked Immunosorbent Assay (ELISA) and Western Blot Immunoassay were selected randomly to be tested for Hepatitis B Virus (HBV) markers. 75% of the sera belonged to intravenous drug abusers with the remaining from hospitalised patients, blood donors and persons attending the Sexually Transmitted Disease (STD) Clinics. HBsAg was positive in 12.5%, HBeAg in 3%, anti-HBs in 34.5% and anti-HBc in 86% of the test group. Since the epidemiology of HIV and HBV infections are similar regarding the modes of transmission (parenteral, perinatal and sexual), the risk groups affected and the occurrence of chronic asymptomatic infection creating a reservoir for further spread, HIV infected persons can be assumed to have a high infection rate for HBV. In our study, the infection rate for HBV was very high being 92%.

#### INTRODUCTION

In Myanmar, the prevalence of Human Immunodeficiency Virus (HIV) infection in blood donors was 0.39% and the intravenous drug abusers (IVDA) comprised the major group of people affected by this infection with a 51.3% positivity rate (1). Hepatitis B Virus (HBV) infection is also highly endemic in our country with a Hepatitis B surface antigen (HBsAg) carrier rate of 9 - 11% in the general population (2) with an infection rate of 35% in the control group and 60% in health care personnel (3).

HIV and HBV infections share common epidemiological features, including modes of transmission, the risk groups affected and the occurrence of chronic asymptomatic infection resulting in an inapparent source of further spread.

Because of these similarities, the anti-HIV positive cases provide a useful group in which to study the spread of both infections. This study examines the HBV infection rate in persons with HIV infection.

#### MATERIALS AND METHODS

##### Study group

A total of 200 sera positive for HIV antibody by Enzyme Linked Immunosorbent Assay (ELISA) and Western Blot Immunoassay were selected randomly to be tested for HBV markers. The specimens were collected during 1989 from patients in various hospitals and Sexually Transmitted Disease (STD) Clinics in Yangon, IVDAs from Insein Jail, Psychiatric Hospital, Wet-Htee-Kan (Pyay) and blood donors from Central National Blood Bank.

### Laboratory methods

The selected sera were screened for HBV markers to evaluate the infection rate by using Haemagglutination Test Kits from Green Cross Company (Japan). Reverse Passive Haemagglutination (RPHA) Kits were used to detect HBsAg (Anti-hebscell) and Hepatitis B(e) Antigen (HBeAG)(e-cell). Passive Haemagglutination (PHA) Kits were used to detect the antibodies, anti-HBs (Hebgenscell) and anti-HBc (Core-cell).

### RESULTS

Table 1 showed that of the 200

HIV antibody positive cases, there were 94 IVDAs of which 90.4% were also infected by HBV. Of 59 hospitalised cases, the majority being IVDAs, the infection rate for HBV was 94.9%. Patients and contacts from the STD Clinics comprised 20 in number with a 90% infection rate for HBV. Among the 27 blood donors, 92.6% had markers for HBV infection. The overall infection rate for HBV in HIV infected cases was 92%.

The prevalence of the four HBV markers in HIV infected cases was presented in Table 2. HBsAg was positive in 12.5%, HBeAg in 3%, Anti-HBs in 34.5%

Table 1. Infection rate of HBV in HIV antibody positive cases

| Sr.No. | Type of HIV antibody positive cases | Number tested |        | No.(+)for HBV markers | Infection rate for HBV (%) |
|--------|-------------------------------------|---------------|--------|-----------------------|----------------------------|
|        |                                     | male          | female |                       |                            |
| 1      | Intravenous drug abusers            | 94            |        | 85                    | 90.4                       |
| 2      | Hospitalised cases                  | 57            | 2      | 56                    | 94.9                       |
| 3      | STD patients and contacts           | 19            | 1      | 18                    | 90.0                       |
| 4      | Blood donors                        | 27            |        | 25                    | 92.6                       |
| Total  |                                     | 197           | 3      | 184                   | 92.0                       |

Table 2. Prevalence of HBV markers in HIV antibody positive cases

| Total tested | percentage % |       |          |          |                         |
|--------------|--------------|-------|----------|----------|-------------------------|
|              | HBsAg        | HBeAg | Anti-HBs | Anti-HBc | One or more HBV markers |
| 200          | 12.5         | 3     | 34.5     | 86       | 92                      |

and Anti-HBc in 86%.

Figure 1 illustrated the age/sex specific prevalence of HBV markers in the HIV infected cases, the majority (86%) being in the age range of 20-34 years. The peak incidence (30%) was within 30-34 years. Of the 200 cases in this study, only 3 were females and all 3 of them had HBV markers.

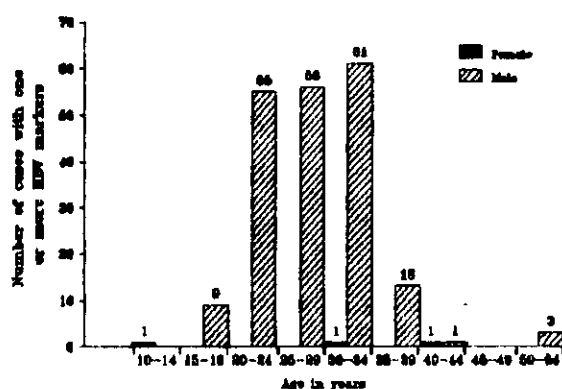


Figure 1. Sex and age specific prevalence of HBV markers in HIV-antibody positive cases

The four HBV markers presenting alone or in combination with one or more of the remaining markers were shown in Table 3. Both HBsAg and anti-HBc were present in 9.5% of cases indicating acute or chronic infection or a carrier state. In addition to these markers, the presence of HBeAg marked an increase infectivity as seen in 3% of the cases. When anti-HBc alone was detected as in 45% of cases, it could be one of the two stages - either during active infection (when HBsAg declined but before the appearance of anti-HBs, the

window period) or recovery after anti-HBs declined. Presence of anti-HBs alone (6%) or in combination with Anti-HBc (28.5%) signified the stage of recovery. No markers were detected in 8% of cases indicating susceptibility to HBV infection.

Table 3. Hepatitis B markers presenting alone or in combination in 200 HIV antibody positive cases

| Sr. No. | Hepatitis B markers      | HIV infected cases |      |
|---------|--------------------------|--------------------|------|
|         |                          | Number             | %    |
| 1       | HBsAg + Anti-HBc         | 19                 | 9.5  |
| 2       | HBsAg + Anti-HBc + HBeAg | 6                  | 3.0  |
| 3       | Anti-HBc                 | 90                 | 45.0 |
| 4       | Anti-HBs                 | 12                 | 6.0  |
| 5       | Anti-HBs + Anti-HBc      | 57                 | 28.5 |
| 6       | One or more markers      | 184                | 92.0 |

## DISCUSSION

The prevalence of HBV markers was very high in HIV antibody positive cases being 92% in our study. This is in accordance with findings by Schorr *et al.* in 1985 who stated that many patients with AIDS showed serologic evidence of prior infection with HBV (4). In our study, HBsAg was positive in 12.5% of the study group, which was slightly higher than the usual carrier rate of 9-11% in the general population (2). Schneiderman *et al.* in 1988 stated that although evidence of remote HBV infection is extremely common, the HBsAg carrier rate, chronic active hepatitis or cirrhosis occur no more frequently in AIDS patients than in the general population (5). Evidence of remote HBV infection was signalled by

34.5% of cases being positive for anti-HBs and also 45% of cases being present for anti-HBc alone.

The majority of the HBV infected cases was grouped within 20-34 years of age (85.5%), the same age range where blood donors and drug addicts infected by HIV in Myanmar were highest (1).

Lane et al. in 1989 stated that in their study of 85 patients with AIDS, abnormal liver function tests were found in 48.2% although only one patient became jaundiced clinically (6). Liver disease although usually asymptomatic is a frequent accompaniment of AIDS. In our study of 59 hospitalised patients who were positive for HIV antibody, 3 presented with viral hepatitis.

Several studies have been conducted concerning the correlation between HBV and HIV infections. Schneiderman in 1988 published that liver involvement generally does not directly cause morbidity or result in death. Biochemical liver tests are commonly elevated in the AIDS population, and alkaline phosphatase has proved to be the most specific enzyme for infiltrative processes (5).

Twu et al. in 1989 reported that HIV-I tat III gene and the HBV X gene together increased HIV-I long terminal repeat (LTR) regulated chloramphenicol acetyl transferase (CAT) expression above that observed with either gene alone, showing a synergistic

effect of the X gene and tat (7).

Novick et al. in 1988 stated that in their study on IVDAs positive for HBsAg, 58% of them were also positive for anti-HIV (8). In another study on IVDAs in 1988, Lange et al. concluded that dual infection with these 2 viruses may be responsible for the development of fulminant hepatitis B in IVDAs (9).

In another study by Hirshman et al., it was stated that 68 of 73 patients (93.2%) with HIV infection were positive when tested for the presence of HBV-related DNA sequences in peripheral blood mononuclear cells (PBMCs) by the dot blot method. The data showed that the extrachromosomal DNA fragment that hybridized to HBVDNA was a natural recombinant between HIV and HBVDNA and this DNA played a role in the pathogenesis of AIDS (10).

WHO published an interesting report on the reactivation of HBV infection in AIDS patients as shown in a study by Vento et al. in 1989 in which 3 anti-HBs positive cases who had recovered fully from acute hepatitis B, presented with clinical acute hepatitis due to HBV reactivation after the development of AIDS (11).

Prof. Hollinger in 1987 reviewed data relating to HBV and AIDS and attributed immunologic defects to HBV infection. A reduction of the helper-to-suppressor T cell ratio and depressed Inter-

leukin-2 levels were noted in patients with acute hepatitis B and in asymptomatic carriers. This functional defect of the helper/inducer cells may predispose individuals to infection with HIV (12).

In conclusion, the effects of HIV infection on the expression of hepatitis B and related liver diseases and on the development of chronicity of hepatitis B infections, deserve attention. Since theoretically HIV and HBV are transmitted by similar parenteral, sexual and perinatal routes, these baseline data confirm that HIV has the potential to cause a serious effect on the susceptible population of Myanmar which has a high infection rate for HBV.

#### ACKNOWLEDGEMENTS

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Relationship of symphysio-fundal height  
and abdominal circumference to  
gestational age

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Symphysio-fundal height (SF-height), abdominal, circumference (AC), anterior abdominal wall skinfold thickness were measured serially in (506) women with uncomplicated pregnancies in Mandalay General Hospital. A total of (2513) readings were made. The standard graphs of above measurements in relation to gestational age of fetus were drawn. The correlation coefficient (r) values were calculated. Both SF height and AC were increased as gestational age but the numerical values were not equivalent to weeks of gestation. The engagement of head and anterior abdominal wall skinfold thickness did not change the relationship significantly. The normgravidogram, resulting from this study may be useful in detection of high risk pregnancies in antenatal clinics, although it cannot reflect the gestational age accurately. Further research work is necessary to detect reliability of this normgravidogram in screening of high risk cases.

#### INTRODUCTION

Assessment of gestational age is an important part of obstetric care as it plays a major role in decision making with regards to obstetric management. The most commonly accepted clinical indicators of gestational age include, last menstrual period (L.M.P) and assessment of fetal size by palpation of the uterus.

Anderson et al. (1981) reported from a study on (417) pregnant women that fundal height measurements were assumed to be directly equivalent to gestational age in weeks. However, there is no universal agreement as to whether symphysio-fundal height and abdominal circumference measurements

accurately reflect the maturity of the fetus. Besides, the accuracy of clinical measurements is influenced by the size of the fetus, its presentation, the engagement of the presenting part, the position of the uterus, weight and height of the pregnant woman, position of the woman, abdominal wall skin-fold thickness, amniotic fluid volume and full bladder.

This study is therefore undertaken:

- (1) to determine the relationship of symphysio-fundal height (with or without consideration of engagement of head) to gestational age.
- (2) to determine the relationship between abdominal

circumference (with or without consideration of anterior abdominal wall skinfold thickness) and gestational age.

(3) to plot a normgravido-gram in Myanmar pregnant women.

## MATERIALS AND METHODS

Pregnant mothers attending antenatal care clinic in M.G.H from 1-10-1987 to 30-4-1989 were enrolled into the study if they have uncomplicated singleton pregnancies with known LMP and previous regular cycles without use of hormonal contraceptives during the last 3 cycles preceding the LMP.

Elderly primigravidae, multiple gestation, complications of pregnancy and chronic maternal diseases were excluded.

The selected mothers-to-be were seen at fortnightly intervals within (36) weeks and thereafter weekly until delivery. For all selected mothers, the following measurements were made, by two of the four authors only.

(1) Symphysio-fundal height (SGH) was measured in centimetres by using a non-elastic metric tape with the subject in supine position, legs extended and her bladder empty. The position of the uterus was centralized and the upper border of the uterine fundus was defined by using the ulnar edge of the examiner's left hand and marked with a pen. The end of a non-elastic tape measure was held against the symphysis pubis and the distance to the mark on the uterine fundus was

recorded. Each measurement was taken to the nearest 0.5 cm.

(2) Abdominal circumference (AC) was measured in inches at the level of the umbilicus.

(3) Abdominal wall 'skinfold thick-ness' was measured at the site which was two inches away from the center of the umbilicus on the right and left side by using 'Haltain skinfold caliper'. The mean skinfold thickness was noted and subtracted from the measured abdominal circumference measurement.

(4) If the head was in the pelvis, the portion of the head above the brim was noted in fifths and measured in centimeters from the upper border of the symphysis pubis. The length of the portion of the head in pelvis (in cms) was calculated from the measured length of the non-engaged portion of the head above the brim of the pelvis. e.g. if one-fifth of the head above the brim was 2cms, the remaining four-fifths of the head in the pelvis would be 8 cms. This calculated length of the head in the pelvis was added to the measured symphysio-fundal height.

An unselected subsample of (79) pregnant women were followed from (28) weeks fortnightly till (36) weeks and then weekly till delivery. Their gravidograms were also drawn.

### Statistical analysis

The Mean  $\pm$  1 SD values were calculated for each variable. Correlation coefficients were calculated. Regression lines were drawn and the Mean  $\pm$  1 SD values were plotted against the appropriate gestational age (Figure 1,2,3 & 4).

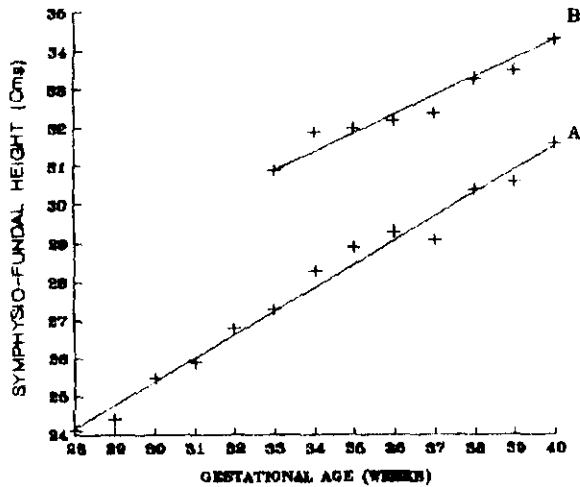


Figure 1. A: Relationship between Symphysio-fundal height (mean) and gestational age ( $r=0.99$ )  
 B: Relationship between Symphysio-fundal height plus length engaged portion of head and pelvis (mean) and gestational age ( $r=0.95$ )

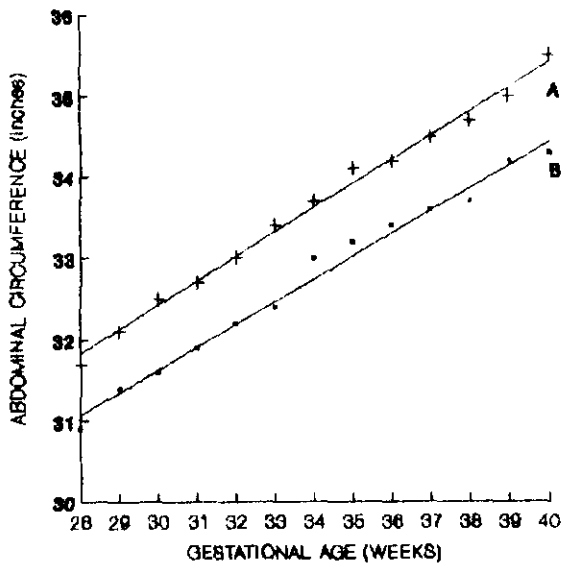


Figure 2. A: Relationship between abdominal circumference (mean) and gestational age ( $r=0.95$ )  
 B: Relationship between abdominal circumference minus skinfold thickness (mean) and gestational age ( $r=0.96$ )

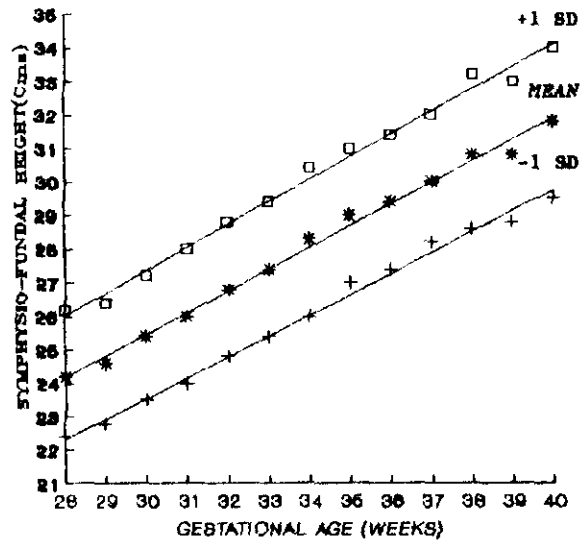


Figure 3. Relationship between symphysio-fundal height and gestational age in Cohort Study ( $r=0.99$ )

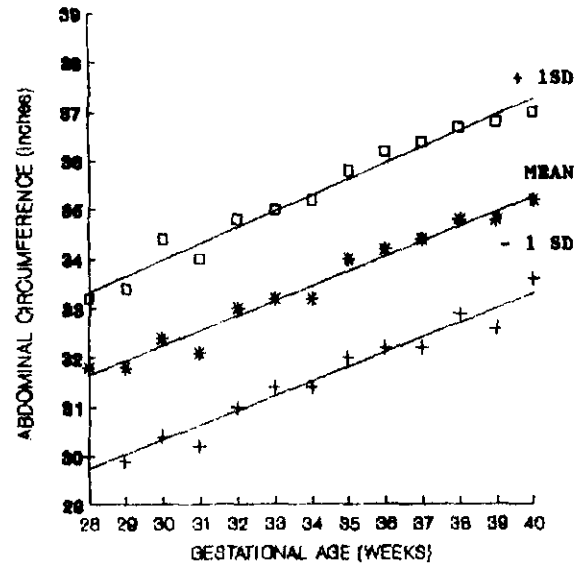


Figure 4. Relationship between abdominal circumference and gestational age in Cohort Study ( $r=0.95$ )

## RESULTS

The total number of pregnant women studied was (506), out of which, (274) women delivered at Mandalay General Hospital (M.G.H) and (232) women did not do so. The number of patients attending antenatal clinics regularly and delivered at M.G.H was (79) out of (274) total patients delivered at M.G.H.

In this study, the patient ranged from age 15-45 years, the highest percentage was in the (21 to 25) years age group (35.70%).

The gravidity distribution was from primigravida to gravida 5 and above. Primigravida formed 38.33% of total and maximum group was in gravida 2 to 4 range. (50.69%).

Regarding the educational status, most of the subjects were educated up to primary level. (45.56%). Most of the cases (59.96%) belonged to the low income (less than 500 kyats) group. The mean height of cases in this study was (5) feet.

## DISCUSSION

In recent years new technologies have contributed much to the estimation of gestational age. The accuracy of ultrasound in gestational age assessment has been widely studied and increasing role of ultrasound in obstetrics is established. But routine ultrasonography of all pregnant patients is practi-

cally not possible. In many obstetric practices, history taking and clinical examination during pregnancy are major methods of gestational age assessment.

Anderson *et al* (1981) suggested that combination of several clinical estimators including quickening (QIK), fetal heart tone first audible with an unamplified stethoscope (FHT), uterus at the level of umbilicus (UMB) and fundal height measurements (F-H) can provide a prediction of delivery date as precise as that of a known LMP. They reported that at least two obstetric landmarks (FHT), (QIK) or (UMB) or three or more (F-H) measurements are necessary to predict delivery date. The use of fundal height measurements has been disregarded by some authors because of the wide variability found in different measurements. (Beazley & Underhill 1970).

In 1953, Rumbolz and Mc Googan showed the association between reduced growth of the uterine fundus and intrauterine growth retardation (IUGR). In the last decade, the difficulty of diagnosing intrauterine growth retardation on abdominal palpation has been recognised. In an attempt to facilitate the diagnosis of intrauterine growth retardation, several charts of the symphysis-fundus measurement have been made. (Westin 1977: Belizan *et al* 1978, Quaranta *et al* 1981). Despite this, the technique is still not popular, probably

because of the lack of agreed principles (Table 1), the suggested need for an individual curve for each maternity unit or each country and the anticipated problem of observer variation.

In the present study, the inter-observer variation was eliminated as far as possible as observations were made by only two persons. The birth-weights varied between 3 lbs and 8 lbs 15 ozs. The (8) babies belonging to the 3 lbs to 5 lbs group were all premature and had no signs of IUGR nor fetal abnormality. The mean birth weight in this study was 6 lbs and 6 ozs which was comparable to mean birth weight of 6 lbs 15 ozs for males and 6 lbs 9 ozs for females, reported by Khin Mg Lwin and Mya Tu, 1967.

Table 2 showed comparison of values obtained from symphysio-fundal height charts of Westin (1977), Belizan (1978) and Quaranta (1981) with the present study.

As shown in Table 1, Myanmar values are much less than the Western values and therefore it is apparent that each population should have its own chart.

In this study, correlation coefficient (r) between SE height and weeks of gestation was 0.99 and when engagement of head was taken into account, (r) value decreased to 0.95. Therefore SF height measurement is reliable whether head is engaged or not (Figure 1 & 3).

The correlation coefficient

for AC was 0.95 and when

Table 1. Different principles used in different charts of symphysio-fundus measurements for diagnosis of IUGR

| Reference              | Derivation of chart                           | Value to diagnose IUGR                                                           |
|------------------------|-----------------------------------------------|----------------------------------------------------------------------------------|
| Westin (1977)          | Mean birth weight $\pm$ 1SD                   | value of more than 2 cm below mean (or) 3 consecutive static or declining values |
| Belizan et al. (1978)  | Birth weight between 10th & 90th percentile   | Value less than 10th percentile                                                  |
| Quaranta et al. (1981) | Birth weight between 25th and 90th percentile | 2 consecutive or 3 isolated values less than 10th percentile                     |

Table 2. Comparison of symphysio-fundal height values (Mean in cms)

| Gestational age(weeks) | Westin (1977) | Belizan (1978) | Quaranta (1981) | Present Study |
|------------------------|---------------|----------------|-----------------|---------------|
| 20                     | 18.0          | 18.5           | 20.2            |               |
| 24                     | 22.0          | 22.5           | 24.1            |               |
| 28                     | 26.0          | 26.5           | 28.1            | 24.1          |
| 32                     | 29.6          | 30.5           | 31.8            | 26.7          |
| 36                     | 33.0          | 33.5           | 34.7            | 29.4          |
| 40                     | 35.5          | 34.5           | 36.3            | 31.6          |

skinfold thickness was subtracted, r value become 0.95, the difference being 0.01 (Figure 2 & 4). Hereagain, abdominal wall skinfold thickness is not found to influence the AC measurement and weeks of gestation significantly. SF-height and AC measurements were found to be much less than actual weeks of gestation at each visit. Therefore the universal teaching that SF-height in cms and AC in inches is equivalent to gestational age in weeks, now seems not to be true. One can not rely on these values to predict gestational age in cases who do not know their last menstrual date.

There are some reports showing high specificity (84 - 88%) and sensitivity (78 - 84%) rate of fundal height measurements in screening of light for gestational age babies (Matthews Mathai, 1987).

The authors suggest that this simple, cost-effective and convenient measurement be utilised at the primary health care centres in detecting IUGR for early referral to centres where facilities are available for proper management.

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**The pathognomonic value of symptoms and signs  
in the management of acute respiratory  
infections in young children**

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1826 children attending the out patient department of Mandalay General Hospital, from 1st February 1986 to 31st January 1987 with acute respiratory infection (ARI) related symptoms were classified by the primary health workers as mild, moderate and severe ARI cases (i.e initial classification). These children were again examined by the investigator (doctor) and appropriate treatment was given. The cases were followed up till they died or were well. The final classification was done considering the clinical presentation, response to the treatment and the outcome of the patient. To determine the pathognomonic value of clinical signs and symptoms in classification of the severity of ARI cases, different criteria for classification of severe ARI were laid down, the initial and final classification were compared and their respective sensitivity and specificity were calculated. It is concluded that out of the clinical signs and symptoms of respiratory distress, tachypnoea, chest indrawing and inability to drink were found to be important clinical signs and symptoms in determination of the severity of ARI. In addition to the criteria proposed by the WHO, very fast breathing (i.e respiratory rate over 70/min) with or without wheeze should be one of the criteria in classification of severe ARI.

**INTRODUCTION**

Acute respiratory infections (ARI), diarrhoeal diseases and malnutrition are the principal causes of illness and death in children in developing countries. Where as diarrhoeal diseases and malnutrition have received considerable attention, acute respiratory infections have often been ignored until recently.

The magnitude of the problem of ARI in Myanmar is similar to that of other developing countries. ARI is one of the leading causes of morbidity and mortality particularly in young infants and children. Although the morbidity rate of diarrhoeal disease is slightly higher than that of ARI, the mortality of ARI is the highest among the five leading causes.

In developing countries in areas, where there are only primary health workers to look after the children, the outcome of children with ARI depends upon early detection and referral of severe cases. There were evidences to show that the application of standardised case management and antimicrobial treatment through an intensive programme of training and supervision of primary health workers can produce a measurable impact of up to 50% reduction in the mortality from childhood pneumonia in rural areas of developing countries. WHO working group on case management of ARI (Geneva 25-29 March 1985) recommended that a community health worker should consider ARI severe if he observes chest indrawing with cough, stridor, wheeze or fever, or cough or fever in a

child too ill to drink. Such a child was to be given antimicrobial and referred to a health centre.

A child breathing fast (over 50 breaths per minute) with cough, wheeze or fever but no chest indrawing would have moderate ARI and could be treated at home with an antimicrobial and supportive measures. Children with mild ARI eg. with only cough, hoarseness, fever, running nose or a red throat should not be given antimicrobials but only supportive measures. Although such a scheme may improve the prognosis for young children with ARI, there is no precise information to show how effective it is. This study is to have an answer in quantitative terms and to find out the relationship of the clinical signs and symptoms with the severity of ARI so as to determine the pathognomic value of symptoms and signs in the management of ARI in young children.

#### PATIENTS AND METHODS

The study was done at the out patient department and paediatric wards of Mandalay General Hospital. The study began with the training of the primary health workers, PHWs (Health Assistants) for a week during the last week of January 1986. The health assistants who took part in the study were from the rural health centres near Mandalay and they were arranged to work in the out patient department of the Mandalay General Hospital in turn. Altogether, eight health assistants took

part in the study. They were trained to examine the children and to fill in the individual record consisting of presenting clinical signs and symptoms and classification. They were found to be competent after a week's training and cases were taken in beginning from February 1986.

All children from easily accessible areas attending the out patient department of Mandalay General Hospital with ARI related symptoms were included in the study. The PHWs (health assistants) received the children presenting with ARI related symptoms and the individual record was filled in by observing the symptoms and the history given by the accompanying person. Then a physical examination was performed for relevant clinical signs and these as well as the classification made by the primary health workers (initial classification) were recorded according to the scheme proposed by the WHO working group with slight modification. There was some misunderstanding in interpreting WHO classification and those children with very fast breathing i.e. respiratory rate  $>70/\text{min.}$  were classified as severe by the health workers.

The child was then examined by a physician (Assistant investigator) who according to his own finding, filled in the clinical record, made a provisional diagnosis and treatment was prescribed.

Chest X'ray examination was done as soon as possible when there is the possibility of pneumonia and when crepitations were heard on auscultation. The individual records and the corresponding clinical records were numbered serially. No corrections were made on individual records filled in by the health workers. The assistant investigator issued an appointment card for each follow-up examination and the date on the clinical record was noted. If the patient did not return to the hospital on the date due, a home visit was done. The patients were followed up till they were well and the results were recorded. When treatment was terminated, the final diagnosis with its appropriate classification (final classification) was made by the paediatrician (Principal investigator) and were recorded. The final classification was done according to the criteria shown in Table 1.

During the first twelve months of the study 2171 children with ARI from easily accessible areas were collected. Chest X'ray could not be done in some of the pneumonia cases due to unfavourable circumstances e.g X'ray machine out of order, electricity failure etc. When the data collected during periods when X'ray examination was not feasible were excluded from the study there were a total of 1826 cases and the analysis was done on these 1826 cases.

## RESULTS

To determine the pathognomonic value of clinical signs and symptoms in classification of the severity of ARI cases, different criteria for classification of moderate and severe ARI were laid down, the initial and final classification were compared and their respective sensitivity and specificity were calculated.

Table 1. Criteria for final classification

### *Moderate ARI*

Presence of one or more of the following:-

1. Positive chest X'ray finding involving up to 50% lung parenchyma.
2. Initially classified mild ARI cases who required antibiotics because of the worsening of signs and symptoms.
  - (a) Crepitations on auscultation.
  - (b) Persistence of high fever  $>38^{\circ}\text{C}$  for more than 5 days without antibiotics.

### *Severe ARI*

Presence of one or more of the following:-

1. Positive chest X'ray finding involving more than 50% of lung parenchyma.
2. Respiratory distress needing oxygen therapy.
3. Cyanosis.
4. Worsening of the condition with injection Procaine Penicillin or oral co-trimoxazole or oral amoxycillin or ampicillin within or after 4 days of therapy.
5. Expired from ARI

Table 2 was prepared using the proposed WHO classification. Table 2a was prepared with the classification used in this study. Table 2b and 2c were prepared by the addition of nasal flaring as one of the criteria for severe to

the proposed WHO classification and the classification that was used in this study respectively. Table 2d was prepared by omitting 'inability to drink' as one of the criteria for 'severe' in the proposed WHO classification.

**Table 2. Comparison of initial and final classification  
(W.H.O Classification)  
February 1986 - January 1987**

*Criteria for severe*

1. Cough and chest indrawing.
2. Cough or wheeze plus not able to drink.
3. Cough with wheeze plus very fast breathing (>70/min.)

| Final<br>Classification | Total | Initial<br>Severe | classification |      |
|-------------------------|-------|-------------------|----------------|------|
|                         |       |                   | Moderate       | Mild |
| Severe                  | 139   | 119               | 20             | 0    |
| Moderate                | 394   | 12                | 291            | 91   |
| Mild                    | 1293  | 0                 | 13             | 1280 |
| Total                   | 1826  | 131               | 324            | 1371 |

|          | Sensitivity (%)     | Specificity (%)                  |
|----------|---------------------|----------------------------------|
| Severe   | $119/139 = 85.61$   | $(1293+394-12)/(1293+394)=99.29$ |
| Moderate | $291/394 = 73.86$   | $(1293+139-33)/(1293+139)=97.70$ |
| Mild     | $1280/1293 = 98.99$ | $(394+139-91)/(394+139)=82.93$   |

**Table 2a. Comparison of initial and final classification  
(Mandalay)  
February 1986 - January 1987**

*Criteria for severe*

1. Cough and chest indrawing
2. Cough and not able to drink
3. One or more respiratory symptoms and signs plus very fast breathing (>70/m)

| Final<br>classification | Total | Initial classification |          |      |
|-------------------------|-------|------------------------|----------|------|
|                         |       | Severe                 | Moderate | Mild |
| Severe                  | 139   | 138                    | 1        | 0    |
| Moderate                | 394   | 26                     | 277      | 91   |
| Mild                    | 1293  | 0                      | 13       | 1280 |
| Total                   | 1826  | 164                    | 291      | 1371 |

|          | Sensitivity (%)     | Specificity (%)                  |
|----------|---------------------|----------------------------------|
| Severe   | $138/139 = 99.28$   | $(1293+394-26)/(1293+394)=98.46$ |
| Moderate | $277/394 = 70.30$   | $(1293+139-14)/(1293+139)=99.02$ |
| Mild     | $1280/1293 = 98.99$ | $(394+139-91)/(394+139)=82.93$   |

Table 2b. Comparison of initial and final classification  
February 1986 - January 1987

Criteria for severe

1. Cough and chest indrawing
2. cough and unable to drink
3. Cough with wheeze plus very fast breathing (>70/min.)
4. Cough and nasal flaring

| Final classification | Total | Initial classification |          |      |
|----------------------|-------|------------------------|----------|------|
|                      |       | Severe                 | Moderate | Mild |
| Severe               | 139   | 120                    | 19       | 0    |
| Moderate             | 394   | 16                     | 287      | 91   |
| Mild                 | 1293  | 3                      | 13       | 1277 |
| Total                | 1826  | 139                    | 319      | 1368 |

|          | Sensitivity (%)     | Specificity (%)                  |
|----------|---------------------|----------------------------------|
| Severe   | $120/139 = 86.33$   | $(1293+394-19)/(1293+394)=98.67$ |
| Moderate | $287/394 = 72.84$   | $(1293+139-32)/(1293+139)=97.77$ |
| Mild     | $1277/1293 = 98.76$ | $(394+139-91)/(394+139)=82.93$   |

Table 2c. Comparison of initial and final classification  
February 1986 - January 1987

Criteria for severe

1. Cough and chest indrawing
2. Cough and not able to drink
3. One or more respiratory symptoms plus very fast breathing (>70/min.)
4. Cough and nasal flaring

| Final classification | Total | Initial classification |          |      |
|----------------------|-------|------------------------|----------|------|
|                      |       | Severe                 | Moderate | Mild |
| Severe               | 139   | 138                    | 1        | 0    |
| Moderate             | 394   | 29                     | 274      | 91   |
| Mild                 | 1293  | 3                      | 13       | 1277 |
| Total                | 1826  | 170                    | 288      | 1368 |

|          | Sensitivity (%)     | Specificity (%)                  |
|----------|---------------------|----------------------------------|
| Severe   | $138/139 = 99.28$   | $(1293+394-32)/(1293+394)=98.10$ |
| Moderate | $274/394 = 69.54$   | $(1293+139-14)/(1293+139)=99.02$ |
| Mild     | $1277/1293 = 98.76$ | $(394+139-91)/(394+139)=82.93$   |

Table 2d. Comparison of initial and final classification  
February 1986 - January 1987

Criteria for severe

1. Cough and chest indrawing
2. Cough with wheeze plus very fast breathing (>70/min.)

| Final classification | Total | Initial classification |          |      |
|----------------------|-------|------------------------|----------|------|
|                      |       | Severe                 | Moderate | Mild |
| Severe               | 139   | 104                    | 32       | 3    |
| Moderate             | 394   | 7                      | 293      | 94   |
| Mild                 | 1293  | 0                      | 13       | 1280 |
| Total                | 1826  | 111                    | 338      | 1377 |

|          | Sensitivity (%)     | Specificity (%)                    |
|----------|---------------------|------------------------------------|
| Severe   | $104/139 = 74.82$   | $(1293+394-7)/(1293+394) = 99.59$  |
| Moderate | $293/394 = 74.37$   | $(1293+139-45)/(1293+139) = 96.86$ |
| Mild     | $1280/1293 = 98.99$ | $(394+139-97)/(394+139) = 81.80$   |

### DISCUSSION

It was found that the sensitivity and specificity were similar for criteria that was used in this study and the criteria proposed by the WHO except for the sensitivity for severe ARI in which the result was better with the criteria that was used in this study. (99.28% Vs 85.61%). It shows that with increasing respiratory distress, there is increasing tachypnoea. Therefore very fast breathing (i.e. respiratory rate >70/min.) should be one of the criteria for classification of severe ARI.

Nasal flaring is another clinical sign which should be useful in classification of ARI cases. Out of 77 children presenting with nasal flaring, 68 children (88.3%) were finally classified as severe ARI. However in this study, it appeared that no practical advantage can be gained by

considering nasal flaring. It was present together with one or more of the other determining clinical signs or symptoms (i.e chest indrawing, inability to drink and very fast breathing) in severe cases. No case of severe ARI presented with cough and this sign alone. On the other hand, it was present in 6 moderate and 3 mild ARI cases so that no practical advantage could be gained by considering this clinical sign.

Table 2d revealed that 'inability to drink' is one of the important criteria to classify severe ARI and when the symptom was omitted there was decline in the percentage of sensitivity in severe ARI from 85.61% to 74.82%.

Therefore, it can be concluded that out of the clinical signs and symptoms of respiratory distress, tachypnoea, chest indrawing and inability to

drink were found to be the important clinical signs and symptoms in determination of the severity of ARI and in addition to the criteria proposed by the WHO, very fast breathing (i.e. respiratory rate over 70/min.) with or without wheeze should be one of the criteria in classification of severe ARI.

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Contraceptive prevalence survey in  
urban Mandalay

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A study of K A P ( knowledge, attitude and practice) on contraceptive in Myanmar females was undertaken in Mandalay. Two thousand five hundred ever married females between the age of 15 and 49 years were interviewed. It was found that 97.8% of these women possessed knowledge regarding contraceptives. Oral contraception was the most knowledgeable recognised method, followed by the injectable type. Sterilization and vasectomy were the third and fourth recognised methods. The total ever users were 48.92% and 20.88% were current users. Oral pills were used by 28.08%, injectables by 20.53% and sterilization by 5.27% of knowledgeable persons. The reasons for not using the fertility regulation methods were fear of side effects, desiring to have more children, ignorance regarding contraception, not wanting to interfere with nature, financial problems and religious beliefs.

#### INTRODUCTION

Fertility regulation by the use of various contraceptive methods is carried out voluntarily by individual family in Myanmar. There is no family planning programme at the national level because of the need for increased manpower for the country. People choose their own method of contraception when they have decided the need for spacing their family.

Contraception has the greatest importance in the field of preventive medicine being essential to the health and welfare of individuals, families and whole communities. It would seem to be a fundamental human right that parents should control their fertility and limit the

size and spacing of the family. Involuntary parenthood creates many problems, social and financial as well as physical and emotional. To establish a family is still the earnest desire of most married couples although the modern idea is to limit the number of children.

The study on the prevalence of contraception in Myanmar females is one of the project activities of the Clinical Research Unit (Obstetrics and Gynaecology) Mandalay. The objectives of the study were (i) to determine the knowledge, attitudes and practices about contraception in females living in urban community in Mandalay; and (ii) to determine the availability of contraceptive methods.

## MATERIALS AND METHODS

Study areas were the antenatal and gynaecological clinics of Mandalay General Hospital and some wards (Aung-mya-e-thar-zan, Maw-ra-gi-wa, Pyae-gyi-myet-shin, May-ga-gi-ri, Chan-mya-tha-zi.) of Mandalay. The study population consisted of 2500 ever married females aged 15 to 49 years old.

The survey instrument was an interview-administered questionnaire (1) containing 75 items including identification, socio-economic status, reproductive behavior and intentions, knowledge of fertility regulation methods, current and past contraceptive use, availability of contraceptive methods and reasons for not using contraceptives. Data were collected by 2 project medical officers from 1st August 1988 till March 1990. The data were analysed in the computer division of the Department of Medical Research.

## RESULTS

### Characteristics of respondents

The age range stretched from 15 to 49 years. The largest distribution being seen between the age of 25 and 29 years (25.6%), followed by the 20 and 24 years of age (24.99%) and the smallest number (1.89%) fell between the ages of 45 and 49 years. The mean age in this study was 28.49 years.

Of the women interviewed, only 7% had never attended school. The largest proportion (52%) had studied up to the primary

school level. Twenty five, 11 and 5% of the women had middle school, high school and university education respectively. Only 35.2% of the respondents were employed at the time of study.

As this survey included subjects attending antenatal clinics, 1088 cases (43.52%) were pregnant and 292(11.68%) were lactating at the time of study.

Among the respondent, women with no children constituted 19.6%, those with one child 19.76%, those with two children 18.44%, those with three children 15.68%, those with four children 11.24%, those with five and above children 15.4%. The mean parity for urban setting was 2.8.

### Knowledge

In this survey, knowledge about various methods of contraception and that about available sources of contraception were also studied and shown in Figure 1.

### Attitudes

Among the respondents, 1998 women (79.92%) agreed to space their families; 11.42% did not agree because they were desirous of more children and did not want to interfere with nature; 2.28% decided to have children depending upon conditions; and 6.38% were undecided about spacing their families.

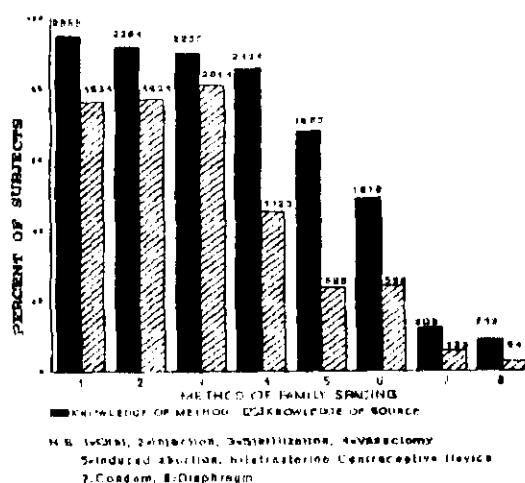


Figure 1. Comparison of knowledge of methods & sources of contraception

Regarding the questionnaire 'desire for more children', 42.4% did not want any more children, 10% wanted one more child, 17.2% two more children, 17.48 % three children, and very few women (12.44%) wanted four children and more. The mean number of children desired being 1.9.

### Practices

The percentage of respondents practising contraception among those who have knowledge are shown in Table 1.

Although 97.84% of the women possessed knowledge concerning contraception, percentage of current usage was only 20.88%.

Table 1. Percentage of respondents (n=2500) having knowledge about various methods of contraception and the percentage of subject practised contraception among those who have knowledge.

| No. | Method                            | subjects having knowledge | Subjects Practising | Percentage               |                                           |
|-----|-----------------------------------|---------------------------|---------------------|--------------------------|-------------------------------------------|
|     |                                   |                           |                     | subject having knowledge | subject practised by knowledgeable person |
| 1   | Oral                              | 2369                      | 664                 | 94.76                    | 28.03                                     |
| 2   | Injectables                       | 2294                      | 471                 | 91.76                    | 20.53                                     |
| 3   | Sterilization                     | 2277                      | 120                 | 90.08                    | 5.27                                      |
| 4   | Vasectomy                         | 2134                      | 146                 | 85.36                    | 6.84                                      |
| 5   | Induced Abortion                  | 1696                      | 5                   | 67.84                    | 0.29                                      |
| 6   | Intrauterine contraceptive device | 1218                      | 40                  | 48.72                    | 3.28                                      |
| 7   | Rhythm                            | 687                       | 238                 | 27.48                    | 34.64                                     |
| 8   | Withdrawal                        | 439                       | 73                  | 17.56                    | 16.63                                     |
| 9   | Condom                            | 300                       | 28                  | 12.0                     | 9.33                                      |
| 10  | Diaphragm                         | 213                       | 4                   | 8.52                     | 1.88                                      |
| 11  | Others                            | 60                        | 5                   | 2.4                      | 8.33                                      |

The causes of not using contraceptives were pregnancy and lactation at the time of interview (54.8%); fear of side effects (13.84%); desire of more children (8.57%); lack of knowledge (3.87%); reluctance of interfere with nature (2.8%); and financial problem (3%).

For the preferred type of contraception to be used in future, sterilization was found to be most preferable method chosen by 24.44%, followed by oral pills 17.2%; injectables 18.24%; vasectomy 9.68%; rhythm and withdrawal 1.8%; and intrauterine contraceptive device methods, 1.04%. There were 13.84% of the subjects who did not want to use any methods and 13.12% did not know the methods to be use in the future.

## DISCUSSION

Knowledge regarding contraception in the urban population of the Mandalay was found to be 97.84% a little higher than in the semi-urban population of the Yangon which was 89.35% (2). This difference may be due to the Kyaw-Tint *et al.* study being conducted in semi-urban areas or to the time difference of 5 years. Women of all ages, educational level and geographical regions knew of at least one family planning method (3).

Of the contraceptive methods available, oral pills are the

most known; 94.76% in this study, 98.2% in the Thailand study and 84.05% in Kyaw-Tint *et al.* study. This is followed by the injectable 91.76%, 87.4% and 77.25% respectively in the three studies. The third and fourth ranking known methods were female sterilization and vasectomy in this and the Thailand study. In the Kyaw-Tint series these methods were not included for scrutiny. Similarly in all these studies the barrier methods were known only to a few percentage of women: 12.5%, 19.4% and 5% respectively. (Mya-Mya *et al.*, Thailand and Kyaw-Tint *et al.*).

On correlating the knowledge concerning contraception and available source, for the four most popular methods, more than 80% of the sources. This finding conforms with the two other studies. When compared to the World Fertility Survey, the rural portion of the 1974 Korean Survey revealed a 86% accessibility whereas the Mexican rural Survey varied from 25% to 53%.

Even though 97.84% of women are knowledgeable concerning contraception, only 21.16% were practising them at the time of study. The data for Kyaw-Tint *et al.* study was 89.35% and 19.28% for the knowledge and practice of contraception. The authors have tried to elucidate the cause of the gap in the knowledge and the practice of contraception. In this study, of women were found to be either pregnant or lactating

during the time of interview. If these women were deleted from the total number the percentage of current users are 47.23%. The rest of the interviewees gave reasons as being fearfulness of side effects, wanting more children, not wanting to interfere with nature, due to financial conditions and religious beliefs.

The preferred type of contraceptive use in order is sterilization, injectables, oral pills and vasectomy. Surgical contraception may be considered to be the best method of contraception of informed, adequately counselled couples, since termination of fertility rather than pregnancy spacing is the usual fertility regulation objective. concerning attitude towards family spacing, 80% of the mothers possess a favourable attitude; only 11.42% were not agreeable towards spacing and 6.38% were undecided.

This study is the first phase of the project which undertaken in an urban setting. It will soon be followed by the second half of the project to cover the rural community, as only 9.92% of females were currently practising contraception in the Myaungtaga and Yegyaw village tracts of Hmawbi Township (5). Further information correlation studies from the 75 variables in the questionnaire of this

project will be processed and presented later.

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## Effect of acute diarrhoea on the bioavailability of chloroquine in Myanmar subjects

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Considering the possibility of altered bioavailability of drugs during acute diarrhoea, there is a need to determine the therapeutic status of chloroquine as an antimalarial prophylactic during an attack of acute diarrhoea and to develop a reasonable basis for dose adjustment if necessary, in such clinical situations. Chloroquine 300 mg base was administered orally to 16 adult diarrhoeal patients from the Infectious Disease Hospital, Yangon and 12 healthy non-diarrhoeal volunteers. The drug serum levels at various time intervals up to 96 hours were analysed fluorometrically and the pharmacokinetic profile studied. Acute diarrhoea was found to decrease the rate, but did not alter the extent of absorption of chloroquine. Since the overall bioavailability of chloroquine remains unchanged, it was concluded that if there is no vomiting, dosage adjustment is not necessary in acute diarrhoea.

### INTRODUCTION

Despite the relentless advance of drug resistance in *Plasmodium falciparum*, chloroquine still remains the treatment of choice for sensitive strains in Myanmar and many of the developing countries. This is because chloroquine is inexpensive, widely available, well tolerated and cures malaria with relatively few doses (Mya-Oo, 1980, White et al., 1988).

Although development of resistance is fully recognized and has caused the loss of what was once a very effective drug in *falciparum* malaria, the possibility of occurrence of a relative resistance to chloroquine due to reduced bioavailability, causing a

strain of parasite fully sensitive to standard dosage to persist and be recorded as resistant, also needs to be considered (WHO, 1965).

Out of many factors influencing bioavailability, diarrhoea, a disorder very common in Myanmar, must also be taken into account since it has been suggested by WHO (1965) that bowel disease *per se* will affect absorption, and by Botha (1974) that a double dose prophylaxis of chloroquine may be necessary in cases with travellers diarrhoea, to prevent suboptimal serum levels occurring in areas where malaria is highly endemic.

The objectives of this study are: (1) to investigate the effect of acute diarrhoea on

the bioavailability of chloroquine, (2) to develop a reasonable basis for dose adjustments, if necessary, in such clinical situations and (3) to establish a standard pharmacokinetic data of chloroquine in Myanmar which can be used for comparative studies with other countries.

## MATERIALS AND METHODS

### 1. Drugs and chemicals

- Chloroquine phosphate tablets, 150 mg base (Chlorofos, Myanmar Pharmaceutical Factory, Yangon)
- Pure chloroquine standard, (provided by the Myanmar Pharmaceutical Factory, Yangon)
- Sodium hydroxide (5 mol/l)
- Sodium hydroxide (0.1 mol/l)
- Hydrochloric acid (0.1 mol/l)
- 1,2 Dichloroethane
- Isoamyl alcohol
- Ammonia solution (1 mol/l)

### 2. Subject selection

Sixteen patients of both sexes from the Infectious Disease Hospital, aged 20 to 60 years, were selected on the basis of a satisfactory history and medical examination. All diarrhoeal patients included in the study were considered healthy except for having diarrhoea; have not been taking medication for the past two weeks; and fulfil the criteria for acute diarrhoea according to the WHO/CDD (1984). The control group consisted of ten healthy non-diarrhoeal volunteers from the DMR.

### 3. Experimental design

A controlled parallel group design was employed. Following a fasting state of about 10 hours, control 0-hour blood and urine samples were taken after which, a single dose of chloroquine 300 mg base (2 tablets) was administered orally to both patients and controls. Blood sample collection was continued at 1,3,6,24,48 and 96 hours. A second urine sample was collected at approximately 3 hours after drug administration and was analysed together with the control urine sample by Lelijveld and Kortmann test (1970) for spot detection of chloroquine. If the test result was positive in the control or negative in the 3-hour sample, the patient was excluded from the study. In diarrhoeal patients, IV and oral rehydration therapy was allowed but smoking and other drug medications were restricted up to 12 hours, after which appropriate antibiotic therapy was started if there was indication for it. Hourly monitoring of heart rate and BP, and careful observation for possible adverse effects was done up to 6 hours after chloroquine treatment, followed by four-hourly monitoring up to 96 hours. The serum was separated immediately and stored at - 20°C and was analysed within two weeks.

### 4. Analytical methods

The samples were assayed for serum chloroquine levels by a modification of the double extraction fluorometric technique of Ensor, 1966. All samples were determined in

duplicates using a computerised fluorescent spectrophotometer, model 850, Hitachi, Japan.

### 5. Pharmacokinetic analysis

The serum concentration-time data of each patient and healthy volunteer was plotted on a semilogarithmic graph paper and the pharmacokinetic parameters of each individual calculated. The serum concentration-time profile of chloroquine, up to the sampling time of 96 hours, was found best-fitted to a two compartment open model with the elimination phase of the blood level curve showing a non-linear biexponential decay due to the simultaneous occurring distribution. This was peeled-off by using the method of residuals and the hybrid constant thus obtained were used to calculate the elimination rate constant ( $K_{el}$ ) by :

$$K_{el} = \frac{A+B}{A/\alpha + B/\beta}$$

Where alpha and beta were the exponential coefficients during the two phases. 'A' and 'B' are the intercepts of alpha and beta at time zero respectively. The area under concentration-time curve ( $AUC_{0-t}$ ) was calculated according to the trapezoidal rule up to the time of the last sample and extrapolating to

infinity by integrating  $\frac{C_t}{K_{el}}$  ;

where  $C_t$  is the last drug concentration value. The apparent volume of distribution ( $aVd$ ) and the total clearance ( $Cl$ ) were calculated by:-

$$aVd = F \times \frac{\text{dose}}{C_0}$$

and

$$Cl = F \times \frac{\text{dose}}{AUC_{0-\infty}}$$

Where  $C_0$  was the theoretical serum concentration at the time of administration.  $F$  is the bioavailability factor of chloroquine, which was taken as 0.9, according to White (1985).

Chloroquine concentration up to the 7th day (168 hours) was then extrapolated by using the standard formula for a two compartment model;

$$C_p = A.e^{\alpha t} + B.e^{\beta t}$$

Where  $C_p$  is the plasma concentration at 't' (168 hours).

### 6. Statistical consideration

Statistical analysis of group data was performed by unpaired Student's 't' test and the significance and association between the various kinetic parameters in health and diarrhoea were examined by linear regression analysis using a computer IBM-PC-XT.

## RESULTS

A total of 16 diarrhoeal patients and 12 healthy volunteers took part in the study and their clinical and laboratory data shown in tables 1 and 2 were in line with the criteria for selection of subjects.

**Table 1. Clinical and laboratory data of healthy volunteers and diarrhoeal patients (Mean values)**

|                                 | Data                 | Units     | Reference values | Control group (n=12) | Diarrhoea group (n=16) |
|---------------------------------|----------------------|-----------|------------------|----------------------|------------------------|
| Haemoglobin                     | M                    | (gm %)    | 13 - 18          | 12.6                 | 12.4                   |
|                                 | F                    |           | 11.5 - 16.5      |                      |                        |
| Haematocrit                     | M                    | ( % )     | 40 - 54          | 40.7                 | 41.3                   |
|                                 | F                    |           | 37 - 47          |                      |                        |
| Total and differential proteins | Total                | (g/l)     | 60 - 82          | 67.8                 | 71.1                   |
|                                 | Albumin              |           | 36 - 52          | 38.5                 | 34.8                   |
|                                 | Globulin             |           | 24 - 37          | 29.4                 | 36.3                   |
| Serum electrolytes              | Na                   | (mmol/l)  | 136 - 148        | 140.6                | 137.3                  |
|                                 | K                    |           | 3.8 - 5          | 4.2                  | 3.9                    |
|                                 | Cl                   |           | 95 - 105         | 100.3                | 100.0                  |
|                                 | HCO <sub>3</sub>     |           | 24 - 32          | 23.3                 | 18.5                   |
| Urea                            |                      | (mmol/l)  | 2.5 - 6.6        | 2.5                  | 6.0                    |
| Liver Function Tests            | Bilirubin            | (μmol/l)  | 3 - 21           | 11.3                 | 12.6                   |
|                                 | - Total              |           |                  | 8.4                  | 8.4                    |
|                                 | - Conjugated         |           |                  | 2.8                  | 4.2                    |
|                                 | - Unconjugated       | (IU/l)    | 25 - 92          | 50.8                 | 52.0                   |
|                                 | Alkaline phosphatase |           |                  | 10.3                 | 19.4                   |
|                                 | SGOT                 |           |                  | 8.4                  | 15.0                   |
| Heart rate                      | SGPT                 |           |                  |                      |                        |
|                                 |                      | beats/min | 70 - 80          | 89.4                 | 99.5                   |
| Blood pressure                  | Systolic             | mmHg      | 90 - 120         | 108.8                | 100.9                  |
|                                 | Diastolic            | "         | 60 - 80          | 71.9                 | 69.4                   |

**Table 2. Data of patients from the Infectious Disease Hospital, Rangoon. (Mean values)**

|   |                                        |                    |        |
|---|----------------------------------------|--------------------|--------|
| 1 | Age (in years)                         | -                  | - 29.2 |
| 2 | Body Weight (Kgm)                      | -                  | - 46.0 |
| 3 | No. of stools passed                   | - before drug adm. | - 12   |
|   |                                        | - after drug adm.  | - 8    |
| 4 | Dehydration (on admission)             | - Grade IV         | - 7    |
|   |                                        | - Shock*           | - 9*   |
| 5 | Amount of IV fluid therapy (in litres) |                    | - 5.1  |
| 6 | Diarrhoea checked                      | - within 48 hours  | - 12   |
|   |                                        | - within 72 hours  | - 4    |
| 7 | Culture                                | - Cholera positive | - 8    |
|   |                                        | - Cholera negative | - 8    |
| 8 | Medication                             | - Tetracycline     | - 13   |
|   |                                        | - Chloramphenicol  | - 3    |

\*Note: In these patients, the study was conducted only after recovery from shock state through initial rehydration therapy.

Figure 1 showed the comparison of the mean serum concentration-time profile of the two groups studied.

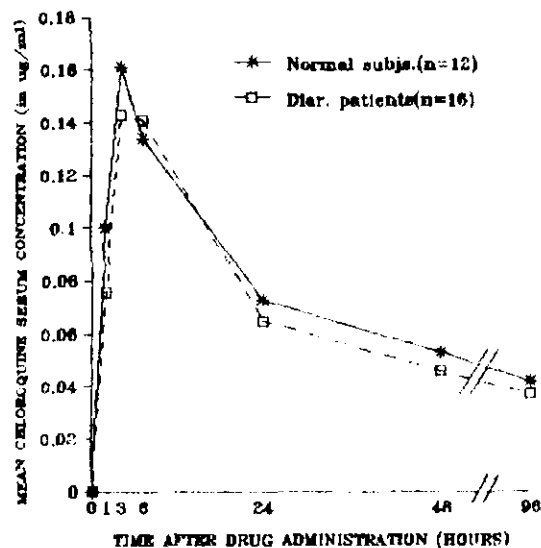


Figure 1. Comparison of mean serum chloroquine concentration between different groups

From the time of oral administration of 300 mg chloroquine base, the serum levels in normal healthy volunteers (the control group) increased rapidly with the absorption half-life of 0.79 hours ( $K_{ab}=0.89 \text{ hr}^{-1}$ ), reaching the peak serum level of 0.175 µg/mg at 4.77 hours and declining with an elimination half-life of 48.16 hours ( $K_{el}=0.014 \text{ hr}^{-1}$ ). The elimination phase was biexponential which include a rapid alpha phase with the half-life of 6.33 hours ( $\alpha=0.1 \text{ hr}^{-1}$ ) and the terminal elimination beta phase decli-

ning slowly with the half-life of 79.09 hours ( $\beta=0.0083 \text{ hr}^{-1}$ ). The apparent volume of distribution ( $aVd$ ) was 31.30 l/Kg (1513.10 litres) and the total clearance ( $Cl$ ) was 519.34 ml/min., giving the AUC of 9.23 µg/ml.hr.. The pharmacokinetic parameters of chloroquine in healthy Myanmar subjects did not differ significantly with those reported in other countries by different authors. (Gustafsson *et al.* 1983; Frisk-Holmberg *et al.* 1984; White, 1985; White, 1988).

In the group of diarrhoeal patients, the serum levels of chloroquine increased more slowly than the controls with the absorption half-life of 1.14 hours ( $K_{ab}=0.65 \text{ hr}^{-1}$ ), reaching peak serum level of 0.180 µg/ml at 6.09 hours and declining with an elimination half-life of 44.26 hours ( $K_{el}=0.0163 \text{ hr}^{-1}$ ). The elimination phase was also biexponential which include a rapid alpha phase with the half-life of 5.89 hours ( $\alpha=0.129 \text{ hour}^{-1}$ ) and a slow terminal beta phase with the half-life of 79.47 hours ( $\beta=0.009 \text{ hr}^{-1}$ ). The  $aVd$  was 32.18 litre/Kg (1481.24 litres) and the  $Cl$  was 572.95 ml/min., giving the AUC of 8.17 µg/ml. hr..

Comparison of mean pharmacokinetic data of the two groups above is shown in table 3. Statistical comparison between the parameters showed that the  $K_{ab}$  of diarrhoeal patients was significantly lower than healthy subjects ( $p < .001$ )

Table 3. Comparison of mean pharmacokinetic parameters of chloroquine in the healthy volunteers and diarrhoeal patients.

| Pharmacokinetic parameters | Units               | Control group<br>(n = 12) | Diarrhoeal group<br>(n = 16) | Level of<br>significance<br>(P value) |
|----------------------------|---------------------|---------------------------|------------------------------|---------------------------------------|
| Co                         | µg/ml               | 0.1918 ± 0.0402           | 0.2005 ± 0.0667              |                                       |
| K <sub>ab</sub>            | hours <sup>-1</sup> | 0.8904 ± 0.1573           | 0.6508 ± 0.1763              | < 0.001                               |
| T <sub>½ab</sub>           | hours               | 0.7921 ± 0.1338           | 1.1431 ± 0.3235              | < 0.01                                |
| Alpha                      | hours <sup>-1</sup> | 0.1144 ± 0.0319           | 0.1289 ± 0.0528              | > 0.5                                 |
| T <sub>½α</sub>            | hours               | 6.5300 ± 1.8654           | 5.8887 ± 2.0087              | > 0.5                                 |
| Beta                       | hours <sup>-1</sup> | 0.0088 ± 0.0007           | 0.0090 ± 0.0017              | > 0.5                                 |
| T <sub>½β</sub>            | hours               | 79.0958 ± 6.1468          | 79.4660 ± 15.6048            | > 0.5                                 |
| K <sub>el</sub>            | hours <sup>-1</sup> | 0.0147 ± 0.0025           | 0.0163 ± 0.0041              | > 0.5                                 |
| T <sub>½el</sub>           | hours               | 48.1571 ± 7.8695          | 44.2614 ± 11.8252            | > 0.5                                 |
| T <sub>max</sub>           | hours               | 4.7701 ± 0.5668           | 6.0914 ± 1.3092              | < 0.01                                |
| C <sub>max</sub>           | µg/ml               | 0.1747 ± 0.0395           | 0.1802 ± 0.0562              | > 0.5                                 |
| AUC <sub>0-a</sub>         | µg/ml               | 9.2325 ± 2.3297           | 8.1789 ± 1.6605              | > 0.5                                 |
| Cl                         | ml/min.             | 519.34 ± 166.94           | 572.95 ± 124.12              | > 0.5                                 |
| aVd                        | Litres              | 1513.10 ± 358.32          | 1481.24 ± 441.28             | > 0.5                                 |
|                            | Litres/Kg           | 31.2984 ± 7.4794          | 32.1773 ± 9.0347             | > 0.5                                 |

which result in a corresponding significant increase in the absorption half-life (T<sub>½ab</sub>) (p<0.01) and the time to reach the maximum serum concentration (T<sub>max</sub>) (p<0.01). A high degree of correlation was also seen between K<sub>ab</sub> and T<sub>max</sub> (r=0.9301, p<0.001) and between T<sub>½ab</sub> and T<sub>max</sub> (r = 0.9360, p<0.001).

The serum chloroquine concentration, on extrapolation to day 7 (168 hours), was 24.2±5.2 µg/l for healthy subjects (95% confidence intervals, 21.2-27.2 µg/l) and

22.3±5.0 µg/l for diarrhoeal patients (95% confidence intervals, 19.8-24.8 µg/l) and there was no statistical difference between the two groups studied.

#### DISCUSSION

The optimal therapeutic effect of a drug can be achieved only when sufficient concentration of the active drug reaches the site of action at the appropriate moment (Levy and Gibaldi, 1972) This concept also provides the basis of multidrug resistance in malarial patients since it is

well accepted that there is a failure of drug to reach toxic levels within the parasitized red cells. But before we conclude that the resistance is due to the resistant cells having acquired the capacity to actively extrude drug from the cell interior through multi-drug resistant transporter (Martin et al., 1987), one also need to make sure that the resistance is not due to other factors influencing drug bioavailability, of which the most important being drug absorption. Similarly, doubling the dose without knowledge of drug kinetics cannot be expected to improve the quality of treatment and may lead to hazards of overmedication.

The findings in the present study showed that chloroquine was rapidly absorbed from the gastrointestinal tract ( $K_{ab} = 0.89 \text{ hr.}^{-1}$ ,  $T_{1/2ab} = 0.75 \text{ hr.}$ ) and was also rapidly distributed ( $K_{el} = 0.11 \text{ hr.}^{-1}$ ,  $T_{1/2el} = 6.53 \text{ hr.}$ ) into various compartments in the body. A very large  $aV_d$  of more than 1000 litres denotes extensive tissue distribution and binding, and this is also responsible for the extremely slow metabolism and elimination of chloroquine. Because of rapid distribution and a very slow elimination, a biexponential curve was obtained when serum chloroquine concentration was plotted against time on a graph paper, Fig.(1).

The rate of absorption of chloroquine ( $K_{ab}$ ) was significantly reduced in

diarrhoeal patients leading to a significant increase in ( $T_{1/2ab}$ ) and a significant delay in  $T_{max}$ , the relationship indicated by a strong correlation between the parameters. However, the results obtained with other parameters indicated that neither the bioavailability (i.e.  $AUC_{0-\infty}$ ), nor the disposition of chloroquine was significantly altered by acute diarrhoea.

It has been stated that diarrhoea is not the result of decreased absorption of water and electrolytes from the gut, but is due to active secretion exceeding the intestinal capacity to reabsorb (WHO/CDD Ser. No. 80.2, 1984). The mechanism of absorption, including the active transport of sodium and glucose and the passive diffusion of water and other electrolytes, remains largely intact (Cutting and Langmuir, 1980). Chloroquine, being a weak base, is absorbed by passive diffusion, the speed of passage depending on lipid solubility and the degree of ionization of the drug. Since the passive diffusion mechanism remains intact, the extent of absorption probably remains unaffected. In contrast, the rate of absorption can be altered by many factors seen in diarrhoea. Change in pH in the gut lumen may quantitatively influence the degree of ionization and the rate of disintegration and dissolution of the drug (Gibaldi, 1979), and the altered intestinal motility is still believed to play some part by influencing gastric emptying, which is

often delayed in diseases of the gastrointestinal tract (Nimmo, 1979), and by accelerating the intestinal transit, which may lead to a decrease in the drug-mucosal contact time (Parsons and David, 1979). Thus, one or both factors may be involved in decreasing the rate of absorption of chloroquine, but the exact mechanisms involved and the resultant clinical significance remains to be clarified.

It has been reported that plasma levels as low as 20 µg/l (equal to approximately 0.6 µmol/l) was adequate for treatment of *P. falciparum* malaria (McLendon strain) (Berliner et al., 1948). Plasma levels of 20 µg/l during six days of treatment were also reported to be effective in 20 non-immune volunteers; (W.H.O Tech. Rep. Ser. No. 296, 1965), but recrudescence occurred when plasma levels were lower than 20 µg/l. At present, effective plasma level in man is accepted as 30 µg/l against drug-sensitive *P. falciparum* and 15 µg/l against *P. vivax* (W.H.O Monograph Series No. 27, 1981).

The effectiveness of chloroquine on malaria parasites with such low concentrations is believed to be due to the preferential accumulation of chloroquine within the parasitized erythrocytes to the extent of at least two orders of magnitude greater than the concentration outside (Polet and Barr, 1968).

In the present study, the serum chloroquine reached a mean concentration of 90 µg/l (4.5 times that of the MIC) in the first hour and persisted well above the MIC up to 96 hours in all patients studied. The  $C_{max}$  obtained in all groups was also well within the maximum tolerable dose of 1.25 µmol/l (approximately 400 µg/l) and thus, unlikely to give side effects (Fachlmann et al., 1981). But the serum concentration at day 7 was seen to fall below the suppressive concentration of 30 µg/l in both groups indicating that effective prophylaxis may be questionable during this period. A similar finding was reported by Frisk-Holmberg et al., (1984) that a weekly prophylactic dose of 300 mg chloroquine produced an initial plasma concentration below the desired level at the end of the first week of treatment but adequate levels were reached in subsequent weeks due to the cumulative effect of chloroquine. Thus, the present study seem to support the proposal of the above authors that, if the 300 mg regime is to be kept, it should be started earlier (about two weeks) before an entrance to an infected area, if not, one should take a loading dose of 600 mg on the day of arrival followed by the weekly regime for a period for which exposure is anticipated. But it should be noted that diarrhoea is not the reason for dose adjustment if there is no vomiting.

Since different strains of *P.*

*falciparum* are not uniformly sensitive to antimalarial drugs (W.H.O Tech. Rep. Ser. No. 296, 1965), a wider study on Myanmar patients with malaria is necessary to obtain data regarding serum levels of the drug and the sensitivity of different strains in order to assure optimum treatment.

A similar study consisting of serum and erythrocyte concentration of chloroquine in patients with acute diarrhoea was conducted by Rombo *et al.*, (1982) in Sweden. No significant difference was reported between the controls and diarrhoeal patients. It was suggested that additional dose of chloroquine is not necessary for malaria prophylaxis in patients suffering from acute diarrhoea if there is no vomiting. These findings were in agreement with those in the present study.

In conclusion, the findings in the present study showed that:

(1) acute diarrhoea was found to decrease the rate, but not the extent of absorption of chloroquine.

(2) apart from the rate of absorption and the time taken for achieving maximal drug concentration, serum concentration of chloroquine achieved was not significantly different between diarrhoeal and non-diarrhoeal subjects throughout the period of study. Thus, dosage adjustment is not necessary in acute diarrhoea, if there is no

vomiting.

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Effect of cholera toxin on intestinal lysosomal enzymes in the suckling rat

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Lysosomal function, namely lysosomal enzyme activities and lysosomal membrane integrities were investigated in suckling rats which received cholera toxin intragastrically and in shamoperated controls using two typical lysosomal enzymes, acid phosphatase and cathepsin D. It was found that the maximal specific activities of both the enzymes were decreased in the infected group when compared with the control suggesting a reduction in the ability of intracellular digestion by lysosome. On the other hand, the value of the ratio between maximal and basal activity (an expression of the degree of retention of enzyme by lysosome or lysosomal membrane stability) of acid phosphatase was significantly increased suggesting lesser extent of leakage of the enzyme into cytoplasm when compared with the control. However, there was no difference in the lysosomal membrane stability when measured in terms of cathepsin D. This discrepancy between the two enzymes was explained by their latencies.

INTRODUCTION

*Vibrio cholerae* has been identified as one of the enteric pathogen isolated from stools of patients with acute diarrhoea in a suburban community in Myanmar (1). *V. cholerae* produces an enterotoxin (cholera toxin-CT) which has the properties and action closely related to those of heat labile toxin (LT) of enterotoxigenic *Escherichia coli* (2). Although there have been many studies on the mechanism of fluid secretion due to the toxins of *V. cholerae* and *E. coli* (2), relatively little is known about the effects of the toxins on subcellular organelles of intestinal epithelial cells. Recently, Aye-Kyaw et al. (3) have indicated that the heat stable toxin of *E. coli* mainly

affects lysosome by increasing the intestinal lysosomal enzyme activities and lysosomal membrane stability. Walker and Isselbacher (4) also indicated that lysosome may play an important role in the uptake and transport of macromolecules by intestinal cells. In this paper, we have studied the alterations of lysosomal enzymes in the intestine of suckling rat infected with cholera toxin.

MATERIALS AND METHODS

Experimental

Five suckling Wistar rats, which served as controls, were given 0.1 ml intragastric injections of 0.8% (w/v) Evans blue dye while another five suckling Wistar rats, which showed positive toxin reaction and constituted the infected

group were given 0.1ml of cholera toxin (Inaba 5698, obtained as lyophilized form from Cherno-sero Therapeutic Research Institute, Japan) solution (containing 25 µg cholera toxin per ml) prepared in the same dye solution. The milk filled stomach of the rats could be identified below the left lung and between the liver and the spleen as a white patch. The proper delivery of the toxin solution coloured by the blue dye could be seen through the abdominal wall. After 3 hours, the animals were sacrificed by neck/head dislocation and the stomach were examined to confirm the existence of dye in the gut lumen. The intestines were then removed, and the gut weight and the remaining carcass weight were taken. For each rat, the ratio of gut weight to carcass weight was calculated and a ratio of more than 0.065 was taken as showing a positive effect (i.e. demonstrating a toxic secretory diarrhoea) according to Dean *et al.* (5) and Baselski *et al.* (6). The intestines were washed with chilled 0.25M sucrose containing 0.5% of 0.2M Tris-HCL buffer (pH 7.4) and then homogenized in (20 x weight of tissue ) ml of 0.25M sucrose with a Potter-Elvehjem homogenizer. The homogenates were used for lysosomal study.

#### Lysosomal study

In this study, the experimental design was primarily devoted to measuring lysosomal enzyme activity and lysosomal membrane integrity, using two

typical lysosomal enzymes, acid phosphatase (EC 3.1.3.2) and cathepsin D (EC 3.4.23.5). For each enzyme studied, the homogenate was divided into two equal portions marked 'maximal' and 'basal' and then equilibrated in 0.1 M acetate buffer (pH 5.0). To the maximal portion, Triton X-100 was added at a final concentration of 0.1% in incubation mixture (the optimal concentration for the complete destruction of latency of lysosomal enzymes) as recommended by Bjorkerud *et al.* (7) and Aye-Kyaw *et al.* (3,8). An equal volume of distilled water was added to the basal portion. They were incubated at 37°C for 1hr with gentle shaking and centrifuged at 11,000g for 30min to remove unlysed particles, and the enzymes released into supernatant were assayed. All steps were carried out at 4°C whenever necessary.

#### Enzyme assays.

Acid phosphatase was assayed at pH 5.0 according to the method described by Aye-Kyaw (9), using β-glycerophosphate as substrate and the released inorganic phosphate after termination of the reaction with 10%(w/v) perchloric acid was measured by the method of Aye-Kyaw *et al.* (10) Cathepsin D was assayed according to the method described by McDonald and Chen (11). Protein was determined by the method of Miller (12).

Enzyme activities were expressed as milliunits (mU) per mg protein in the

Table 1. Gut; Carcass ratio values of the control and the infected animals

| Group                           | Gut weight (g) | Carcass weight (g) | Gut;carcass ratio |
|---------------------------------|----------------|--------------------|-------------------|
| Control 1                       | 0.404          | 8.029              | 0.050             |
| 2                               | 0.389          | 7.718              | 0.050             |
| 3                               | 0.291          | 7.376              | 0.039             |
| 4                               | 0.434          | 8.449              | 0.051             |
| 5                               | 0.390          | 7.576              | 0.051             |
| Mean $\pm$ SEM                  |                |                    | 0.048 $\pm$ 0.002 |
| Infected 1                      | 0.512          | 6.530              | 0.078             |
| 2                               | 0.570          | 6.120              | 0.093             |
| 3                               | 0.582          | 8.665              | 0.067             |
| 4                               | 0.590          | 7.687              | 0.076             |
| 5                               | 0.425          | 6.442              | 0.066             |
| Mean $\pm$ SEM                  |                |                    | 0.077 $\pm$ 0.011 |
| Significance (Control-Infected) | P<0.05         |                    |                   |

homogenate where 1 U is equal to 1  $\mu$ mole of substrate transformed or product produced per minute. Statistical analyses were performed using Students unpaired  $t$  test.

## RESULTS

Table 1 shows the values of gut weight, carcass weight and gut: carcass ratio of each and individual rat in the control and the infected group. All the animals in the infected group showed gut: carcass ratio more than 0.065 and therefore could be taken as showing positive secretory effects according to Dean et al. (5) and Baselski et al. (6). Besides, the group-wise comparison of gut: carcass ratio showed a significant increase in the infected group making the group-wise comparisons of lysosomal data in the present study valid.

Figure 1 shows the maximal specific activities of intes-

tinal lysosomal acid phosphatase and cathepsin D (released by Triton X-100) in the control and the infected groups.

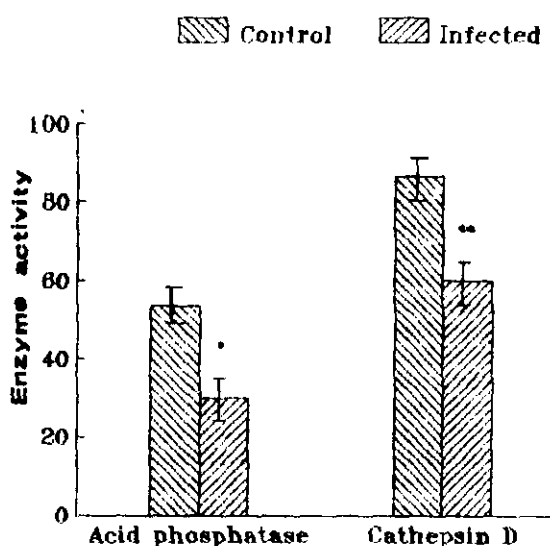


Figure 1. Intestinal total (maximal) lysosomal enzyme levels (released by triton X-100) in the control and infected groups. Each value represents the mean  $\pm$  SEM. Enzyme activities are expressed as millunit per milligram protein \*P < 0.01 (compared with the respective control). \*\*P < 0.05 (compared with the respective control)

It was found that the maximal specific activities of the enzymes were significantly decreased in the infected group when compared with those of the control group probably indicating the reduction in the ability to digest macromolecules held within the cell by intestinal lysosomes after infection with cholera toxin.

Figure 2 shows the ratio between maximal and basal (latent) activity of lysosomal acid phosphatase and cathepsin D.

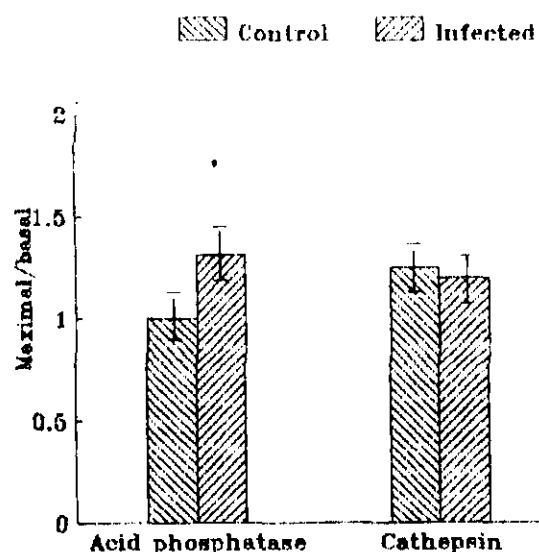


Figure 2. The ratio between maximal and basal (latent) activity of lysosomal enzymes in control and infected groups. Each value represents the mean  $\pm$  SEM. \* $P < 0.01$  (compared with the respective control)

It was found that the maximal : basal ratio of acid phosphatase was significantly increased in the infected group when compared with the control whereas cathepsin D did not show any significant changes. This ratio has been

interpreted as an indicator of the stability of lysosomal membrane (3,7,8) and an increase in this ratio would probably suggest an increased stability of lysosomal membrane.

## DISCUSSION

Although there have been many studies on the clinical aspect and the mechanism of secretion in acute diarrhoea caused by the toxins carried out on the effect of the toxins on subcellular organelles. Recently, Aye-Kyaw et al. (3) reported that *E. coli* stable toxin (molecular weight approx.5000) mainly affects intestinal lysosomes in suckling rat by increasing their enzyme activities and membrane stability, which is consistent with the concept that lysosomes may be involved in the uptake and transport of macromolecules by intestinal cells (4). However, administration of another toxin of *E. coli* namely labile toxin to adult rat intestine did not show any changes in the subcellular organelles on intestinal mucosa (13).

In the present study, lysosomes seem to be affected again by cholera toxin since alterations in lysosomal enzymes and membrane stabilities were observed. The reason for the discrepancy in the effects of cholera toxin, *E. coli* stable toxin and *E. coli* labile toxin on intestinal cell may lie in the difference in the animal models used. In the present study and in the study with

*E. coli* stable toxin (3) the suckling rats were employed in contrast to the mature rats used in the study of *E. coli* labile toxin infection (13). Moreover, it should be noted that infant intestinal cells are more permeable to macromolecules than mature intestinal cells (4). In contrast to the study with *E. coli* stable toxin (3), the decrease in maximal activities of the lysosomal enzymes in the present study could be due to the inhibitory effect of cholera toxin on lysosomal enzymes.

The decrease in intestinal lysosomal enzyme activities found in the present study may suggest a decrease in intracellular digestion by the lysosome. For lysosomal membrane stability, a discrepancy was found between acid phosphatase and cathepsin D, the reason for which may probably lie in that latency of cathepsin D even under normal conditions is much more easily destroyed than acid phosphatase (that is, cathepsin D is not as latent as acid phosphatase and could be released much more easily than acid phosphatase from lysosome) (14). However, it should be noted that acid phosphatase is more specific for lysosome and is usually used as the marker enzyme for lysosome.

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Detection of Russell's viper venom antigen in human  
snake bite victims during the course  
of antivenom therapy

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Venom levels before and after administration of 40 ml of liquid and lyophilised monovalent antivenom in 67 cases of human Russell's viper bite were measured by Enzyme-linked immunosorbent assay (ELISA) technique. A venom level of 60 ng/ml or more was detected in most patients (41/46) who presented with nonclotting blood. In 13 out of 16 cases treated with 40 ml of liquid preparation and 10 out of 25 cases treated with the same dose of lyophilised antivenom, venom level of 60 ng/ml or more were not neutralised within 2 hr. of therapy, indicated that 40 ml antivenom was not sufficient in treating 50% of the patients with nonclotting blood. The neutralising ability of lyophilised preparation is superior to that of the liquid antivenom used in the study.

## INTRODUCTION

Russell's viper bite is an important health hazard of our farmers, the case fatality is about 10%. The specific treatment of snake bite is the early administration of a sufficient quantity of specific potent antivenom, aiming at an immediate neutralisation of venom. The choice of antivenom depends on the species of snake. The current practices in Myanmar is to give 40 ml of antivenom to any snake bite victim who gives a convincing history of Russell's viper bite. The assumption is that the maximum yield of venom through milking is 500-700 mgs and that one bite of snake will deliver 1/10 of the maximum yield. Since 1 ml of antivenom can neutralise

2 mgs of venom, the current dose of 40 ml antivenom is assumed to be sufficient for every case of snake bite (1). Using ELISA technique described by Theakston *et al.* (2) we were able to measure Russell's viper venom (RVV) antigen levels in serum and urine of RV bite patients (3). It has been used for monitoring the dose of antivenom administered as well as assessing the efficacy of different antivenom preparations.

## PATIENTS AND METHODS

### Patients

A total of 67 patients admitted to four township hospitals ( Kyauktaga,

Tharrawaddy, Nyaunglaybin and Insein) were studied during 1982-83.

### Methods

Of 67 cases, 27 received 40ml of lyophilised antivenom (one ampoule of antivenom is reconstituted with 10ml of sterile distilled water) and the rest were treated with the same dose of liquid antivenom. Blood samples were collected before and after at one hr and two hourly intervals after antivenom up to 6 hours after therapy. A simple bedside clotting test was done according to the method described by Warrell, et al.(4).

The micro-ELISA technique used was the one described by Theakston et al. (2). Antigen used in the assay was pooled crude vacuum dried venom of Myanmar *Vipera russelli* and antivenom was the lyophilised and liquid preparations of ammonium sulphate precipitated, enzyme (pepsin) refined equine antiserum. Both preparations were supplied by the biological laboratories of Myanmar Pharmaceutical Industries (MPI), Yangon. Alkaline phosphatase type I (Sigma) was used for conjugation of antivenom according to the method described by Engvall and Perlmann, (5). Flat bottom polystyrene microtitre plates were supplied by Dynatech Co. and Chromoscan EIA (BIOTEK) was used for measuring optical density of the final reactive product. Venom concentration was measurable to a limit of 10 ng/ml. The coefficient of

variation of the method in our laboratory is 13%.

### RESULTS

Of 67 patients studied 46 had nonclotting blood at the time of admission. Nine patients with nonclotting blood were treated with 40ml of liquid antivenom initially, six received an additional 20ml of the same antivenom after 2hr., two after 4hr., and one after 6hr., following the initial dose. The time elapse between bite and treatment ranged from 0.30hr. to 34hr. Initial serum venom levels ranged from 15ng to 175ng/ml.

#### Venom levels following anti-venom therapy

It is observed that among the patients with nonclotting blood in 13 out of 16 cases treated with 40ml of liquid antivenom and in 10 out of 25 cases treated with the same dose of lyophilised antivenom, venom levels of 60ng/ml or more were not neutralised within 2 hr, following therapy.

#### Venom level vs initial clotting test

Nonclotting blood was observed initially in 46/67 cases we studied. 41 out of 46 patients with nonclotting blood had venom levels of more than 60ng/ml (Fig. 1). The remaining five with nonclotting blood had venom levels less than 60ng/ml, four being late arrivals (3.20hr. to 21.00hr. after bite) and one who arrived 1.30hr after bite.

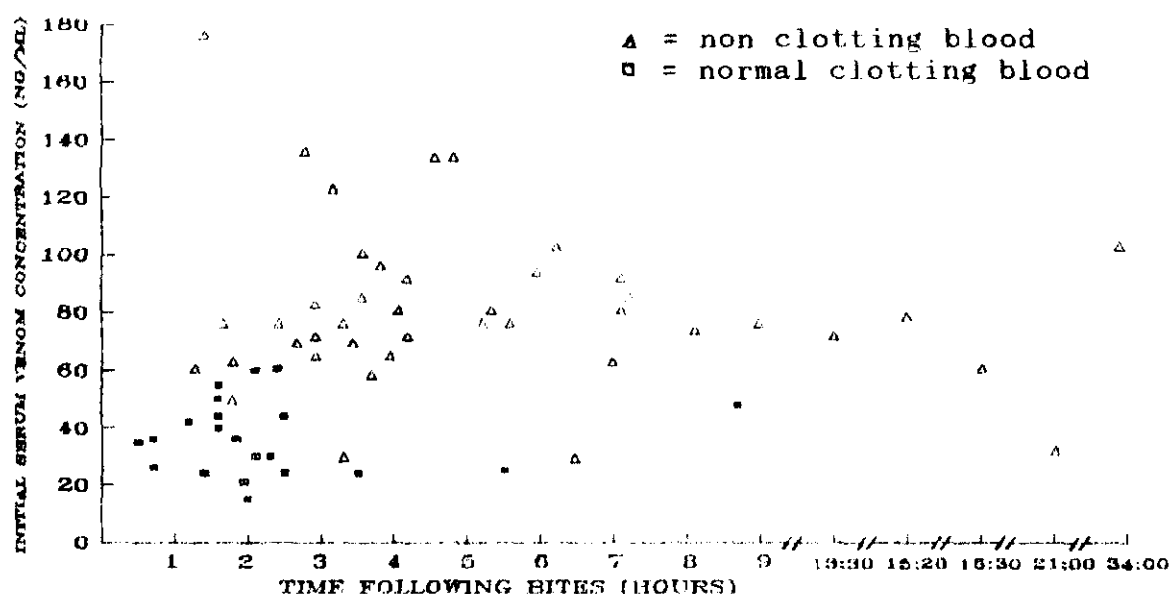


Figure 1. Initial serum venom levels of Russell's viper bite victims

Of 21 cases presenting with normally clotted blood all except two had venom levels less than 60ng/ml.

#### Subsequent venom levels vs antivenom dose

No venom antigen could be detected in the 6hr samples following therapy in groups treated with a single 40ml dose of lyophilised antivenom, whereas venom levels ranging from 20ng to 60ng/ml could still be detected in sera of patients who received 40ml initially and an additional 20ml of liquid antivenom at 2hr, following initial therapy.

#### DISCUSSION

Initial venom levels following bite ranged from 15 ng to 175 ng/ml indicating that the

amount of venom injected by bites is variable. The initial venom level is also influenced by the time elapsing between bite and measurement and the rate of absorption of venom which inturn depends on the motility of the bitten limb and whether venom was deposited superficially or deeply in the muscles (3). The role of tourniquets is uncertain.

Venom levels measured following administration of 40 ml of liquid and lyophilised antivenoms to patients with nonclotting blood are shown in Figure 2. It was found that a venom level of  $15 \pm 5$  ng/ml (SE) was detected in 6 hr. sample following administration of 40 ml of liquid antivenom in contrast to the results obtained by our previous study (3) where venom

level of  $13 \pm 5$  ng/ml (SE) could still be detected 54 hr. following antivenom therapy.

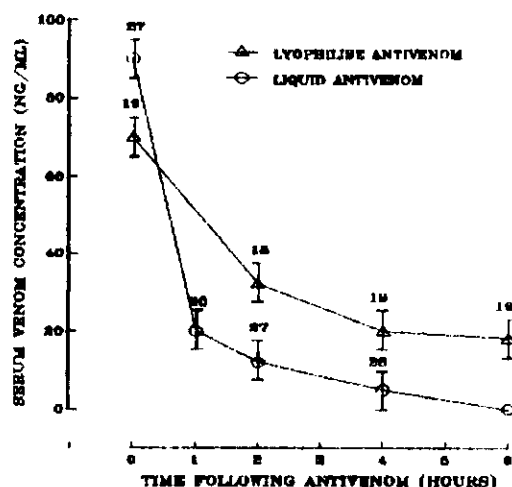


Figure 2. Venom clearance in Russel's viper bite victims following administration of 40 ml of liquid and lyophilised monospecific antivenom. Mean  $\pm$  1 Standard error are given with the number of patients studied at each time after antivenom therapy

Significant quantitative differences in constituent of venom of snakes was observed within species, geographically and even from the same locality (6,7). The antigenic differences in RVV from different localities might explain the variable potency of the antivenom. However, use of improperly stored liquid antivenom resulted in poor neutralisation of venom could not be ruled out.

In 10 out of 25 cases treated with 40 ml of lyophilised antivenom, venom levels of 60 ng/ml or more were not neutralised in 2 hr. following therapy suggested that more

antivenom would be needed to achieve prompt neutralisation of venom since this dose was based on neutralisation of maximum yields of venom. Such tests may be misleading in predicting antivenom performance (8).

46/67 cases we studied had nonclotting blood; evidence of systemic envenomation. 41 out of 46 patients with nonclotting blood had venom levels of more than 60 ng/ml indicated that the nonclotting state is associated with initial venom levels of 60 ng/ml or more. Four late arrivals of 46, who presented with nonclotting blood, had venom levels ranged from 25 ng to 57 ng/ml may well have had venom levels exceeding 60 ng/ml or more earlier in the clinical course. A venom level of 50 ng/ml associated with nonclotting state was observed in one early arrival of 1.30 hr. after bite.

The persistence of venom antigen following repeated doses of liquid antivenom in patients with nonclotting blood (Figure 2) supports the impression that the neutralising performance of liquid antivenom was inferior to that of lyophilised antivenom. Since correct storage at  $+4^{\circ}$  C is essential for the maintenance of the potency of liquid antivenom (9), it is unlikely that most rural health centres and hospitals will meet this requirement. However, production and use of lyophilised antivenom should be encouraged.

## ACKNOWLEDGEMENT

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Detection of pathogenic bacteria in children  
with diarrhoea

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86 children ranging from one to three years old with diarrhoea from Yangon Children's Hospital and respective controls were included in this study. Isolation rates of major pathogens responsible for acute diarrhoea in children were Enterotoxigenic *Escherichia coli* (ETEC) 30.2 per cent, Enteropathogenic *E. coli* (EPEC) 8.1 per cent, *Salmonella* 1.2 per cent, *Shigella sonnei* 1.2 per cent, *Plesiomonas* 1.2 per cent and vibrios 1.2 per cent. In the control group the prevalence was found to be ETEC 9.3 per cent, EPEC 7.0 per cent, *Salmonella* 12.0 per cent, *S. Sonnei* 1.2 per cent, *Plesiomonas* 7.0 per cent and vibrios 1.2 per cent. The total pathogen isolated from diarrhoea and control cases were 46.5 per cent and 38.4 per cent respectively. It was also noted that *Salmonella* and *Plesiomonas* isolation rates were higher in the control group.

## INTRODUCTION

During the Multicentre Hospital based control study on the aetiological agents responsible for acute diarrhoea in children during the years 1982 and 1983, it was found that most of the bacterial agents were Enterotoxigenic *E. coli* out of the 7 major pathogens tested. There was a remarkable decrease in the isolation rate of other pathogenic organisms besides Enterotoxigenic *E. coli*. Thus, we are again aiming to find out a new pathogen besides 7 major pathogens which might be responsible for acute diarrhoea.

## MATERIALS AND METHODS

The study period ranged from 30-7-85 to 20-9-85. The study was conducted at Yangon

Children's Hospital and children between the ages of 1 and 35 months presenting with a history of acute diarrhoea of 72 hours or less was taken as diarrhoea cases. Diarrhoea was defined as a sudden increase in the frequency of stools (3 times/day) and/or increase in the liquidity of stools. An interval of 24 hours was designated to divide one episode from another. For each diarrhoea case, a child of the same sex, age ( $\pm 30$  days for cases 1 - 11 months of age;  $\pm 60$  days for cases 12 - 35 months of age), socio-economic status, geographic area and ethnic group who has not had diarrhoea within the past month was included as a control. During the study period 58 cases and controls were included in the less-than 12 months age group. The 12-35 months age group consisted of 28 cases and 28 controls.

### Collection of stool samples

From each case and control at least 5 ml of faeces was collected after admission. If the child was unable to excrete stool within one hour of admission, a specimen was obtained by insertion of a short lubricated rubber tube into the rectum. Specimens were placed in sterile screw-capped containers.

### Bacteriological methods

Bacteriological processing was done within 2 hours. Stools were plated directly onto MacConkey agar, *Salmonella shigella* (SS) agar, Deoxycholate Hydrogen Sulfide Lactose (DHL) agar (Nissui, Japan), TCBS (Thio-sulphate Citrate Bile salts Sucrose) agar, *Vibrio* Agar and Blood Agar. In addition, part of each specimen was cultured in enrichment broth of Selenite F and alkaline peptone water, and inoculated onto solid media for isolation after appropriate incubation. All these plates were examined for *Salmonella*, *Shigella* and *Vibrio* species by standard methods. *Salmonella*-like and *Shigella*-like colonies were further characterized biochemically and by serotyping. *Vibrio*-like colonies were further identified biochemically, and by serotyping and determining their salt tolerance properties.

For *E. coli* isolation, 3 - 10 lactose fermenting colonies with typical *E. coli* morphology were picked from

among the colonies grown from each stool sample. The other non-lactose fermenting organisms and suspected pathogens from different media were picked and further characterized by biochemical tests and by serotyping. For identification of Enterotoxigenic *E. coli* (ETEC) as heat labile (LT) toxin strains it was done by Chinese Hamster Ovary cell assay and BIKEN assays. For heat stable (ST) toxin strains it was done by using suckling mice as reported previously by Tin Aye et al. (1).

### RESULTS

Major bacterial pathogens were isolated from 46.5 per cent and 38.4 per cent of diarrhoeic and control stools respectively. When examined in different age groups it was observed that 37.9 per cent and 64.3 per cent of the pathogens were isolated from less than 1 year age group and from 1 - 3 year age group, respectively (Table 1). The major pathogen responsible for diarrhoea was found to be Enterotoxigenic *E. coli* 30.2 per cent and 9.3 per cent from diarrhoea and control groups respectively and found to be significant between the two groups studied. It was noted that in the diarrhoea group, 12 (14.0 per cent) were ST, 9 (10.5 per cent) were LT and 5 (5.8 per cent) were STLT. In the control group, 3 (3.5 per cent) were ST, 4 (4.7 per cent) were LT and 1 (1.2 per cent) were STLT. Thus ETEC was the commonest bacterial agent encountered in this study.

Table 1. Isolation of major pathogens responsible for acute diarrhoea in children (Yangon Children's Hospital)

|                                        | < 1 yr   |             | 1 - 3 yrs |             | Total<br>< 1-3 yrs | Total<br>< 1-3 yrs |
|----------------------------------------|----------|-------------|-----------|-------------|--------------------|--------------------|
|                                        | Diar (A) | Control (B) | Diar (C)  | Control (D) | Diar (A+C)         | Control (B+D)      |
| No of cases tested                     | 58       | 58          | 28        | 28          | 86                 | 86                 |
| Enterotoxigenic <i>E. coli</i> (ETEC)  | 16(27.6) | 6(10.3)     | 10(39.3)  | 2(7.1)      | 26(30.2)           | 8(9.3)             |
| ST                                     | 8(13.8)  | 3(5.2)      | 3(10.7)   | 0           | 12(14.0)           | 3(3.5)             |
| LT                                     | 7(12.1)  | 3(5.2)      | 2(7.1)    | 1(3.6)      | 9(10.5)            | 4(4.7)             |
| STLT                                   | 1(1.7)   | 0           | 5(17.9)   | 1(3.6)      | 5( 5.8)            | 1(1.2)             |
| Enteropathogenic <i>E. coli</i> (EPEC) | 5(8.6)   | 4(6.9)      | 2(7.1)    | 2(7.1)      | 7( 8.1)            | 6(7.0)             |
| <i>Edwardsiella</i>                    | 0        | 1(1.7)      | 1(3.6)    | 0           | 1( 1.2)            | 1(1.2)             |
| <i>Salmonella</i>                      | 1(1.7)   | 8(13.8)     | 0         | 2(7.1)      | 1(1.2)             | 10(11.6)           |
| <i>Shigella sonnei</i>                 | 0        | 0           | 1(3.6)    | 1(3.6)      | 1(1.2)             | 1(1.2)             |
| <i>Plesiomonas</i>                     | 0        | 3(5.2)      | 1(3.6)    | 3(10.7)     | 1(1.2)             | 6(7.0)             |
| <i>Vibrio</i>                          | 0        | 0           | 3(10.7)   | 1(3.6)      | 3(3.5)             | 1(1.2)             |
|                                        | 22(37.9) | 22(37.9)    | 18(64.3)  | 11(39.3)    | 40(46.5)           | 33(38.4)           |

Figures in parenthesis denote percentages.  
Diar = Diarrhoea

It was noted that Enteropathogenic *E. coli* (EPEC) and *Edwardsiella* isolation rates did not show significant difference between the two groups studied. It was observed that *Salmonella* isolation rate was higher in the control group (11.6 per cent), than in the diarrhoea group (1.2 per cent) and found to be significant between the two groups studied. *Shigella sonnei* was isolated from the 1 - 3 years age group, one from the diarrhoeal patient and one from the control group.

*Plesiomonas* was isolated from 1 case of diarrhoea (1.2 per cent) and 6 cases of control (7.0 per cent) and thus it was significantly higher in the control group than in the diarrhoea group. Of the 3 strains or vibrios isolated from diarrhoea cases (3.5 per cent), 2 were *V. cholerae* Inaba and 1 was *V. fluvialis*.

One strain of *V. mimicus* was isolated from the control group (1.2 per cent).

Serotypes of Enterotoxigenic *E. coli* (ETEC) Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E. coli* (EIEC) were shown in Table 2. Out of 60 strains, 39 strains were

Table 2. Serotypes of *Escherichia coli* isolated

| Group        | Serotype | A | B | C | D | Total |
|--------------|----------|---|---|---|---|-------|
| ETEC<br>(39) | 08 H9    |   |   | 2 |   | 2     |
|              | 08 H11   |   |   |   | 1 | 1     |
|              | 023 H-   | 1 |   |   |   | 1     |
|              | 025 H-   | 2 |   | 2 |   | 4     |
|              | 027 H20  | 1 |   |   |   | 1     |
|              | 058 HUT  | 1 |   |   |   | 1     |
|              | 078 H-   | 1 |   |   |   | 1     |
|              | 086 H11  | 1 |   |   |   | 1     |
|              | 086 H18  |   | 1 |   |   | 1     |
|              | 0126 H12 | 1 |   | 3 |   | 4     |
|              | 0126 HUT |   |   | 1 |   | 1     |
|              | 0128 H27 | 1 |   |   |   | 1     |
|              | 0153 H10 | 1 |   |   |   | 1     |
|              | 0159 H16 |   |   | 2 |   | 2     |
|              | 0159 H34 | 1 |   | 1 |   | 2     |
|              | OUT H7   | 1 |   |   |   | 1     |
|              | OUT H9   | 1 |   |   |   | 1     |
|              | OUT H10  | 1 |   |   |   | 1     |

|                                         |      |     |    |    |    |    |
|-----------------------------------------|------|-----|----|----|----|----|
|                                         | OUT  | H12 | 1  |    |    | 1  |
|                                         | OUT  | H18 | 1  | 1  |    | 2  |
|                                         | OUT  | H41 |    | 2  |    | 2  |
|                                         | OUT  | H48 | 1  |    |    | 1  |
|                                         | OUT  | HUT |    | 1  | 2  | 3  |
|                                         | OUT  | H-  | 1  | 1  |    | 1  |
| <hr/>                                   |      |     |    |    |    |    |
| EPEC<br>(19)                            | 026  | H36 | 1  |    |    | 1  |
|                                         | 044  | H18 |    | 1  |    | 1  |
|                                         | 055  | H27 |    |    | 1  | 1  |
|                                         | 086  | H21 | 1  |    |    | 1  |
|                                         | 0111 | H4  |    | 1  |    | 1  |
|                                         | 0111 | H45 |    | 1  |    | 1  |
|                                         | 0114 | H2  | 1  |    |    | 1  |
|                                         | 0114 | H32 |    | 1  |    | 1  |
|                                         | 0119 | H6  | 1  |    |    | 1  |
|                                         | 0125 | H12 | 1  | 3  |    | 4  |
|                                         | 0125 | H21 |    | 1  |    | 1  |
|                                         | 0125 | H27 |    | 1  |    | 1  |
|                                         | 0125 | H-  |    | 1  |    | 1  |
|                                         | 0126 | H31 |    | 1  |    | 1  |
|                                         | 0127 | HUT |    | 1  |    | 1  |
|                                         | 0146 | H19 |    |    | 1  | 1  |
| <hr/>                                   |      |     |    |    |    |    |
| EIEC                                    | 0112 | H-  |    | 1  |    | 1  |
|                                         | 0124 | H-  |    | 1  |    | 1  |
| <hr/>                                   |      |     |    |    |    |    |
| Total                                   |      |     | 23 | 16 | 17 | 4  |
|                                         |      |     |    |    |    | 60 |
| <hr/>                                   |      |     |    |    |    |    |
| A= diarrhoea < 1 yr B= control < 1 yr   |      |     |    |    |    |    |
| C= diarrhoea 1-3 yrs D= control 1-3 yrs |      |     |    |    |    |    |

EPEC, 19 strains were EPEC and 2 strains were EIEC. Among ETEC the serotypes sought were 08H9, H11, 023H-, 025H-, 027H20, 058HUT, 078H-, 086H11, H18, 0126H12 HUT, 0128H27, 0153H10, 0159H16, H34. Among EPEC, the serotypes sought were 026H36, 044H18, 055H27, 086H21, 011H4, H45, 0114H2, H32, 0119H6, 0125H12, H21, H-, 0126H31, 0127HUT and 0146H19. Among EIEC the serotypes sought were 0112H-, 0124H-. The serotypes of *Salmonella* and *Shigella* isolated were shown in Table 3. The species included *S. krefeld*, *S. senftenberg*, *S. stanley*, *S. paratyphi B*, *S. derby* and *S. enteritidis*.

Mixed pathogens isolated from diarrhoea and control cases

were shown in Table 4. *Plesiomonas* mixed with other bacteria such as ETEC, or EPEC or *Vibrio* was found in 4 cases 1 from diarrhoea and 3 from

Table 3. Serotypes of *Salmonella* and *Shigella* isolated

| Genus             | Serotype           | A | B | C | D | Total |
|-------------------|--------------------|---|---|---|---|-------|
| <i>Salmonella</i> | <i>paratyphi B</i> |   | 1 |   |   | 1     |
|                   | <i>stanley</i>     | 1 | 2 |   |   | 3     |
|                   | <i>derby</i>       |   | 1 |   |   | 1     |
|                   | <i>typhimurium</i> |   |   |   | 1 | 1     |
|                   | <i>enteritidis</i> |   | 1 |   |   | 1     |
|                   | <i>krefeld</i>     |   | 2 |   |   | 2     |
|                   | <i>senftenberg</i> |   | 1 |   | 1 | 2     |
|                   |                    |   |   |   |   |       |
| <hr/>             |                    |   |   |   |   |       |
| <i>Shigella</i>   | <i>sonnei</i>      |   |   |   | 1 | 1     |
|                   |                    |   |   |   |   | 2     |

A = diarrhoea < 1 yr  
B = control < 1 yr  
C = diarrhoea 1 - 3 yrs  
D = control 1 - 3 yrs

control cases. *Salmonella* with EPEC was found in 3 of the control cases. *Shigella* mixed with ETEC was found in one and ETEC mixed with EPEC was found in 3 diarrhoea cases. Thus a total of 11 cases had mixed infection, 5 from diarrhoea cases and 6 from the control cases.

Relationship between ETEC and EPEC was shown in Table 5. A total of 16 strains producing ST were serotyped and 4 strains showed 0126 serogroups (25.0 per cent) with different H serotypes (3 were H12 and one untypable H). The other serotypes include 08H9, 025H-, 027H20, 078H-, 086H11,

Table 4. Mixed pathogens isolated from diarrhoea cases

|                             | < 1 yr    |              | 1 - 3 yrs |              | Total<br>Diar | Total<br>Control |
|-----------------------------|-----------|--------------|-----------|--------------|---------------|------------------|
|                             | Diar<br>A | Control<br>B | Diar<br>C | Control<br>D |               |                  |
| <i>Plesiomonas</i> + others | 0         | 2            | 1         | 1            | 1             | 3                |
| <i>Salmonella</i> + EPEC    | 0         | 3            | 0         | 0            | 0             | 3                |
| <i>Shigella</i> + ETEC      | 0         | 0            | 1         | 0            | 1             | 0                |
| ETEC + EPEC                 | 1         | 0            | 2         | 0            | 3             | 0                |

Diar = Diarrhoea

H18,0128H27 and 0153H10. 16 strains producing LT were

and the other serotypes identified were 0126H12, 08H9.

Table 5. Relationship between ETEC and EPEC

|         |          | < 1 yr     |            | 1 - 3 yrs |             |
|---------|----------|------------|------------|-----------|-------------|
|         |          | Diar(A)    | Control(B) | Diar(C)   | Control (D) |
| ST(16)  | 025 H-   | 086 H18    | 08 H9      |           |             |
|         | 027 H20  | OUT H-     |            |           |             |
|         | 078 H-   | OUT HUT    | 0126 HUT   |           |             |
|         | 0126 H12 |            |            |           |             |
|         | 0128 H27 |            |            |           |             |
|         | 0153 H10 |            |            |           |             |
| LT(16)  | OUT H18  |            |            |           |             |
|         | OUT H48  |            |            |           |             |
|         | 023 H-   | OUT H18    | 025 H-(2)  | 08 H11    |             |
|         | 025 H-   |            |            |           |             |
|         | 058 HUT  | OUT H41(2) | 0159 H34   |           |             |
|         | 0159 H34 |            |            |           |             |
| STLT(6) | OUT H7   |            | OUT HUT    |           |             |
|         | OUT H9   |            |            |           |             |
|         | OUT H12  |            |            |           |             |
|         | OUT H-   |            |            |           |             |
|         | OUT H10  |            |            |           |             |
|         |          |            | OUT H-     |           |             |

Figure in parentheses denote number of cases detected

Diar = Diarrhoea OUT = 0 untypable  
HUT = 8 untypable

serotyped, of which 2 strains showed 0159 serogroups with H34 serotypes (12.5 per cent).

The other serotypes identified were 08H11, 022H-, 025H- and 058HUT. 6 strains producing both ST and LT were serotyped and 2 of them showed 0159H16

## DISCUSSION

Diarrhoeal diseases which are often caused by microorganism or their toxins, constitute the greatest mortality and one of the greatest cause of morbidity on a global scale and depending on age and living conditions, average attack rates of diarrhoeal illnesses exceed one to six episodes per person per year and diarrhoea illnesses account for the greatest portion of the staggering childhood mortality and exceed 20 per cent in the poorest areas (2.7). Although the classic secretory enterotoxin of *V. cholerae* causes the most dramatic disease, *E. coli* that produce one or more enterotoxins are the commonest recognized causes of diarrhoea in tropical developing areas (7,8). As indicated from earlier reports ETEC is an important pathogen in tropical and developing countries particularly in infants under 5 years of age(1,2,3,4). From this study also it was noted that 30.2 per cent and 9.3 per

cent of ETEC were isolated from diarrhoea and control cases respectively. The higher isolation rate of ETEC as compared to other pathogens indicates that it plays an important role in the causation of diarrhoea in children. It was reported by various authors that 60 per cent of ETEC could be serotyped (9 - 14). In this study it was found that 61.5 per cent of ETEC could be serotyped and the serotypes identified were, 08,023, 025, 058, 078, 086, 0126, 0128, 0153 and 0159. It was also observed that isolation rate of *Salmonella* and *Plesiomonas* was higher in the control than in the diarrhoea group. The isolation rate of *Salmonella* and *Plesiomonas* was found to be 11.6 per cent respectively in the control group. *Plesiomonas shigelloides* was the new pathogen identified in this study. It was reported by various workers that *Plesiomonas* was isolated from samples of diarrhoeic stools in subtropical and tropical countries as Mali, Zaire, Kenya, Madagascar, Tahiti, Cuba, India; (15,16), Japan (17); Australia (18) and occasionally Europe and the United States (19). Carriers are quite rare in Europe (19) but was reported that it was more frequently observed in association with epidemics (17,18). The isolation rate of *Vibrio* was 3 per cent in the diarrhoea group and 1 per cent in the control group. 2 of them were found to be *V. cholerae* Inaba and one *V. fluvialis* from the diarrhoea group and they were from 1 - 3

years age group. From the control group also 1 *V. mimicus* was isolated and it was also from 1 - 3 years age group. One of the causes of the control group having a high isolation rate of pathogens might be the fact that in most developing countries the adult foods were heavily contaminated with bacterial pathogens (20). In Myanmar, food, currency notes, flies and water are highly contaminated with enteric pathogens (21,22,23) and may contribute to the dissemination of these pathogens.

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## Effect of dietary iron deficiency on some enzyme levels in growing rats

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Primary derangement in the levels of iron enzymes in iron deficiency have been reported by various workers. The present study was undertaken to determine whether secondary derangements in the levels of other non-iron enzymes could accompany in the primary derangement. Three groups of rats were fed synthetic diet for 85 days. Group I received the iron deficient diet *ad libitum*, group II was pair fed with group I but received supplemented iron and group III received the iron supplemented diet *ad libitum*. The following were the mean values of some biological data at time of sacrifice. Body weight of group I, II and III were 82.5 g, 96.5 g, 108.5 g and haemoglobin, 7.2 g%, 14.1 g% and 14.8 g% respectively. Enzyme levels were measured in different tissues of the three groups of rats. The levels of hepatic iron enzyme, catalase, as well as non-iron enzymes, glucose 6 phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GD) and malate dehydrogenase (MD) were significantly decreased in the iron deficient group. The decrease was more pronounced in the levels of catalase. However, lactate dehydrogenase did not show any significant changes. The enzymes were unaffected in kidneys and spleen except catalase, which decreased in group I.

### INTRODUCTION

Iron deficiency anemia is one of the main nutritional problems in the world today. Some investigators have pointed out a preponderance of underweight children among those with nutritional iron deficiency anemia. Several surveys among the various population groups of Myanmar showed that iron deficiency and iron deficiency anemia is a widespread health problem in our country(1,2). Accumulated evidence over the past years revealed for optimum growth and development and therefore, the impact of this element insufficiency on the physical and mental development needs urgent elucidation. With these views in mind, the effect of dietary iron deficiency on some vital enzymes in cellular functions and development are

investigated in this study.

Depression of iron containing enzymes(catalase and cytochrome oxidase) and aconitase, which requires iron cofactor for its activity, in iron deficient rat tissues have been reported (3,4,5). The derangement in the levels of iron dependent enzymes were found to be associated with derangement in the levels of iron independent enzymes, such as xanthine oxidase, glucose 6 phosphate dehydrogenase (G-6-PD) and glutamate oxaloacetic transaminase (GOT) (6,7). In this study, therefore, is extended the measurement of dehydrogenases, which contribute the formation of cellular energy adenosine triphosphate(ATP) by generating substrates for Kreb's (Citric Acid) cycle or by feeding reduced coenzymes (pyridine

nucleotides) into cytochrome system. Activities of catalase and G-6-PD were reexamined. In order to explore the role of degradative process as a contributing factor in lowering enzyme levels, lysosomal activity was examined by measuring the marker enzyme acid phosphatase activity.

## MATERIALS AND METHODS

### Enzyme determination

Enzyme assays were made in tissue homogenates prepared in 0.25 M sucrose. After being minced, the chilled tissues were homogenized in 8 volumes of 0.25 M sucrose containing 0.01 M EDTA for one minute in a Potter Elvehjem electric homogenizer at top speed. The cells were lysed by freezing and thawing the homogenates ten times, and were centrifuged at 100,000 x g for 1 hour in a Hitachi Ultracentrifuge, 55 P 2. The sedimented pellets were resuspended in 0.1 M NaCl, 0.01 M with respect to phosphate buffer pH 7 and sonicated for 1 minute. Large particles from the sonicated pellets were removed by centrifugation at 6,000 x g for 20 minutes. The enzymes in the supernatants obtained from before and after sonication were separately assayed for enzyme activities. It was found after freezing and thawing more than 50% of the enzyme activities were associated with insoluble cell pellet fraction and, therefore, sonification step was found to be necessary for complete extraction. All the steps were carried out at 4°C whenever necessary.

Catalase activity was measured

by the method used by Thin-Thin-Hlaing and Villela(8) using hydrogen peroxide as substrate. Glucose 6 phosphate dehydrogenase (G-6-PD) was assayed by the method of Horecker and Smyrniotis(9), glutamate dehydrogenase (GD) by the method of Strecker(10), lactate dehydrogenase (LD) by Neilands(11) and malate dehydrogenase (MD) by Lowry(12). Acid phosphatase was assayed by the method described by Aye-Kyaw (13) and protein concentration by Lowry(14), as modified by Miller(15).

### Haematological measurements

Standard methods described by Dacie and Lewis (16) were used. Hepatic non-heme iron concentration was determined by the method described by Torrance and Bothwell (17).

### Diet and experimental

Post-weanling Albino rats aged 30 days were used for the experiment. The iron deficient diet was prepared according to the method described by Vitale, *et al.*(18). The iron deficient diet comprised the following: glucose 658 g, casein (vitamin free)200 g, choline chloride 5 g, peanut oil 100 g, salt mixture 22 g and vitamin mixture 5 g per Kg of the diet (19). The iron supplemented diet was prepared by the method of McCall, *et al.*(20) by adding 240 mg ferrous sulphate ( $\text{FeSO}_4 \cdot 12 \text{H}_2\text{O}$ ) to 1 Kg of iron deficient diet.

The rats were divided into three groups, each group consisting of four animals, and fed on a diet for 85 days. Iron deficient rats received the iron deficient diet, the amount was isocaloric with that of the iron deficient rats. Control rats received the iron supplemented diet *ad libitum*. This experiment was designed to differentiate the

under-nutrition induced in rats by restricted food intake without associated iron deficiency anemia.

The rats were placed in individual stainless steel metabolic cages. Iron contamination was avoided by using glass water bottles with rubber cap and stainless steel spout. Deionized water was allowed *ad libitum*.

Weights of the animals were recorded daily. Haemoglobin concentration (Hb) and packed cell volume (PCV) were determined weekly using tail blood samples. Reticulocyte count were made on the day of sacrifice. When the iron deficient animals became definitely underweight (i.e. 85 days on the diet) the animals were sacrificed by stunning and decapitation after being starved overnight. Liver, spleen and kidney were removed, rinsed in cold normal saline, blotted to dry and weighed. Tissue homogenates were prepared for enzyme assays and portions were taken for non-heme iron concentration determination as described under 'enzyme determination' and 'Haematological measurements'.

## RESULTS

### Haematological observations

Some haematological and biological data of the three groups of rats were shown in Table 1. It can be seen that the mean Hb and PCV values in the iron deficient group were 7.2 g% and 33.2% respectively, which were significantly lower than Hb 14.1 g% and PCV 45% in the pair fed group and Hb 14.8 g% and PCV 47.1% in the control group. The mean MCHC value and reticulocyte count of the iron deficient rats were 23.1% and 23.9% respectively, compared with MCHC 32% and reticulocyte count 2.95% of the pair fed rats and MCHC 32% and reticulocyte count 3.7% of the control respectively. It can be seen that hepatic non-heme iron

concentration of the iron deficient rats was 23ug/g, that of the pair fed rats was 137.8ug/g and of the controls was 997 ug/g, indicating an exhaustively depleted iron stores in liver of iron deficient rats. Thus, it is evident that severe iron deficiency anemia was induced in rats fed with iron deficient diet.

### Body weights

At the time of sacrifice, as had been shown in Table 1, the body weight of the iron deficient rats was 82.5 g of the controls (P is less than 0.02). Although mean weight of the pair fed rats appeared to be lower than that of the control group, it was not statistically significant. It is therefore, suggested that growth retardation is superimposed by specific iron deficiency.

### Liver enzymes

Total specific liver enzyme activities of three groups of rats were shown in Table 2. The total specific activities of the enzymes, catalase, G-6-PD and malate dehydrogenase (MD), in the iron deficient group and in the pair fed group decreased significantly when compared to the control group. Glutamate dehydrogenase (GD) was depressed in the iron deficient group only. No significant changes were found between the three groups in lactate dehydrogenase and acid phosphatase specific activities. One interesting finding is that no significant changes were observed between iron deficient group and the pair fed group in the specific activities of all the enzymes measured, although there is an apparent decrease in the iron deficient group in the catalase, G-6-PD and glutamate dehydrogenase (GD) total specific activities.

# Kidney enzymes

The enzyme levels of kidney were shown in Table 3. In the kidneys,

only catalase and malate dehydrogenase levels were affected. In the kidneys, catalase levels of the iron deficient rats and

Table 1. Some biological and haematological data in different groups of at the time of sacrifice (i.e. after 85 days on the diet)

| Group                     | Body wt.<br>(g) | Hb<br>(g%) | PCV<br>(%) | MCHC<br>(%) | Reticulocyte<br>count (%) | Hepatic non-haem.<br>iron<br>(µg/g liver) |
|---------------------------|-----------------|------------|------------|-------------|---------------------------|-------------------------------------------|
| Group I<br>(Fe deficient) | 82.5±1.5        | 7.2±0.81   | 33±1.9     | 23±1.5      | 23.9±0.09                 | 23.0±1.06                                 |
| Group II<br>(Fair-fed)    | 96.5±0.8        | 14.1±0.50  | 45±1.4     | 32±1.1      | 2.9±0.22                  | 137.8±9.30                                |
| Group III<br>(Control)    | 108.5±3.5       | 14.8±0.60  | 47±1.2     | 32±0.9      | 3.7±0.57                  | 97.0±14.20                                |
| Significance              |                 |            |            |             |                           |                                           |
| I : II                    | N.S.            | P<0.001    | P<0.01     | P<0.05      | P<0.01                    | P<0.01                                    |
| I : III                   | P<0.02          | P<0.001    | P<0.01     | P<0.05      | P<0.01                    | P<0.02                                    |
| II: III                   | N.S.            | N.S.       | N.S.       | N.S.        | N.S.                      | N.S.                                      |

Each value represents the mean ± standard error for four rats. Probability 'P' is calculated by using Student's 't' test.

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

Hb, PCV and MCHC represent haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration respectively.

Table 2. Enzyme levels in extracts of liver homogenates in different groups of rats

| Enzyme             | Supernatant       | Pellet extract | Total      |            |
|--------------------|-------------------|----------------|------------|------------|
| C <sup>a</sup>     | Group I           | 3.75±0.26      | 2.72±0.75  | 6.47±0.80  |
|                    | Group II          | 5.50±0.27      | 2.73±0.43  | 8.23±0.55  |
|                    | Group III         | 8.30±0.43      | 3.44±0.35  | 11.74±0.55 |
|                    | Significance I:II | P < 0.01       | N.S.       | N.S.       |
|                    | I:III             | P < 0.05       | N.S.       | P < 0.01   |
|                    | II:III            | P < 0.05       | N.S.       | P < 0.05   |
| G-6PD <sup>b</sup> | Group I           | 11.82±1.85     | 15.10±2.10 | 26.92±2.50 |
|                    | Group II          | 17.04±1.90     | 11.45±1.60 | 28.49±3.30 |
|                    | Group III         | 19.85±0.60     | 16.72±0.60 | 36.57±1.20 |
|                    | Significance I:II | N.S.           | N.S.       | N.S.       |
|                    | I:III             | P < 0.05       | N.S.       | P < 0.05   |
|                    | II:III            | N.S.           | P < 0.05   | P < 0.05   |
| GD <sup>c</sup>    | Group I           | 22.55±3.10     | 46.54±12.5 | 69.09±11.0 |
|                    | Group II          | 37.86±3.60     | 47.05±7.0  | 84.94±8.0  |
|                    | Group III         | 44.49±3.50     | 58.74±11.0 | 103.23±6.0 |
|                    | Significance I:II | P<0.05         | N.S.       | N.S.       |
|                    |                   | P<0.05         | N.S.       | P<0.05     |
|                    |                   | N.S.           | N.S.       |            |

Table 2. (continued)

| Enzyme          |                   | Supernatant       | Pellet extract   | Total             |
|-----------------|-------------------|-------------------|------------------|-------------------|
| MD <sup>d</sup> | Group I           | 1.43 $\pm$ 0.31   | 2.08 $\pm$ 0.37  | 3.51 $\pm$ 0.57   |
|                 | Group II          | 1.46 $\pm$ 0.01   | 2.00 $\pm$ 0.20  | 3.46 $\pm$ 0.60   |
|                 | Group III         | 1.93 $\pm$ 0.23   | 3.00 $\pm$ 0.38  | 4.93 $\pm$ 0.50   |
|                 | Significance I:II | N.S.              | N.S.             | N.S.              |
|                 | I:III             | N.S.              | N.S.             | P<0.50            |
|                 | II:III            | N.S.              | N.S.             | P<0.05            |
| LD <sup>e</sup> | Group I           | 208 $\pm$ 15.5    | 181 $\pm$ 18.0   | 389 $\pm$ 29.0    |
|                 | Group II          | 189 $\pm$ 10.0    | 179 $\pm$ 6.5    | 368 $\pm$ 14.5    |
|                 | Group III         | 223 $\pm$ 18.0    | 180 $\pm$ 8.5    | 403 $\pm$ 8.1     |
|                 | Significance I:II | N.S.              | N.S.             | N.S.              |
|                 | I:III             | N.S.              | N.S.             | N.S.              |
|                 | II:III            | N.S.              | N.S.             | N.S.              |
|                 | Group I           | 98.07 $\pm$ 6.50  | 49.33 $\pm$ 5.00 | 147.40 $\pm$ 6.50 |
|                 | Group II          | 97.75 $\pm$ 5.00  | 57.37 $\pm$ 1.50 | 155.12 $\pm$ 4.50 |
|                 | Group III         | 117.40 $\pm$ 9.50 | 42.47 $\pm$ 4.50 | 159.87 $\pm$ 4.00 |
|                 | Significance I:II | N.S.              | N.S.             | N.S.              |
|                 | I:III             | N.S.              | N.S.             | N.S.              |
|                 | II:III            | N.S.              | N.S.             | N.S.              |

Table 3. Enzyme levels in extracts of kidney homogenates in different groups of rats

| Enzyme             |                   | Supernatant      | Pellet extract   | Total            |
|--------------------|-------------------|------------------|------------------|------------------|
| CA                 | Group I           | 1.40 $\pm$ 0.21  | 1.10 $\pm$ 0.08  | 2.50 $\pm$ 0.18  |
|                    | Group II          | 1.40 $\pm$ 0.14  | 1.14 $\pm$ 0.16  | 2.54 $\pm$ 0.11  |
|                    | Group III         | 1.95 $\pm$ 0.06  | 1.78 $\pm$ 0.46  | 3.73 $\pm$ 0.11  |
|                    | Significance I:II | P N.S.           | N.S.             | N.S.             |
|                    | I:III             | P < 0.05         | N.S.             | P < 0.05         |
|                    | II:III            | P < 0.05         | N.S.             | P < 0.05         |
| G-6PD <sup>b</sup> | Group I           | 20.18 $\pm$ 2.80 | 13.25 $\pm$ 1.10 | 33.43 $\pm$ 2.20 |
|                    | Group II          | 15.27 $\pm$ 2.40 | 11.79 $\pm$ 1.70 | 26.90 $\pm$ 1.40 |
|                    | Group III         | 15.32 $\pm$ 2.60 | 25.29 $\pm$ 7.00 | 40.61 $\pm$ 7.00 |
|                    | Significance I:II | N.S.             | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | N.S.             | N.S.             |
| GD <sup>c</sup>    | Group I           | 88.00 $\pm$ 8.50 | 97.82 $\pm$ 6.70 | 185.82 $\pm$ 24  |
|                    | Group II          | 108.00 $\pm$ 5.5 | 89.40 $\pm$ 5.83 | 147.40 $\pm$ 21  |
|                    | Group III         | 113.00 $\pm$ 6.0 | 48.50 $\pm$ 9.99 | 161.56 $\pm$ 19  |
|                    | Significance I:II | N.S.             | N.S.             | N.S.             |
|                    | I:III             | N.S.             | P < 0.01         | N.S.             |
|                    | II:III            | N.S.             | N.S.             | N.S.             |
|                    | Group I           | 1.09 $\pm$ 0.23  | 2.08 $\pm$ 0.45  | 3.17 $\pm$ 0.52  |
|                    | Group II          | 1.08 $\pm$ 0.21  | 5.63 $\pm$ 1.49  | 6.71 $\pm$ 1.50  |

Table 3. (continued)

| Enzyme          |                   | Supernatant     | Pellet extract   | Total           |
|-----------------|-------------------|-----------------|------------------|-----------------|
| MD <sup>d</sup> | Group III         | 1.86 $\pm$ 0.20 | 2.49 $\pm$ 0.45  | 4.55 $\pm$ 1.50 |
|                 | Significance I:II | N.S.            | P < 0.05         | P < 0.05        |
|                 | I:III             | P < 0.02        | N.S.             | N.S.            |
|                 | II:III            | P < 0.01        | N.S.             | N.S.            |
| LD <sup>e</sup> | Group I           | 341 $\pm$ 19    | 42.54 $\pm$ 1.60 | 383.54 $\pm$ 14 |
|                 | Group II          | 325 $\pm$ 17    | 41.58 $\pm$ 1.40 | 366.58 $\pm$ 13 |
|                 | Group III         | 361 $\pm$ 16    | 65.06 $\pm$ 1.10 | 426.06 $\pm$ 28 |
|                 | Significance I:II | N.S.            | N.S.             | N.S.            |
| AP <sup>f</sup> | I:III             | N.S.            | N.S.             | N.S.            |
|                 | II:III            | N.S.            | N.S.             | N.S.            |
|                 | Group I           | 53.68 $\pm$ 4.5 | 61.82 $\pm$ 3.0  | 115.50 $\pm$ 18 |
|                 | Group II          | 45.35 $\pm$ 9.5 | 81.86 $\pm$ 8.0  | 127.21 $\pm$ 15 |
| AP <sup>f</sup> | Group III         | 61.98 $\pm$ 7.5 | 79.74 $\pm$ 5.0  | 141.73 $\pm$ 78 |
|                 | Significance I:II | N.S.            | N.S.             | N.S.            |
|                 | I:III             | N.S.            | N.S.             | N.S.            |
|                 | II:III            | N.S.            | N.S.             | N.S.            |

Each value represents the mean  $\pm$  S.E. for four animals.

Probability is calculated by Student's 't' test. N.S. means not significant at the 5% probability level. Group I - Iron deficient, Group II- Pair-fed, Group III Control.

- (a) Specific activity of catalase (C) is expressed as millimoles H<sub>2</sub>O<sub>2</sub> per second per mg protein.
- (b) Specific activity of glucose-6-phosphate (G-6-PD) is expressed as enzyme units per mg protein.
- (c) Specific activity of glutamate dehydrogenase (GD) is expressed as enzyme units per mg protein.
- (d) Specific activity of malate dehydrogenase (MD) is expressed as micromoles of NAD reduced per hour per mg protein.
- (e) Specific activity of lactate (LD) is expressed as nanomoles NADP per mg protein.
- (f) Specific activity of acid phosphatase (AP) is expressed as nanomoles per minute per mg protein.

so also of the pair fed rats were found to be significantly lower than those of the controls. However, malate dehydrogenase shows a different result in which a significant decrease was found in the iron deficient group rats compared to the pair fed rats. No significant difference was found in other enzymes.

#### Spleen enzymes

The enzymes associated with

the extracts of spleen were also investigated and the results are shown in Table 4.

No significant differences were observed in the enzymes investigated except acid phosphatase, in which the specific activities in the iron deficient rats and the pair fed rats increased, compared to the controls. Unlike liver enzymes, kidney and spleen enzymes were relatively little affected by dietary stress employed in our study.

Table 4. Enzyme levels in extracts of spleen homogenates in different groups of rats

| Enzyme             | Supernatant       | Pellet extract   | Total            |
|--------------------|-------------------|------------------|------------------|
| C <sup>a</sup>     | Group I           | 0.14 $\pm$ 0.05  | 0.21 $\pm$ 0.02  |
|                    | Group II          | 0.16 $\pm$ 0.02  | 0.18 $\pm$ 0.02  |
|                    | Group III         | 0.14 $\pm$ 0.04  | 0.20 $\pm$ 0.03  |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | N.S.             |
| G-6PD <sup>b</sup> | Group I           | 15.57 $\pm$ 4.10 | 8.98 $\pm$ 2.50  |
|                    | Group II          | 18.38 $\pm$ 1.50 | 4.01 $\pm$ 0.80  |
|                    | Group III         | 15.05 $\pm$ 2.60 | 13.76 $\pm$ 3.00 |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | P<0.05           |
| MD <sup>d</sup>    | Group I           | 45.65 $\pm$ 4.0  | 29.14 $\pm$ 2.80 |
|                    | Group II          | 70.20 $\pm$ 5.0  | 32.51 $\pm$ 4.00 |
|                    | Group III         | 51.70 $\pm$ 7.0  | 31.99 $\pm$ 1.30 |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | N.S.             |
| MD <sup>d</sup>    | Group I           | 2.15 $\pm$ 0.49  | 0.97 $\pm$ 0.04  |
|                    | Group II          | 2.86 $\pm$ 0.23  | 1.37 $\pm$ 0.18  |
|                    | Group III         | 2.90 $\pm$ 0.50  | 0.87 $\pm$ 0.12  |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | N.S.             |
| LD <sup>e</sup>    | Group I           | 57.98 $\pm$ 0.5  | 63.98 $\pm$ 0.5  |
|                    | Group II          | 76.47 $\pm$ 0.0  | 56.32 $\pm$ 8.5  |
|                    | Group III         | 42.70 $\pm$ 3.5  | 57.42 $\pm$ 0.5  |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | N.S.             |
| AP <sup>f</sup>    | Group I           | 11.40 $\pm$ 0.45 | 12.88 $\pm$ 0.9  |
|                    | Group II          | 9.78 $\pm$ 1.20  | 12.49 $\pm$ 1.7  |
|                    | Group III         | 8.79 $\pm$ 0.60  | 8.36 $\pm$ 0.9   |
|                    | Significance I:II | N.S.             | N.S.             |
|                    | I:III             | N.S.             | N.S.             |
|                    | II:III            | N.S.             | P < 0.02         |

## DISCUSSION

As we have observed in our present study, growth retardation of iron deficient rats have already been reported by many workers(21, 22). Other studies indicated that bacteria and cultivated tissue cells need iron for optimum growth and mitosis (23, 24), and also showed that

iron appeared to be worked through nucleic acid metabolism. Therefore, lower Hb (anemia) alone dose not explain the impaired growth and function occurred in iron deficient subjects.

Beutler and Blaisdell (25, 26) in their early study reported that they could not detect any decrease of catalase activity

in iron deficient rat liver, but in later studies (4, 5) indicated that iron containing enzymes and proteins, cytochrome, cytochrome oxidase, catalase were found to be depressed in iron deficiency. Aconitase, an enzyme which requires iron for its activity, was found to be low in iron deficient human red cells (3). On the other hand, iron independent enzymes, xanthine oxidase, G-6-PD and glutamate oxalotransaminase (GOT) were also reportedly low in iron deficiency. In our present study, as has been mentioned, out of six liver enzymes (catalase, G-6-PD, GD, MD, LD and AP) measured four of them (i.e. catalase, G-6-PD, GD and MD) were found to be lowered in iron deficient rats. But, similarly low activities were found in rats fed with isocaloric amount of iron supplemented diet. Therefore, it is assumed that iron deficiency depressed many liver enzymes through impaired food intake. In iron deficient man, red blood cell glutamate oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) were found to be low and the reason being pyridoxine phosphate coenzymes depletion (27). The dehydrogenases we have studied in the present investigation require pyridine nucleotide coenzymes (NAD and NADP) for their activities. Therefore, status of these coenzymes in iron depletion needs further elucidation.

The implication of such impairment of cellular enzyme activities on the function and development of an animal should not be over speculated. But it has to be pointed out that these dehydrogenases are

directly involved in cellular energy (ATP) synthesis mainly by feeding substrates into Kreb's Citric Acid Cycle and by feeding reduced coenzymes (NADPH) in to cytochrome system, or are involved in many biosynthetic processes where reduced pyridine nucleotide coenzymes are indispensable.

The observation that iron depletion, not through food restriction lower glutamate dehydrogenase activity. The specific action of iron on the metabolism of glutamate acid needs attention because of this amino acid unique role in central nervous system, through the formation of  $\gamma$ -amino butyric acid which is normal regulator of neuronal activity.

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Short Report

The changing incidence of *plasmodium vivax* infection  
in subjects with malaria

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Malaria is still the most important parasitic disease in the tropics. Malaria is caused by a protozoan parasite of the genus *Plasmodium*, of which three species are commonly known to infect people living in Union of Myanmar. They are *P. falciparum*, *P. vivax* and *P. malariae*. Although *vivax* and *malariae* infections are not generally life threatening, they can cause a sometime severe acute illness. *Vivax* forms of malaria are characterized by relapses i.e. reappearance of symptoms of parasitaemia following a "latent" or symptomless period of up to five years. Chronic or repeated *vivax* malaria infection often causes impaired growth in children and loss of productive activity in adults. Therefore our findings will suggest closer supervision and effective measures against increasing

incidence of *vivax* malaria in some areas of Myanmar Naing-Ngan.

Hospital study

1832 patients from (1) Lashio Divisional Hospital, (2) Lashio Military Hospitals (100-bedded and 40-bedded) and (3) Tharyarwady General Hospital were included in the study. The time of collection of blood samples were between May to July during 1985, 1986, 1987 and 1989.

Village study

1514 subjects with malaria from villages and three sectors of Lashio Township were included in the study. The first study was in May 1984 and the second study was in July 1989.

## Examination of blood films

Taking of blood smears and staining with Giemsa were done according to standard methods. 50 thick fields were examined under oil immersion (100x) and thin smears were again checked for speciation of malaria parasites.

the same hospital.

The great increase of migration into the township and the population shifts within that area have led to a considerable rise in the transmission of malaria in Lashio Township. The same is true in Amazon region of

Table 1. Changing incidence rates of malaria in Lashio Township

| Year         | Villages & sectors              | Subjects examined | Per cent positive with malaria | Plasmodium species |       |       |
|--------------|---------------------------------|-------------------|--------------------------------|--------------------|-------|-------|
|              |                                 |                   |                                | Falciparum         | vivax | mixed |
| 1984<br>May  | Ho-paik<br>Naung-mon            | 800               | 30.3%                          | 21.6%              | 6.1%  | 2.6%  |
| 1989<br>July | Ho-paik<br>Naung-mon<br>9,10,11 | 714               | 48.8%                          | 28.0%              | 14.8% | 6.0%  |

The incidence of *P. falciparum* and *P. vivax* infections in subjects living in Lashio Township was summarized in Table 1. Out of 800 subjects examined, the incidence rates of *falciparum*, *vivax* and mixed infections were found to be 21.6%, 6.1% and 2.6% in 1984. But in 1989, the incidence rates of *vivax* and mixed infections were significantly increased to 14.8% and 6.0% whereas *falciparum* is slightly increased to 28.0%.

The same situation is found to be true in malaria positive patients attending to Tharyarwady Township General Hospital. The increasing trend of *P. vivax* infection was recorded in the years 1988 and 1989 with a percentage of 35% of all malaria infected patients (Figure 1). In the years 1985 and 1986, the *vivax* infection rates were less than 10% of the total malaria patients attending to

Brazil where the population growth rate of 5.04% during 1970 to 1980. There were

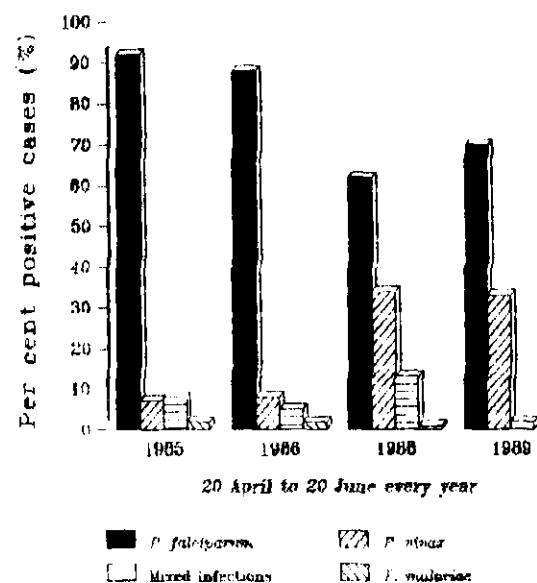


Figure 1. Increasing trend of *Plasmodium vivax* infection in malaria slide positive patients attending to Tharyarwady Township General Hospital (1985-1989)

mostly attracted toward the gold prospecting mining operations, and the settlement of agricultural areas along the Amazon high-way(1). The most majority of the immigrants were non-immunes, entering into regions where anopheline mosquitoes are abundant. Hardly surprisingly, transmission of malaria grow at a steady rate over the last 5 years in Lashio Township. Many travellers, soldiers, merchants, government servants, truck drivers and religious parties etc. can import the parasite, and any geographical changes could stimulate an increase in vector breeding sites (2).

If malaria returns to the country, it will be a disaster for public health and very difficult to re-eradicate. For speciation of malaria parasites, skilful and experienced personnel are needed. On the otherhand, good quality microscopes and better staining methods are also important. To have effective and correct treatment, the results given by the technicians must be precise.

If the results are not reliable, the effective treatment

could not be possible and the relapse of vivax malaria will be a never ending story.

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Abstracts of papers read at  
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**Alcoholic cardiomyopathy in Myanmar.** KHIN-YE-MYINT & THAZIN. Yangon General Hospital, Department of Health.

Early recognition of Alcoholic cardiomyopathy is important because complete abstinence from alcohol during the early stages may prevent diffuse myocardial fibrosis and irreversible chronic congestive heart failure. 31 chronic alcoholics admitted to New Yangon General Hospital over a period of one year were studied for cardiac abnormalities. 22 cases had signs of congestive heart failure and 9 cases did not have any clinical evidence of heart disease and were admitted for alcohol related diseases. 72.7% had evidence of low output cardiomyopathy and 27.3% presented with Beri Beri heart disease, diagnosed by history, clinical findings, electrocardiogram, Chest X-ray and Echocardiogram. Patients with Alcoholic cardiomyopathy presented with poor left ventricular function dilated chambers and reduced ejection function and fractional shortening on Echocardiogram, even after treatment. Cases with Beri Beri heart disease had characteristic electrocardiogram changes and normal or near normal Echocardiograms after treatment with Thiamine. There was no correlation between amount of alcohol consumption, duration of consumption and ejection fraction and fractional shortening. 11.1% of chronic alcoholics without clinical evidence of heart disease were found to have impaired left ventricular function on Echocardiogram. Echocardiogram provides a reliable non invasive means of assessing cardiac abnormalities in chronic alcoholics.

**The drug sensitivity patterns of *Plasmodium falciparum* in Myanmar (1990).** \*MYINT-LWIN, \*KYIN-HLA-AYE, \*MAUNG-MAUNG-MYA, \*YE-HTUT, \*\*KYAW-WIN & \*\*TUN-NYUNT-NAING. \*Parasitology Research Division, Department of Medical Research & \*\*Directorate of Medical Services, Ministry of Defence.

The *in vitro* sensitivity of *P. falciparum* to antimalarials was conducted during 1990 in Mawlamyaing, Lashio and Seik Phyu Township. There were 40 successful *in vitro* tests in Mawlamyaing, 39 in Lashio, 26 in Seik Phyu Township. Chloroquine was found to be highly resistant (95 to 100%) in Mawlamyaing and Lashio areas but 31% sensitivity was detected in Seik Phyu Township. Amodiaquine was found to be much more sensitive (38 to 67%) than chloroquine. EC<sub>50</sub> for chloroquine varies from 7.4 to 8.4 µmol/L for chloroquine and EC<sub>50</sub> for amodiaquine varies from 0.58 to 1.6 µmol/L.

In all the areas studied sulfadoxine/pyrimethamine sensitivity was seen in 65 to 80%. All the isolates from the three areas were 100% sensitive to mefloquine and were also highly sensitive (93 to 100%) to quinine. EC<sub>50</sub> of quinine varies from 1.35 to 2.7 µmol/L and EC<sub>50</sub> of mefloquine varies from 1.5 to 2.2 µmol/L in the areas.

**Hepatitis B virus carrier rate in different blood group of blood donors in Myanmar.** MYO-AYE, THAN-AUNG, KYI-WIN, KHIN-LAY-WAI, KHIN-MG-WIN, KHIN-PYONE-KYI, YEE-YEE-HTWE, KHIN-MAY-OO, ESTHER - OHNMAR-MYINT & NWE-YIN-AUNG. Department of Medical Research, Central National Blood Bank & Institute of Medicine(I).

Total apparently healthy blood donors at National Blood Bank in Yangon from 1 st January to 31 st December 1990 were 31504. Out of these, 2977 donors were Hepatitis B surface antigen positive carriers (Reverse passive haemagglutination method National Lab.). The donors blood groups A, B, O and AB were 25.1%, 32.1%, 34.7% and 7.9% respectively in that year. Hepatitis B virus carrier rate in these donors is 9.4% which is similar to that of the prevalence in general population. Hepatitis B virus carrier rate in A, B, O and AB groups are 9.9%, 9.4%, 9.3% and 8.5% respectively. In this study Hepatitis B virus carrier rate is not much different in different blood groups.

**Experimental therapy of persistent duck hepatitis B virus infection with foscarnet.** MALA-MAUNG, KWESI N. TSIQUAYE, MAREK J. SLOMKA, BO OBERG & ARIE J. ZUCKERMAN. Microbiology Department, Institute of Medicine (I), Yangon. Clinical Sciences Department, London School of Hygiene and Tropical Medicine, London & Antiviral Chemotherapy Department, Astra Alab AB, Sodertalje, Royal Free Hospital School of Medicine, London.

Chronic carriers of Hepatitis B virus (HBV) estimated to be 300 million approximately, serve as a reservoir of the virus in the transmission of infection. In addition, a considerable proportion of these carriers develop chronic liver disease and hepatocellular carcinoma. Therapeutic measures including antiviral chemotherapy and immunomodulation have given variable results and thus experimental models for the development

of effective therapy are essential. Among the animal models, the Duck Hepatitis B Virus (DHBV) carrier Pekin duck serves as an ideal model because DHBV shares similar genetic and biological properties with HBV. Foscarnet, an analogue of pyrophosphate acts as a non-competitive inhibitor of viral DNA polymerase (DNAP) and has in vitro antiviral activity against some herpesviruses, retroviruses and hepadna-viruses. The efficacy of high dose foscarnet was evaluated in DHBV carrier ducks. Plasma levels, DNAP activity and DHBV DNA by dot blot hybridization were monitored during treatment and follow-up periods. Foscarnet suppressed DNAP activity to undetectable levels during therapy in the majority of treated ducks. Hyperlipaemia in ovulating ducks enhanced the suppression of DNAP activity by foscarnet. Although plasma DHBV DNA decreased during drug administration, this reduction did not accompany suppression of DNAP activity in some of the treated animals. This apparent discrepancy between DNAP activity and DHBV DNA was explained by molecular events involved in DHBV replication.

**Is sporadic and epidemic hepatitis E caused by the same or different viruses?**  
 SOE-SOE, PAUL K. NAKANE, F. IIDA, YE-YE-HTKE, AYE-MYINT-SWE, CHO-CHO-HMAN & THAN-SWE. Department of Medical Research, Yangon. Department of Anatomy, Nagasaki University School of Medicine Japan & Department of Pathology, Nihon University School of Medicine, Japan.

The viruses recovered from the bile of experimentally inoculated monkeys with either sporadic or epidemic hepatitis E virus (HEV) stool suspensions were compared by spot hybridization and by electromicroscopy. They have almost the same size and morphology under the electronmicroscope. The two types were also tested by the spot hybridization technique using thymine-thymine dimerized synthetic HEV probe. Both of them hybridized with the same prob indicating that they have common base pairs sequence in some part of their genome.

**A preliminary report on the glutaraldehyde gelification time (GGT).** SAW-NYA-YEE, TIN-HTAY, EI-EI-KHIN & AYE-AYE-THIKE. Department of Pathology, Institute of Medicine(II).

A significant, sensitive, and linear reduction of GGT with increasing concentrations of Total protein, and with somewhat less significance with fibrinogen alone was demonstrated, suggesting the possibility of using GGT as an additional screening tool for chronic infectious and other diseases, where there is considerable increase in immuno-

globulin levels, or increase polypeptides. As a preliminary study we have studied a small sample of primary school children and adults in Yangon. The normal reference value of total and differential protein, were also studied. The GGT for children is  $12.9 \pm 4.4$  minutes, and that of adults  $20.2 \pm 10$  minutes. There is no correlation of GGT with ESR.

**A study of prevention and control of HIV/AIDS infection in selected border Townships.** AUNG-TUN-THET, KYI-SOE, AUNG-KYAING & SOE-MYINT. Department of Planning and Statistics.

Case studied and focus group discussions (FGDs) were conducted. A number of people from health and health related sectors, Communities were interviewed and FGDs were conducted among them. Problem of HIV/AIDS transmission was assessed in four border Townships. Then the profile of people who cross the border and work inside Thailand were studied. The push and pull factors that influence the migration of Myanmar Women who work in Thailand were identified. Through, FGDs interviewing measures were generated to prevent HIV infection among commercial sex workers. Background information were collected to do further research or interventions.

**A study on knowledge, attitude, practice and behaviour (KAPB) of commercial sex workers (CSWs): implications for AIDS.** KHIN-WIN-THIN, NAING-NAING-MYINT, YU-YU-LWIN & MAY-KHIN-SOE. Department of Planning and Statistics.

This paper is a case study of 73 women inmates who are detained at the Adult Training Home for Women, operated by the Ministry of Social Welfare. These women inmates are serving a sentence at this home due to their involvement as Commercial Sex Workers (CSWs). Their personal profile, family history and background, the reasons for becoming a CSW, their practices and health knowledge are covered in this paper. As one of the means of getting AIDS is through hetero-sexual relationships and since CSWs are one of the high-risk groups, it is felt that by undertaking a socio-economic study of CSWs, the necessary information for AIDS prevention, control and management can be guaranteed.

**Knowledge, perception and AIDS related behaviours among current married couples in Yangon Division.** \*AUNG-HTUN, \*\*LE-LE-WIN, \*\*THAN-TUN-SEIN, \*\*THAN-SWE. \*Central Health Education Bureau, Department of Health & \*\*Department of Medical Research.

Knowledge, perception and AIDS related behaviours study was conducted in May and June 1991 in Dagon and Thaketa Townships of

Yangon Division. Samples were randomly selected and psycho-social and behaviour factors were collected by using pretested interview schedule. Samples were current married couples. Knowledge of the samples on the mode of transmission of AIDS, and its sources were presented and discussed. Only very few samples were found stating sexual contact as mode of transmission and different health facilities as sources of transmission. Housewives knew more significantly than husbands that AIDS transmitted from mother to fetus. One out of every ten persons were found stating AIDS as curable disease. Educational level and jobs being engaged by samples determined significantly on the belief that AIDS could be avoided if there was no sexual contact with opposite sex other than spouse. Condom utilization by male while having sex with spouse was found low. Selection of effective communication channels and the need for careful message development for specific behaviour change was discussed.

**AIDS-related knowledge, attitudes and behaviours among Health Care Workers in Yangon (1991).** KHIN-MG-LWIN, SAN-SHWE, MYA-SANDA-KHINE, HLA-SHEIN, THEIN-HLAING & THAN-SWE. Central Health Education Bureau and AIDS/STD Control Program, Department of Health & Department of Medical Research.

AIDS related knowledge, attitudes and behaviours among health care workers was studied in randomized health institutions, including hospitals and Township health centers in Yangon between June and August of 1991. Interviews conducted on 987 health care workers (nursing staffs, technicians and mental workers). 26% of the respondents have seen HIV positive cases. 8.21% have handled HIV positive and HIV with clinical manifestations and 1.72% have experienced about their peers. Who had suffered HIV infection, but 63.12% of them believed that they can stay away from AIDS. Weak body resistance as the causal factor to contract HIV infection was perceived by some of them. Messages on AIDS were not systematically disseminated among health care workers. Their mixed attitudes and complex behaviours were being diagnosed as key elements for AIDS Prevention and Control Programme. The need for organizational development and inter and intrasectional collaboration based on proper Health Education processes was discussed.

**Study of KAP on HIV infection/AIDS among medical doctors in Yangon.** SAN-SHWE, MYASANDA-KHINE, HLA-SHEIN, KHIN-MAUNG-LWIN, THEIN-HLAING & THAN-SWE. Department of Medical Research & Department of Health.

HIV infection/AIDS related knowledge, attitudes and precautionary practices were studied among medical doctors in various health institutions, which included general hospitals, specialist hospitals, Township health centres and GP clinics in Yangon,

between June and August of 1991. The main objective of the study is to provide necessary information that may promote the appropriate use of health education services for the prevention of HIV infection/AIDS in health workers, particularly those at high risk. A total of 425 questionnaires were delivered and 378 (88.9%) returned. The survey results indicated that 99% of the spondents have heard of AIDS and only 74% knew the difference between AIDS and HIV infection. Knowledge concerning aetiology, transmission, diagnosis, control and prevention of the disease were revealed to provide the information for effective management. Positive and negative attitudes towards HIV infected person were also assessed and practices towards prevention of HIV infection/AIDS in health care settings were discussed in detail.

**The prevalence of markers for hepatitis B and human immunodeficiency viruses and malaria parasites in blood donor of Tharyarwady and Yangon General Hospital.** \*PHYU-PHYU-MYINT, \*WIN-MYINT, \*TIN-SHWE, \*\*KO-KO-HLA, \*\*\*KYI-WIN & \*\*\*\*SOE-LWIN. \*Clinical Research Unit (Cerebral and Complicated Malaria), \*\*Department of Medicine, Institute of Medicine (II), \*\*\*National Blood Bank & \*\*\*\*National Health Laboratory.

150 samples of blood collected from potential blood donors of Tharyarwady Civil Hospital were collected and tested for malaria parasites. Of these (80) consensive samples were tested for hepatitis B surface antigen and anti HIV antibodies. 200 blood samples from National Blood Bank, Yangon were also tested for the presence of malaria parasite, Hepatitis B surface antigen and anti HIV antibody. *P. falciparum* malaria parasites were detected in 2% of samples collection from Tharyarwady. Hepatitis B surface antigen was found in 10% of Tharyarwady samples and 11% of samples from Yangon.

**Role of GPs in diarrhoeal management.** SOE-MYINT, TIN-TIN-HTWE & THAN-THAN-WIN. Department of Planning and Statistics.

Diarrhoea diseases kills approximately (4) million children a year around the world. About two and half million of these deaths are the result of dehydration. The tragedy is that most of these can be prevented by available technology i.e. oral rehydration therapy (ORT). It is felt that there is an important role which GPs can play in the use of thesis intervention. Therefore, a convenient sample of (87) General Practitioners from Yangon Municipal Area was requested to answer the self-administrated questionnaire. Objectives of the study are; to identify role of General Practitioners in promotion of homemade oral rehydration solution used in diarrhoea and to estimate

the size of diarrhoea problem, the volume and price structure of ORS sachets used in General Practice. The result of the survey are presented and discussed.

**The study of disability in Yangon.** *SAN-HLA-MU, KYI-WIN-HLAING, HTEIN-WIN-AUNG, KHIN-MAR-THET, KYI-LWIN & SEIN-HLA-AUNG.* Medical Statistics Research Division, Department of Medical Research.

The study is conducted in three Institutions in Yangon; Hospital for disabled, Orthopaedic Hospital and Deaf and Dumb School. The observational, cross-sectional study design is chosen and we collect the data from the hospitals record in the predesigned proformas. It is found that round about 2000 patients are admitted to these institutions annually for treatment and rehabilitation service from all over the country. The orthopaedic Hospital capacitates more patients than the Hospital for disabled. Again the Deaf and Dumb school which gets support from missionary, could accept about 150 to 200 children annually and only a few of them get hearing aids. The causes of disability are mostly injuries and infections, whereas the congenitals, neuromusculars and musculoskeletal are rare. Regarding age, the school going and working age groups are the most effected age groups. However, toddlers are the most burdensome by (?). Assisted severity and dependent severity type of disabled need to have long term rehabilitation and among them, the amputees need more care and rehabilitation, as they have to train for using prosthesis. Out of all the patients, the economically handicapped are sort out from the working age group and found that the males are more than the females with the ratio 2.36: 1. Apart from the educational status of the patients, the health knowledge of the majority of them is not so had that percent distribution of self referrers are higher than the official referrers, 68.65% and 30.55% respectively. As Bed disability days and Restricted activity days are the disability indicators used in the other countries, they are trying to develop in this study to some extent. It is of interest to hope that Bed disability days per person per year are longer than the Restricted activity days in all age groups except in Deaf and Dumb School. The majority of the children in the Deaf and Dumb School are found to be due to maternal cause during pregnancy. The relevant maternal history are Accidents during pregnancy, Rubella, Malaria and other infections and Renal diseases. So far as our study is concerned, all most all of the disabled persons are improved by the comprehensive rehabilitation trainings given in these Institutions.

**Change of sugar in coconut water** \**WIN-MYIST & \*\*AUNG-KHIN.* \*Pharmacology Research Division, Department of Medical Research & \*\*Physiology Department, Institute of Medicine (II).

Coconut water has been claimed to be an

excellent source of water, sugars and minerals and is often used as a replacement fluid. Total content of sugar (about 5 g%) in coconut water depends upon the maturity of the coconut. It is necessary to detect the nature of individual sugar in coconut water according to the age of coconut. Coconuts collected in Yangon were investigated by thin layer chromatographic method 24 numbers of coconuts were analysed for 4 different ages; (3 rd, 5 th, 7 th and 9 th month- 6 for each stage). The intensities of sugar spots on chromatogram were scanned by densitometer and recorded as peak area (percentage). Relative proportion (in percentage) of individual sugar present at 4 different age of coconut were detected and compared. Glucose and fructose were found to be of high concentration in 3 rd and 5 th month coconut and low in 7 th and 9 th month-aged coconut. Sucrose, contradictionally, was lower content in 3 rd and 5 th month and higher in 7 th and 9 th month-aged coconut. Relative total sugar measured by refractometer was observed to be higher in 5 th and 7 th month-aged coconut than 3 rd month (young age) and 9 th month (old age).

**Changes in serum aldosterone levels in response to malnutrition and diarrhoea.** *THET-HTAR-MYINT, AYE-KYAW, THAW-POON & KHIN-MAUNG-U.* Clinical Research Division & Biochemistry Research Division, Department of Medical Research.

The study included 26 children (age 1 to 5 years) who were hospitalized for malnutrition, malnutrition with diarrhoea and acute diarrhoea of less than 48 hrs. duration. The controls, consists of 6 children from the same locality as patients, were apparently healthy and in the same age group. Serum chloride tend to increase in the patients and the increase was more pronounced in the group suffering from both malnutrition and diarrhoea. On the other hand, serum potassium tends to decrease and the decreases were significant in the groups having malnutrition. Serum aldosterone levels were raised in all the three groups of patients namely malnutrition, diarrhoea and malnutrition associated diarrhoea when compared with the control. The increase was still observed even after exclusion of the subjects whose ages lied within the first 1 1/2 year of life for whom the serum aldosterone levels were found to have a sharp rise.

**Preliminary report on immunodiagnostic reagents for hepatitis E.** *KHIN-PYONE-KYI, SOE-SOE, MYO-AYE, YI-YI-HIWE, KHIN-MAY-OO, THAN-AUNG, CHO-CHO-HMAN, SAN-SAN-OO, TOSHIKAZU UCHIDA, FUSAE IIDA, TOSHIO SHIKATA & KHIN-MAUNG-WIN.* Department of Medical Research, Yangon, Myanmar & Nihon University School of Medicine, First Department of Pathology, Tokyo, Japan.

Viral hepatitis E is endemic in Myanmar and epidemic outbreaks are well-known to occur

with sporadic attacks in between the outbreaks. During the collaborative research work on hepatitis E between Myanmar and Japan, bile of experimental monkeys containing the Virus like Particles (VLPs) were collected during animal transmission studies in Myanmar. The Japanese scientists used the virus in bile as a material for molecular cloning of the viral genome to serve for immunoassay of the antibody. Two types of ELISA reagents have been developed namely NE 8 and HT 3. Tests on various types of sera in Myanmar showed that NE 8 ELISA reagents were useful for the seroepidemiological study of the population whereas HT 3 ELISA reagents were better for serodiagnosis of acute cases of hepatitis E.

**A rapid diagnosis of malaria parasites by acridine orange staining method.**

MU-MU-SEIN-MYINT, KAY-THI-KYAW, KYIN-HLA-AYE, MYINT-LWIN & YE-HTUT. Parasitology Research Division, Department of Medical Research & No.2 Military Hospital, Directorate of Medical Services.

The study was designed to find out the diagnostic value of Acridine orange staining method in detection of malaria parasite *Plasmodium falciparum* in compare to the conventional thick and thin blood films. Thirty two *P. falciparum* and two *P. vivax* malaria cases were included in the study and the thin blood smear method was found to be more sensitive in detecting asexual parasites than the thick film and acridine orange staining methods. However, thin film method appeared less sensitive than the other methods in detecting sexual parasites, the gametocytes. It needs 2-4 weeks time to train a technician to be able to examine and diagnose the parasite in thick and thin film methods, but acridine orange staining method can be transferred within one hour. Another advantage of acridine orange staining method is the short duration of examination time, lasting only 45 seconds whereas the thick and thin blood films used 5 minutes and 10 minutes respectively. Hence, it is concluded that the acridine orange staining method is useful for quick diagnosis of malaria although accessory instrument like spectrophotometer is required.

***Aeromonas* and *Plesiomonas* strains isolated from diarrhoea and dysentery cases.**

PHYU-PHYU-WIN & TIN-AYE. Bacteriology Research Division, Department of Medical Research.

This study was carried out to determine the frequency and importance of *Aeromonas* and *Plesiomonas shigelloides* in pathogenesis of gastroenteritis. Stool samples were collected from the diarrhoea and dysentery patients attended to the Infectious Diseases Hospital as well as from the outpatient clinics during August, 1990 to June, 1991. Isolation and identification of the pathogens was processed by standard biochemical tests described by Cowen (1974) and according to the criteria

outlined by von Graevenitz (1985). Of 124 patients studied, *Aeromonas* spp. were isolated from 5 (4.03%) and *Plesiomonas shigelloides* from 2 (1.61%). It is evident that *Aeromonas* and *Plesiomonas* spp. are not an uncommon aetiological agents of acute gastroenteritis in Myanmar. So, it is anticipated to study on these bacteria to expand the knowledge and to provide an information on the epidemiological control of the disease caused by these bacteria.

**Identification of *Escherichia Coli* toxins by**

**Bead ELISA.** MAR-MAR-NYEIN, T. KARASAWA, THUZAR-MYINT & TIN-AYE. Bacteriology Research Division, Department of Medical Research & Department of Microbiology, Kyoto University.

711 isolates of *Escherichia coli* and 21 isolates of other bacteria were tested for the production of toxins as shiga-like toxin 1 SLT I (VT<sub>1</sub>), shiga-like toxin 2 SLT II (VT<sub>2</sub>) and heat labile LT by Bead ELISA method. It was observed that 30 percent of children and 54 percent of adult with diarrhoea and gastroenteritis showed VTEC. It was also observed that VTEC strains belonged to serogroups O26, O55, O86, O125, O142 and O157 and other untypable serogroups.

**Evaluation of differential sugar absorption method for assessment of small**

**intestinal permeability in Myanmar children.** MYO-KHIN, TIN-OO, SANDAR-KYI & KHIN-MAUNG-U. Clinical Research Division, Department of Medical Research.

The differential urinary excretion of lactulose and L-rhamnose after a standard oral dose has been employed as an index of mucosal injury and its feasibility and practicability tested under field conditions. 110 children who were assessed for malnutrition also had intestinal permeability analysis. Weight-for-age were all used as indicators of malnutrition. Moderately malnourished children (75% weight-for-age) had significantly greater lactulose/L-rhamnose ratios ( $p < 0.05$ ) than those children with normal nutrition (90% weight-for-age) ( $0.1495 \pm 0.0134$  vs  $0.1132 \pm 0.0109$ ). No significant differences were found between the differential sugar ratios of those children who had chronic (90% length-for-age) or acute (80% weight-for-height) malnutrition and those children who were normally nourished according to these classifications.

**A preliminary characterization of some isolates of *Entamoeba histolytica* in**

**Myanmar.** THAN-SAW, T. TANAKA, Y. KANEDA, TIN-TIN-AYE, MYINT-LWIN, AYE-THAN & OHMAR-AUNG. Parasitology Research Division, Department of Medical Research & Department of Parasitology, Tokai University, Japan.

In this preliminary study, we aimed to find

out the isoenzyme patterns of *Entamoeba histolytica*, in Myanmar, by using agarose gel electrophoresis technique. The enzyme used were Glucose phosphate isomerase, GPI (EC. 5.3.1.9.), phosphoglucomutase, PGM (EC. 2.7.5.1.), Hexokinase HK (EC 2.7.1.), Malate hydrogenase ME (EC. 1.1.1.40). The enzyme patterns of the isolates of *E. histolytica* obtained from symptomatic and asymptomatic cases, were recorded, taking an RF system in relating isoenzyme band mobility to the migration distance of a standard pathogenic type *E. histolytica*, HK 9 strain. However, no differences in isoenzymes patterns of *E. histolytica* from symptomatic and asymptomatic case were observed using the enzymes of PGM and HK.

**Knowledge, attitudes and practices in relation to snake bite in a snake bite endemic area in lower Myanmar.** MYO-KHIN, THI-HA, KYU-KYU-WIN & KHINE-THAZIN. Clinical Research Division & Snake bite Research Unit, Department of Medical Research.

A community based KAP study was carried out on 226 randomly selected respondents from Tharyarwady Township during the snake bite season of 1990 to determine the prevailing knowledge, attitudes and practices regarding snake bite, poisonous snakes, beliefs, treatment seeking behaviours, attitude towards preventive measures and traditional healers. The majority of respondents (94.3%) believe that snake bite is a priority disease and preventive measures should be undertaken to reduce it. However, only 42.5% of them considered present preventive measure as effective. Most (95%) welcome toxoid immunization as an expansion of preventive measures against snake bite. Males achieved significantly better knowledge scale scores ( $7.64 \pm 1.2$ ) than females ( $6.67 \pm 1.4$ ) ( $p < 0.001$ ), and respected villagers (27 persons) also attained significantly higher knowledge scale scores than the rest of the respondents ( $7.96 \pm 1.2$  vs  $7.4 \pm 1.3$ ,  $p=0.03$ ). The respondents who dwelled at a distance of more than one hour travelling distance from the hospital attained a slightly but not significantly lower knowledge scale scores than those who lived within one hour travelling time ( $7.39 \pm 1.3$  vs.  $7.51 \pm 1.2$ ). The implications of this study to snake bite prevention are discussed.

**King cobra (*Ophiophagus Hannah*) bites in Myanmar.** TUN-PE, TIN-MYINT & AYE-AYE-MYINT. Immunology Research Division, Department of Medical Research, Yangon & Intensive care unit, Yangon General Hospital, Yangon.

Venom and venom antibody levels following king cobra bites in two reptile handlers were measured by enzyme immunoassay. First case received antivenom and the other did not. Case (1) made a complete recovery following bite and produced a high titred short lived antibody. Venom antigen was not detected in 11h. sample after antivenom. Case (2)

experienced recent two minor king cobra bites and received immunisation 4 weeks before the accident. He had local swelling with no neurological symptoms developed throughout the course after bite. Venom antigen measured at 1.45h. after bite was 132 ng/ml which rapidly fell to 45 ng/ml in following 30 min. and was no longer detectable at 14h. after bite. The pattern of antibody response in both cases was comparable except case (2) had its peak level maintained for 13 days instead of one day in case (1) before it gradually fell to low level in 8 weeks following bite. It is likely that previous bites and recent immunisation might have contributed to the boosting of antibody response in case (2).

**Approximate Median Lethal Dose (LD<sub>50</sub>) Determination.** PHONE-KYWE-MYAT, TIN-TIN-AYE & KYI-MAY-HTWE. Immunology Research Division, Department of Medical Research.

Median lethal dose (LD<sub>50</sub>) determination by the classical method requires more mice than the approximate (LD<sub>50</sub>) determination of Meier and Theakston. In order to test the usefulness of the latter technique in biological assays, the (LD<sub>50</sub>) (subcutaneous) of king cobra venom was determined by the two methods in 18 - 20 gm icr mice. The approximate (LD<sub>50</sub>) (103.84 µg/mouse) was about six times the classical (LD<sub>50</sub>) (17.82 µg/mouse) when the mean dose used was 9.75 mg/kg (range 8 - 11.5 mg/kg) and about two and a half times (45.976 µg/mouse) when the mean dose used was 3.75 mg/kg (range 2 - 5.5 mg/kg). It is suggested that the approximate (LD<sub>50</sub>) be determined using a low mean dose.

**Effectiveness of anti-viper venom on experimentally envenomated mice.** WIN-AUNG, SAN-KUN, KHIN-PA-PA-KYAW, AYE-KYAW & HLA-PE. Biochemistry Research Division, Department of Medical Research.

The effectiveness of anti-viper venom (AVV) on envenomated mice by different routes, dosages and time intervals of initiating serotherapy after envenomation was determined. The lethal dose (LD<sub>100</sub>) of Russell's viper venom (RVV) on mice was 5 µg/gm body weight intramuscularly and median effective dose (ED<sub>50</sub>) of AVV intramuscularly was 125 µg/gm body weight, i.e. 25 times more than that of RVV. Following the envenomation with lethal dose, the mice were administered with AVV intramuscularly and intravenously in different time intervals after envenomation. To achieve the equipotency, the intramuscular AVV has to be given approximately 4-fold over the intravenous dosage. Administration of AVV intramuscularly and intravenously were found to be unable to neutralise the effects of

RVV if administered after 2 hours and 4 hours post-venomation respectively.

**Immunogenicity of toxoids of Russell's viper venom in experimental animals.** KHIN-PA-PA-KYAW, THEIN-THAN, HLA-PE, AYE-KYAW, TIN-WIN, MYO-KHIN & NILAR-SEIN. Biochemistry Research Division & Nuclear Medicine Research Division, Department of Medical Research.

This study made to know immunogenicity of formalin treated Russell's viper venom in experimental animals. Russell's viper toxoid was prepared by the mild and step-wise formalinisation. Detoxification was completed on day 10 at 0.8% formalin and adsorbed with aluminium phosphate. Rabbits, Guinea pigs and mice were immunised with toxoid and immune response of the animals tested. The antibody levels of immunised and challenged animals were measured by Radio Immuno Assay method. The immunised rabbits could withstand the challenge of 8 mg to 16 mg (2LD to 3LD) of viper venom by intramuscular injection. The immunised guinea pigs and mice also could withstand the challenge of 3mg (3LD) of viper venom.

**Immune response to Russell's viper venom in immunized monkeys.** NILAR-SEIN, TIN-TUN, MYO-KHIN, MAUNG-MAUNG-THWIN, YIN-YIN-WIN, KHIN-PA-PA-KYAW, WIN-AUNG & HLA-PE. Nuclear Medicine Division & Biochemistry Research Division, Department of Medical Research.

To study the protective effective of Russell's viper venom toxoid, three doses of intramuscular immunization were carried at intervals of 4 weeks in Rhesus monkeys. Circulating Russell's viper venom antibodies were measured throughout the immunization phase. Two weeks after full immunization, a group of Rhesus monkeys was challenged with Russell's viper venom intramuscularly and circulating Russell's viper venom antibody levels in the blood were monitored up to 26 weeks after challenge. For the same period of time, in a control group of Rhesus monkeys, circulating Russell's viper venom antibody levels were determined simultaneously. A solid phase radioimmunoassay, developed at the Nuclear Medicine Research Division laboratory, was used for determination of circulating Russell's viper venom antibody levels. The implications of the findings are discussed.

**Humoral antibody response following Russell's viper bites.** TUN-PE, AYE-AYE-MINT & MYA-MYA-WIN. Immunology Research Division, Department of Medical Research, Yangon & Renal Unit, Yangon General Hospital, Yangon.

The prospective longitudinal study of development, intensity and persistence of antibody in relation to severity of poisoning were studied on 13 Russell's viper bite victims by enzyme immunoassay technique. Retrospective screening of antibody in 91

Russell's viper bite cases reveals that it could be detected from one week to 8 yr. after bite and 55/81 (68%) were positive from antibody. A mild degree of humoral suppression which leads to slow peaking (20 weeks) and waning of antibody was observed in the majority of cases. Intensity of antibody response is related to the initial venom level rather than time between bite and antivenom administration. Uraemia adds further humoral suppressive effect in snake bite renal failure cases.

**Purification of coagulase from Russell's viper (Viper Russellii) venom.** TIN-WIN, AYE-KYAW, KHIN-PA-PA-KYAW, SAN-KUN, SHWE-NI & HLA-PE. Biochemistry Research Division, Department of Medical Research.

Dessicated crude Russell's viper (Viper russelli) venom was fractionated by gel filtration on sephadex G-75. Five fractions were obtained. Fraction I, which was tested to be coagulase rich, was passed through sephacryl S-200 column followed by ion-exchange chromatography on CM-sephadex C-50 column. Five subfractions were obtained. The third subfraction contained coagulase activity of nearly two-fold purification than that of fraction I. SDS Polyacrylamide gel electrophoresis was done and third subfraction showed single band protein. The results suggested that the coagulase enzyme of Russell's viper venom has been purified by this procedure.

**Muscle strength of Myanmar top rowers.** SOE-MIN-THEIN, YE-TINT-LWIN, HLAING-MYINT & MYINT-THEIN. Physiology Research Division, Department of Medical Research.

Eight western type and twenty two traditional type of Myanmar male top rowers were studied. Their explosive power of arm and leg muscles and muscle strength of arm and back muscles were determined. The traditional type of rowers had a significant superior muscle strength in left hand grip, back muscle strength and peak an aerobic power of arm muscles. Reasons for having high muscle strength in arm and back muscles of the traditional type of Myanmar rowers were discussed. Measures for promoting the performance of Myanmar rowers of western type of rowing were also suggested.

**A preliminary report on somatotypes of different national groups of Myanmar.** HTAY-HTAY, MAY-MAY-YI, LILIAN-PO, THIN-THIN-HLAING, SAN-YI, TIN-AUNG, TIN-KHINE-MYINT & THAN-THAN-WIN. Department of Biochemistry, Institute of Medicine (I), Physiology Research Division, Department of Medical Research, Department of Physiology, Institute of Medicine (II), Department of Biochemistry, Institute of Medicine (II) & Department of Physiology, Institute of Medicine (Mandalay).

A total of 286 students (192 males and 94

females) of Academy for Development of National Groups (now, Institute for Development of National Groups), Ywathitkyi, Sagaing, ages 17 to 29, belonging to 8 major national groups of Myanmar, were somatotyped by the Health Center anthropometric rating method as part of the project "Studies on Population Genetics and Health Care in the Union of Myanmar". Somatotype distribution for sex-specific different national groups were compared using analysis of variance to test for differences among the mean somatotypes. Female students were found to be more endomorphic and less ectomorphic than males in all the national groups studied. Male students show a mesomorphic dominance except Bamars and Rakhines, and a low endomorphy rating in all national groups. For female students, there were significant differences between the following; Kachins and Shans; Chins and Shans; and Bamars and Shans. Bamars and Rakhine males were found to be significantly different in somatotype from males of other national groups. Kachin, Naga and Shan male students were found to have similar somatotypes.

**Lipoprotein cholesterol profile in diabetic patients.** MIN-SWE, AUNG-SAN & MYA-MAUNG. Department of Biochemistry, Institute of Medicine (Mandalay).

Measurements of plasma glucose, serum total cholesterol, triglyceride, HDL cholesterol were carried out on 61 diabetic patients and 123 apparently normal subjects. LDL and VLDL cholesterol were calculated from Friedewald's formula modified by Foery and Clement. Mean concentrations of serum total cholesterol, triglyceride, LDL and VLDL cholesterol were found to be significantly ( $p < .001$ ) higher in diabetics and HDL cholesterol level was significantly ( $p < 0.001$ ) higher in normal subjects. Plasma glucose concentrations have no correlation with serum cholesterol, triglyceride, LDL, VLDL and HDL cholesterol in both diabetics and normal controls. However, in normal subjects, the plasma glucose has positive correlation with only LDL cholesterol in both diabetics and controls, serum total cholesterol level were found to be positively correlated with lipoprotein cholesterol and triglyceride. Triglyceride and VLDL cholesterol concentrations were significantly ( $p < 0.05$ ) higher in patients with long duration of diabetic history ( $> 3$  years). Anti-atherogenic index of diabetics was significantly ( $p < 0.001$ ) lower than that of controls.

**Fat and cholesterol changes in exercising rats.** TIN-AUNG & AYE-AYE-THAN. Physiology Research Division, Department of Medical Research.

The changes in fat and cholesterol levels were studied in groups of rats that were forced to exercise on rat treadmill. Results showed that exercise reduced body weight fat pad weight, fat percentage and serum cholesterol level. The exercise induces

effects were more marked on rats eating high-fat diet. However, these effects started to diminish within 6 weeks of exercise termination.

**A conduction study of Median nerve in diabetic patients.** KYAN-MIN, PE-TOE & SAN-YI. Department of Physiology, Institute of Medicine (Mandalay).

There are many reports in the literature on the slowing of motor and sensory conduction of peripheral nerves in diabetic patients. In this study, measurement of conduction velocity of median nerve was done on 83 diabetic patients and 61 age-matched controls with the aim of seeing how it correlates with blood glucose level and duration of disease. The stimulus used was supermaximal delivered through an isolation unit. Muscle and nerve action potentials were amplified by a preamplifier and displayed on a cathode ray oscilloscope. Motor conduction velocity and sensory conduction velocity of median nerve in diabetic patients with or without neuropathy were found to be significantly reduced compared to normal. Significant negative correlation was found between 2-hour postprandial blood glucose level and nerve conduction and between duration of disease and nerve conduction. It is concluded that the risk of developing diabetic neuropathy is greater in poorly controlled diabetics and increases with time.

**A structural investigation of the cartilages associated with human long bones.** \*DAW-KHIN-WIN & \*\*A. J. PALFREY. \*Department of Anatomy, Institute of Dental Medicine & \*\*Department of Anatomy, Charing Cross and Westminster Medical School, London, U.K.

Although most standard text books described all cartilage as avascular, there is much evidence in the literature that some of the hyaline cartilages in the embryo as well as in the adult possess "cartilage canals" containing blood vessels. Even though these canals have been investigated by a number of workers during this century, many points such as the time they first develop, the manner by which they develop and whether they appear in each site at a constant stage of development, remain to be clarified. The most controversial point is the role which they play in the growth of the chondroepiphyses whether they are responsible for nutrition for interstitial growth or whether they are associated with the secondary center of ossification. This paper proposes that these canals play an essential role in the nutrition of the chondroepithysis and in the initiation of the formation of the secondary centre of ossification. Forty four chondroepiphyses of the long bones from the limbs of twenty two human embryos and fetuses (CR 35 mm to 400 mm, age 12 weeks to term) were studied. Serial paraffin sections of appropriate

thickness were cut in various planes, stained with haematoxylin and eosin, and examined by light microscopy. The perimeter and the area of every twentieth section were measured by digitising with an Acorn Archimedes Image Analyser. The cartilage canals present in the same serial sections were also counted. Statistical analyses were made to correlate the size of the epiphysis with the number of cartilage canals present and to investigate the nature of their growth. In this series, the proximal epiphysis of the humerus is the first epiphyseal cartilage to possess cartilage canals by the 35 mm stage (age 12 weeks). A significant positive relationship was established between the size and the number of cartilage canals present in each. As the embryo grows, their pattern changes from simple, blind-ending buds to complex, branching channels with multiple anastomoses. This suggests that they grow by active invasion rather than by passive inclusion. Thus they play an essential role in the nutrition of the developing chondroepiphysis. The structure of the canals in this study does not differ from that previously described by other workers. Microscopic foci of calcification were found on the walls of the canals as early as the 230 mm stage (28 weeks) in the proximal epiphysis of humerus, and at term in the proximal epiphysis of the femur. As the secondary centre of ossification grows the related cartilage canals become incorporated into the secondary marrow cavity. This histological evidence demonstrates an association between the cartilage canals and the initiation of the secondary centre of ossification.

**Blood pressure responses of normal subjects to the lateral recumbent-supine position change.** HLA-YEE-YEE, CHO-CHO-YU-MON, KHIN-NYO-THEIN, THIDA-OO, L.PO & BA-AYE. Department of Physiology, Institute of Medicine (I) & Department of Medical Research.

Measurement of blood pressure, though a routine clinical test, is fraught with problems. The influence of the standing-sitting-lying postures is well-established, but there is a paucity of data regarding blood pressure changes in the horizontal position. Blood pressure responses to the lateral recumbent-supine position has been suggested as a predictor for pre-eclampsia. However, responses of normal, non-pregnant persons have not been documented. We have therefore studied 69 apparently healthy persons (44 females; 25 males), aged 19-60 years in two positions, namely left lateral and supine. Two measurements were taken in the left lateral position, one with the blood pressure cuff on the right arm and the other with the cuff on the left arm: one measurement was taken in the supine position; and Gant's roll-over test was performed to study the response to changing from left lateral to the supine position. Blood pressure recordings with the cuff on the right or left arm showed very good correlation ( $r = +1.000$  for SBP;  $r = +0.92$

for DBP); differences were also not statistically significant. Thus, only one set of values from the left lateral position was taken to compare with that for the supine position. Both systolic and diastolic blood pressures rose in the supine position (SBP being  $96.1 \pm 16.6$  mm Hg in the left lateral position, and rising to  $111.7 \pm 17.3$  mm Hg in the supine position,  $p < 0.001$ ; DBP rose from  $61.2 \pm 11.3$  to  $76.0 \pm 12.2$  mm Hg,  $p < 0.001$ ). Taking a rise of 20 mm Hg and above in DBP from changing left lateral-spine as suggested by Gant, we found a positive response in 24 (34.3%) of subjects. Significantly, the positive rate was similar in the two sexes i.e. 36% (9/25) in the males and 34% (15/44) in females. There was no significant correlation between age and percentage changes in BP. It therefore appears that BP values recorded clinically in the supine position may be over-estimating the true BP. The cut-off point for a positive roll-over test should also be re-evaluated.

**Vitamin "A" status of Myanmar children in coastal region.** MAUNG-MAUNG-CHO, KHIN-MAUNG-NAING, HLA-MIN, PE-WIN, AUNG-NYUNT, WIN-WIN-KHINE, WIN-AUNG, TIN-TIN-OO & HLA-PE. Nutrition Research Division & Biochemistry Research Division, Department of Medical Research.

Recent vitamin A surveys indicate that vitamin A deficiency problem exist in dry, delta and hilly regions and the problem appears to be more serious in dry zone of upper Myanmar. To cover all the geographical regions, a study in the remaining coastal region was conducted during February 1991. Field surveys including clinical, biochemical, dietary and socio-economic status assessment was conducted in some randomly selected villages in Thandwe Township, Rakhine State. The subjects were children of 6 months to 6 years and a total of (1310) subjects were included. Bitot's spot with xerophthalmia (X1B) was used as a clinical criteria. Serum vitamin A level was assessed by LVACG/WHO method. Dietary intake study was conducted by using weightment method. Socio-economic class, height, weight and mid-arm circumferences were recorded. (X1B) was found in (0.68) percent which exceed WHO prevalence limit of (0.5) percent. (9.8) percent of children were found to have serum vitamin A level less than 10 µg/dl and the result also exceed WHO prevalence limit of 5 percent indicating vitamin A deficiency problem. Moreover, the daily consumptions of vitamin A, both in rural and preurban areas were below the recommended daily allowance and the result of food consumption data agree with the clinical and biochemical observations mentioned. Thus, from the clinical, biochemical and dietary assessments, it is concluded that vitamin A deficiency problem also exists in the coastal region of Union

of Myanmar.

**Applicability of WHO criteria by community Health workers for diagnosis of acute lower respiratory infections.** KHIN-MYAT-TUN, TIN-NU-SWE, SOE-AUNG, MYO-KHIN- & TIN-MG-MG. Clinical Research Division & Computer Division, Department of Medical Research.

Applicability of simple clinical signs (WHO 1990 ARI classification) by community health workers (CHWs) for the diagnosis of Acute Lower Respiratory Infections (ALRI) especially pneumonia, were evaluated in out-patient department of Yangon Children's Hospital from November 1990 to February 1991. 944 under five children presenting with ARI related symptoms of less than 7 days duration were included in the study. The percentage agreement of diagnosis status by CHWs with reference to project Medical Officers (gold standard) were Kappa 0.5126 ( $p < 0.001$ ) in less than 2 months old infants and Kappa 0.5442 ( $p < 0.001$ ) in children aged 2 months to 5 years. CHWs were able to detect important clinical signs like chest indrawing and nasal flaring in percentage agreement of 85.1% and 95.4% respectively. However percentage agreement in respiratory rate was found to be 60.2% and there was incorrectness in counting the respiratory rate as the rate increased. Differences in the level of agreement on these important clinical signs misclassified the disease category resulting in low percentage agreement in discriminating the disease severity.

**Widal agglutination test reaction in patients with falciparum Malaria.** TIN-SHWE, AUNG-MIN, KHIN-SWE-OO, KYIN-HTWE, PHU-PU-PU-MYINT & MYAT-MYAT-MAN. Clinical Research Unit (Cerebral and Complicated Malaria), Department of Medical Research & National Health Laboratory, Department of Health.

Serum samples were collected from (49) patients with falciparum malaria during the Summer season. Of these, (30) were followed at Day (7) of admission to hospital. The patients/serum were tested by tube agglutination for antibodies against H and O suspension of enteric fever organisms (*Samonella typhi*, *Samonella paratyphi A* and *Samonella paratyphi B*). 66 serum samples were also collected from patients with falciparum malaria during the rainy season. Of these (41) samples were collected again at Day (7) of admission to hospital. They were tested as above. Out of (49) Day (0) samples collected in Summer, 3 samples collected from patients with parasite density one plus (+) and suffering from uncomplicated malaria showed agglutination titre + 1/40 to (0) antigen of *Samonella typhi* and the same 3 samples showed + 1/40 to antigen of *Samonella typhi*. Out of (66) Day (0) samples collected in rainy season only one sample collected from patients with parasite density two + and suffering from uncomplicated malaria showed agglutination titre + 1/40 to (0) antigen of *Samonella typhi*. All the remaining samples showed - 1/40 agglutination titre to

*Samonella typhi*, *Samonella paratyphi A* and *Samonella paratyphi B*, O and H antigens. There was also no increase in agglutination titres at Day (7) samples. Thus, there may be no relation between widal agglutination reaction and clinical severity or parasite load of patients.

**Studies on the rice field breeding mosquitoes of Yangon.** HTAY-AUNG, SEIN-MIN & THAN-SWE. Medical Entomology Research Division, Department of Medical Research.

Commencing August 1989 this research project was conducted for six months in Thein-Chaung village tract and Kyaung-gone village tract of Yangon Division. Ten species of *Anopheles*, 8 species of *Culex*, 1 species of *Mansonia* and *Aedes aedimophus nigrostratus* were collected. Among *Anopheles* species *An. barbirostris* (6930), were collected in large numbers followed by *An. vagus* (2565), *An. aconitus* (686), *An. annularis* (373), *An. philippinensis* (136). However, *An. aconitus* and *An. annularis* were more abundant after the rice plants reached the maximum height (above 40 inches) of the flowering stage. Four number of primary vector *An. minimus* were also collected from Thein-chaung village tract. Also, four important vectors of Japanese encephalitis viz, *Culex tritaeniorhynchus* (3666), *CX. gelidus* (2224), *CX. vishnui* (5131) and *CX. fuscocephalus* (4623) were abundantly collected from the two study areas.

**Concentration method of detecting *Plasmodium falciparum* using microcentrifuge and heparinised capillary tubes.** YUPAR, KHIN-MYO-NWE, AYE-AYE-CHIT, TIN-SHWE, KO-KO-HLA & WIN-MYINT. Clinical Research Unit (Cerebral and Complicated Malaria), Department of Medical Research & Department of Medicine, Institute of Medicine (II).

Sixty malaria patients were studied. Thick blood film were prepared from the finger tip of the patients as usual to detect malaria parasites in blood. A sample of blood is also collected from the same finger tip with heparinised capillary tubes at the same time. The tubes were centrifuged at 10,000 rpm for 5 minutes. The capillary tube is then broken into three pieces. One upper part of column just below the white cells and the another at the base of the red cells column. Blood is collected from these two samples, fixed and stained together with samples from finger tips usual standard procedure for the detection of malaria parasites. Blood collected from the upper most part of red cell's column yield the highest parasites density. The use of capillary tube is not an extra burden to the work load of the malaria unit and this method may help in the detection of patients

with lower level of parasites in peripheral blood.

**Regional differences in mefloquine response rates in uncomplicated falciparum malaria.** KYAW-WIN, MARLAR-THAN, THAN-OO-LWIN, HTUN-NAING-OO, HLA-MYAT-MON, SAN-SAN-AYE, KYI-KYI-THA, AYE-YU-SOE & THAN-SOE. Clinical Research Unit, Defence Services General Hospital.

One hundred and fifty eight soldiers aged 19 to 33 years, admitted to Clinical Research Unit (D.S.G.H) between November 1990 to September 1991 with uncomplicated falciparum malaria were treated with Tab. Mefloquine 1000 mg stat and observed in hospital for 42 days. Temperature and Blood for MP were taken regularly. Therapeutic response measured was by the WHO criteria for 4-aminoquinolines as S, R 1, R 2, R 3 but using the 9 days instead of 7 days criterion for classification of high grade resistance. The area visited before contracting malaria was specifically noted. Mefloquine response rate in 1990 was 82%. The overall Mefloquine response rate over a nine month period in 1991 is 68.96%. Region-wise response rate showed 36.4% in those returning from Kayin State and 88.9% from other areas combined. Mefloquine response rate of falciparum malaria contracted from Kayin State is lower than those from other areas.

**Monitoring the efficacy of different quinine regimens in uncomplicated falciparum malaria.** KYAW-WIN, MARLAR-THAN, THAN-OO-LWIN, HTUN-NAING-OO, KYI-KYI-THA, AYE-AYE-SOE, THAN-SOE & SAN-SAN-AYE. Clinical Research Unit, Defence Services General Hospital.

One hundred and twenty male patients between ages 18 to 36 years, admitted to clinical research unit (D.S.G.H) between November 1990 to March 1991 with uncomplicated falciparum malaria are treated under supervision with either I/V infusion Quinine dihydrochloride or oral Quinine sulphate (sugar coated tablets) for ten days with and without Tetracycline capsule for 7 days. Their clinical and parasitological features were observed regularly as usual. Therapeutic response was monitored during a 28 days hospital study, using the W.H.O. criteria for 4 aminoquinolines as S, R 1, R 2, R 3 but taking up to include S 2 response for Quinine. The response rates are as follows :- Oral Quinine alone 67.8%, Initial I/V Quinine followed by oral 75.7%. Oral Quinine Plus Tetracycline 92.1% and I/V infusion quinine plus Tetracycline 87.5%. Single drug regimen of sugar coated Quinine sulphate is unsatisfactory in treatment of uncomplicated falciparum malaria. Initial I/V infusion improved the initial parasite clearance, adding Tetracycline further improved the response rates.

**A comparative study of mefloquine, artesunate, and halofantrine in treatment of uncomplicated falciparum malaria.** KYAW-WIN, MARLAR-THAN, THAN-OO-LWIN, HTUN-NAING-OO & HLA-MYAT-MON. Clinical Research Unit, Defence Services General Hospital.

One hundred and thirty patients admitted to Clinical Research Unit, (D.S.G.H) between November 1990 and June 1991 were chosen for study and randomly allocated to the 3 new antimalaria single drug regimens:- Oral Mefloquine, Artesunate, and Halofantrine. They were kept under supervision for 28 days and their clinical features, haematological, biochemical and parasitological parameters were assessed at regular intervals. Fever clearance time, parasite clearance time, cure rate, recrudescences rates and side effects are compared. Cost analysis was also performed. The initial parasite clearance rates are 93.33%, 100%, 98.33% and the cure rates are 78.33%, 75.0%, 76.77% for Mefloquine, Artesunate and Halofantrine respectively. Other data will be presented and discussed. All three drugs have good initial parasite clearance rates but as single drug their cure rates are not fully satisfactory. Artesunate and Halofantrine, although two new drugs, have not superseded Mefloquine in efficacy.

**Two regimens of primaquine as antirelapse treatment for vivax malaria in Myanmar.** THEIN-TUN, FRANCO TIN, LA-WIN-MAUNG & MYINT-HTWE. Department of Health (Malaria Control) & Yangon Division Vector Borne Diseases Control Team.

Two regimens of primaquine as Antirelapse treatment for vivax malaria was studied in 90 cases near Yangon city in Seikkyi-Khanaungto Township during 1990 - 1991. The cases were randomly divided into three groups. Group 1 was administered only chloroquine. Group 2 took chloroquine course plus Primaquine 15 mg daily for five days. Group 3 was given chloroquine course followed by primaquine 15 mg daily for 14 days. Group 1 showed relapse rate of 43.3% (13 out of 30) at the end of 4 months observation period. Relapse rates were 27.6% (8 out of 29) and 9.7% (3 out of 31) in 5 days and 14 days primaquine groups respectively. Four months follow-up was made during non-transmission season 14 days-course of 15 mg daily should be standard antirelapse treatment for vivax malaria in Myanmar. Higher daily doses are more likely to cause severe side effects and needed closed monitoring of patients. Females had lower relapse rates than males. Most of the patients could tolerate 5 days or 14 days course of primaquine. Serious toxic effects of primaquine like intravascular haemolysis was not detected.

**The effect of chloroquine chemoprophylaxis on malaria in Kazunma camps.** MYINT-LWIN, MAUNG-MAUNG-MYA, WIN-MYINT, KYAW-WIN & KHIN-MAUNG-TIN. Parasitology Research Division and Medical Officer, Kazunma Camp.

The study was conducted in November 1990 in the Kazunma village area of the Seik-Phyu Township. The study included 300 villagers of the Kazunma village as the control and the 315 workers of the Kazunma camps. The workers were on chloroquine chemoprophylaxis for more than one month. Among the villagers of the Kazunma village 16% were infected with malaria, *P. falciparum* 64.6% and *P. vivax* 31.3%. The malaria infection rate of the workers from the northern and the base camps were similar to the villagers. The workers from the southern camps have higher level of parasitemia (26%) in spite of the chemoprophylaxis. Though the chemoprophylaxis has no effect on the parasite rate, it seems to have some effect on the parasite density of the workers of the northern and the base camps. The *in vitro* sensitivity of *P. falciparum* isolates were 100% sensitive to Quinine and Mefloquine. They were 30.8% sensitive to chloroquine and 55.6% sensitive to amodiaquine. The isolates were 85.7% sensitive to sulphadoxine/primethamine.

**Effect of diclofenac sodium (odfen) in patients with osteoarthritis.** YE-THWE, SOE-PYE, KHINE-MOE & THIDA-SAN. No.(2) Military Hospital, Yangon.

Efficacy of oral diclofenac sodium on 25 Myanmar osteoarthritis patients were tested at the No. (2), Military Hospital during February to June 1991. Response to drug was assessed by severity of local pain, pain at night, swelling and reduction of joint movement. This study showed that involvement of knee joint is the commonest site in Myanmar OA patients and majority of our patients had more than one joint involved. Total dosage required for the different site involved ranged from 1200 - 1825 mg and total period of treatment also ranged from 17.41 - 19.25 days to subside symptoms. All patients but one (i.e. 4%) tolerated the drug very well and improved score indicated the high effectiveness of the drug. This study supported our previous finding that diclofenac sodium is a potent and safe non-steroidal antiinflammatory drug.

**Preliminary report on the effect of the two different insulin preparations [Protaphane HM (Isophane insulin)] and monotard HM [Insulin zinc suspension] at a fixed dose in type II (NIDDM) diabetic patients with oral hypoglycaemic agent (OHA) Failure.** YE-THWE, WIN-WIN-MYINT, SOE-PYE, CYNTHIE-TIN-OO, HTAY-HTAY-HAN, THIN-THIN-WIN, KHINE-MOE & THIDA-SAN. No. (2), Military Hospital, Ministry of Defence.

Eight diabetic patients are selected for study. Two hourly recording of blood sugar

are performed between 1600 and 0600 hours (8 samples) with and without intermediate acting insulins as a night time supplement to OHA. The results of blood sugar levels are analysed. Although the level of blood glucose is significantly lowered starting from 2400 hours onwards in each study using intermediate acting insulins, the additions of these two insulins at a fixed dose of 10 units does not improve the glycaemic control of the diabetic patients with OHA failure. Furthermore, the effect of these two insulins is also compared and found to have no significance.

**Study of knowledge, perception and practice of patients utilizing traditional medical care at the traditional medical centre in Yangon and Mandalay.** THAW-ZIN, THAUNG-HLA, NWE-NWE-WIN, WIN-KYI, KYIN-THEIN, AYE-LWIN, SOE-MOE, MYA-BWIN, AUNG-NAING, THEIN-HLAING & HLA-PE. Department of Medical Research and Department of Indigenous Medicine.

With recognition of traditional medicine as a great potential in contribution to primary health care, the National Health Plan in Myanmar is, at present, engaged in developing the most appropriate method for inclusion of traditional medicine in the Health Care Strategies for the attainment, by the people of Myanmar, the goal known as "Health for all by the year 2000". For the traditional medicine system to contribute usefully to primary health care, factors such as sociocultural background, strength of traditional belief, economic considerations, distance to be covered in attaining health care, knowledge and compliance in taking traditional remedies, etc., has to be taken in to account if realistic health plan and related training programmes are to be developed. Thus, the study was conducted on 922 patients attending the traditional medicine health centres in Yangon and Mandalay and their socio-cultural background, strength of the traditional belief, their knowledge, perception and practice on traditional medicine was studied so as to identify the target beneficiaries and to develop methods by which Traditional Health care can be delivered effectively to the population for which it is intended. The acceptable role of traditional medicine in Primary Health Care was discussed.

**Study of disease morbidity and drug dispensing patterns on patients utilizing traditional medical care at the traditional medicine centres in Yangon and Mandalay.** THAW-ZIN, THAUNG-HLA, NWE-NWE-WIN, WIN-KYI, KYIN-THEIN, AYE-LWIN, SOE-MOE, MYA-BWIN, AUNG-NAING, THEIN-HLAING & HLA-PE. Pharmacology Research Division & Clinical Research Unit (Traditional Medicine), Department of Medical Research & Department of Traditional Medicine.

With the purpose of narrowing the gap in

health service coverage between urban and rural areas, the National Health Plan in Myanmar envisaged the development and involvement of traditional medicine in the support of the primary health care. For the delivery of the traditional medical care to effectively support the National Health Plan, the first step is the development of a list of essential drugs which will offer the widest possible coverage of the prevalent affecting the population. This study was conducted with the aim to identify the prevalent morbidity pattern encountered in traditional medical care and the drugs most needed to treat such ailment, to study the drug dispensing patterns and to find out the rationale behind such prescribing, and finally, to develop a common treatment schedule for the diseases identified above so as to get an overview of the cost of the traditional medical care. The records and charts of all patients who attended the traditional medicine hospital and dispensaries in Yangon and Mandalay during the specified months, namely January, April and July, 1991 as well as the total amount of drugs used for various illnesses for one year (1 st September, 1990 to 31 st August, 1991) were studied. The amount of drug used in the record was compared with the total amount of drugs that would be needed according to the common treatment schedule which was developed through interview and discussion of 19 well-experience traditional medicine physicians. The association between seasonal variation and disease morbidity, and subsequent drug dispensing pattern, as well as the cost and rationale behind drug use was discussed.

**Comparative effects of vanadate on 2,3-bisphosphoglycerate levels of erythrocytes and lysates from normal controls and patients with  $\beta$ -thalassaemia.** MYINT-OO, G.L. MEND & W.J.O' SULLIVAN. Parasitology Research Division, Department of Medicine Research & The University of New South Wales, Australia.

The vanadate-stimulated hydrolysis of 2,3-bisphosphoglycerate in normal and  $\beta$ -thalassaemic erythrocytes was measured by using  $^{31}$  p Nuclear Magnetic Resonance spectrometry. The differences were observed on the initial rates of decline of 2,3-bisphosphoglycerate in suspensions of intact red cells of  $\beta$ -thalassaemia patients and normal controls in the presence of vanadate. Comparison of the rates of decline of 2,3-BPG in freeze-thawed and sonicated lysates was made to clarify the anion transporting system involved in the intact cells. The results suggested that the transport of vanadate through the membrane becomes the rate limiting step in the stimulation of 2,3-BPG phosphatase in erythrocytes from patients with  $\beta$ -thalassaemia.

**Transforming growth factor beta 1 (TGF $\beta$ 1) is a strong mitogen for human Ito cells in culture.** KHIN-MAUNG-WIN, F. CHARLOTTE, A. MALLAT, AM PREAUX, P. MAVIER, D. DHUMEAUX & J. ROSENBAUM. Experimental Medicine Research Division, Department of Medical Research & INSERM Unit 99, Hospital Henri Mondor, France.

The role of Ito cells in the pathogenesis of liver fibrosis is well established. During the course of fibrogenesis, these cells proliferate, switch to a myofibroblast-like phenotype and synthesize fibrosis components. The signals responsible for Ito cells proliferation are still poorly understood. Moreover, most studies of Ito cells proliferation have dealt with rats cells. In this study, we have investigated the effect of purified growth factors, all expressed in either normal or fibrotic liver, on the growth of cultured human Ito cells in their myofibroblast-like phenotype. Human Ito cells were grown from liver explant and characterized by immunostaining for desmin and alpha-smooth muscle action. For growth experiments, cells were cultured in DMEM with 0.5% human serum (depleted of growth factors over heparin-sepharose) and were counted after 6 days. Basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) stimulated human Ito cell proliferation, as already shown for rat Ito cells. TGF $\beta$  1, known to inhibit rat Ito cell proliferation, was surprisingly a strong mitogen for human Ito cells, more potent than bFGF or PDGF. We investigated whether this effect was mediated by autocrine secretion of growth factors by Ito cells, as shown in other cellular models. We could indeed show that an antibody to PDGF partially blocked the mitogenic effect of TGF $\beta$  1. In contrast, anti-bFGF antibody failed to inhibit the mitogenic effect of TGF $\beta$  1 despite the fact that TGF $\beta$ 1 did induce an accumulation of bFGF in human Ito cells (demonstrated by western blotting). These data show that TGF $\beta$ 1 is mitogenic for human Ito cells and they suggest that results obtained with rat Ito cells cannot be extrapolated to human cells. They also show that the mitogenic effect of TGF $\beta$ 1 is at least in part mediated by an autocrine secretion of PDGF by Ito cells. The role of TGF $\beta$ 1-induced accumulation of bFGF in the mitogenic effect of TGF $\beta$ 1 is unclear. The lack of effect of the anti-bFGF antibody does not completely rule out bFGF mediation as the intracellular localization of bFGF could prevent its interaction with antibody. TGF $\beta$ 1 is well-known for inducing matrix components synthesis. The demonstration that it is also a human Ito cell mitogen makes it a likely key mediator or liver fibrogenesis.

**Study of inhibitory effects of some traditional medicine formulations on adrenaline-induced hyperglycemia in animal models.** AYE-THAN, TIN-MYINT, MU-MU-SEIN-MYINT, WIN-MYINT & MYA-BWIN. Pharmacology Research Division, Department of Medical Research, Yangon.

Traditional Medicine Formulations TMF-27,

TMF-28 and TMF-32 has been used traditionally to treat diabetes in Myanmar. Blood glucose levels of adrenaline-induced diabetic rabbits were determined after oral administration of maximum dose of the traditional medicine formulations TMF-27, TMF-28 and TMF-32 and their watery extracts respectively. From the data obtained, it was concluded that the oral administration of 1.6 g/kg of TMFs did not produce inhibition of hyperglycaemic effect in rabbits. Only the water soluble extract of TMF-32 inhibited the hyperglycaemic blood glucose level on adrenaline diabetic rabbits ( $p < 0.05$ ) and rats ( $p < 0.05$ ). The acute toxicity studies carried out on rats could not reveal any adverse or side effects of these TMFs as the maximum dosage tested. The implications of the findings are discussed.

**Antibacterial activity of honey, propolis and bee pollen: an in-vitro study.** *PHYU-PHYU-WIN & TIN-AYE.* Bacteriology Research Division, Department of Medical Research.

An in-vitro study was carried out to determine the antibacterial activity of honey, propolis (bee glue) and bee pollen. It was found that pure honey is a potent inhibitor of all the pathogens tested. Most pathogenic bacteria failed to grow in honey at a concentration of 30% and above. Propolis was found to have antibacterial activity only against Gram-positive cocci, but limited activity against Gram-negative bacilli. Propolis can eliminate the growth of Gram-positive even at a concentration of 10 percent. Bee pollen has no antibacterial effect on all the pathogens tested. The findings confirm previous reports of antimicrobial properties of these materials.

**Contraceptive marker survey in Myanmar.** *AUNG-TUN-THET, AUNG-KYAING, SOE-MYINT & KYAWT-SAN-LWIN.* Department of Planning and Statistics.

Case study approach was used. The key distributors from major pharmaceutical markets (Mingala and New Bogyoke Market) were interviewed on sources of contraceptives, distribution channels, price and volume of the market. Similarly, small markets and shops from smaller towns and villages were visited and contraceptive retailers were interviewed. Some random purchase of contraceptives were also done. As expected beforehand, many key distributors from major markets in Yangon were worried about taxation on pharmaceutical they sell. After explaining to them about the fact that survey was not related to any tax appropriation, they were more than willing to co-operate. Focus group discussions were conducted with health workers from townships in Yangon and Bago divisions and mothers from villages were also interviewed about contraceptive use. The major source of contraceptives and distribution channels are identified. The

price structure of contraception are mentioned. Precision of contraceptive advice and utilization patterns are described.

**Access to family planning services through GPs.** *LE-LE-WIN, SAN-SHWE, MYAT-LAY-KYIN, BO-KYWE & THAN-TUN-SEIN.* Department of Medical Research & Department of Health.

In Myanmar, private medicine still plays a significant role in delivery of health care as well as in the provision of family planning services. Thirty nine GPs - practising in Sangyaung Township, Yangon Division were surveyed, to investigate the present situation of services with regards to family planning services, provided by them. Though family planning services being provided by GPs was not too much in the study area, the majority thought of the importance of the family planning services and believed that, they could help people for birth spacing. They felt that, they needed more information on social/economic impact of the family planning. They also suggested on opening of government operated clinics. The findings will be provided for the future planning of Family Planning Services in Myanmar.

**The cost of abortion.** *\*KATHERINE-BA-THIKE & \*\*KHIN-THET-WAI.* \*Department of Obstetrics and Gynaecology, Institute of Medicine II & \*\*Medical Education Unit, Institute of Medicine I.

This study was carried out to highlight the costs of unsafe abortion. The costs borne by twenty patients with unsafe abortion were compared with 40 patients each of spontaneous abortion and spontaneous vaginal delivery. The mean duration of hospital stay was 1.95 days, 2.8 days and 12.9 days respectively for vaginal delivery, spontaneous abortion and unsafe abortion. Antibiotic usage was 100% in unsafe abortion and 52% and 82% in normal deliveries and spontaneous abortions. Usage of intravenous infusions and blood transfusions were 80% and 35% in patients with unsafe abortion, being used less in the comparison groups. Minor surgery (evacuation of the uterus) was performed in 82% of spontaneous abortion patients, 35% with unsafe abortion required evacuation of the uterus why 40% required operative drainage of pus. The cost of unsafe abortion was calculated as expenditure before admission (mean - kyats 400/-) medical and non-medical costs during hospitalisation (mean - kyats 748/- and 129/-) and loss of income during admission (mean - kyats 388/-). The average total cost borne by patients with unsafe abortion was kyats 1408/-, approximately nine times and three times more than normal delivery and spontaneous abortion. It is evident that abortion places a heavy cost both on patients and the health services.

**Maternal mortality and morbidity and surgical operations in hospitals.**  
*SAN-SAN-MYINT, AYE-MI-SAN & AYE-MOE-MOE-LWIN.* Department of Planning and Statistics.

In this paper, we shall analyse the data and discuss the information concerning the maternal mortality and morbidity and surgical operations performed in the reporting hospitals for 5 years (from 1985 to 1989). The objectives of the study are as follow :-

- (1) To determine the trend of institutional MMR.
- (2) To examine the geographical distribution of maternal mortality and morbidity.
- (3) To ascertain the leading causes of institutional maternal mortality and morbidity.
- (4) To determine the situation of surgical operations in different hospitals and it's trend.
- (5) To examine the pattern of higher rate surgical operations.

Using the routine health information system, data was analysed and the results are discussed to uncover the underlying trends.

**Utilization of gynaecologic beds at the Central Women's Hospital (1990).**  
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A one year situational analysis of utilization of gynaecological beds at Central Women's Hospital (Unit II) is presented. The pattern of admission, details of treatment, duration of hospital stay and bed occupancy were determined. There were 667 admissions of which 564 were elective admissions and 103 were emergencies. Of all admissions, 94.1 percent were operated on. Of the abdominal operations, hysterectomy (135) was the most common operation followed by female sterilization (120). Anterior and posterior colporrhaphy (56) was the commonest vaginal operation performed. The mean hospital stay for cases of female sterilization, myoma uteris, ovarian tumour and uterovaginal prolapse was 7 days, 26 days, 22 days and 19 days respectively while the mean waiting time was 5 days, 14 days, 14 days and 11 days respectively. Recommendations whereby the waiting time and the hospital stay and indirectly the bed occupancy can be reduced are presented. It is hoped that quality medical care can be rendered by optimal use of existing resources.

**Relative albumin clearance in high risk pregnant women for pregnancy-induced hypertension (P.I.H.).***MYA-MYA, HLA-YEE-YEE & SI-LAY-KHAING.* Department of Obstetrics and Gynaecology and Department of Physiology, Institute of Medicine I & Central Women's Hospital, Yangon.

A study on "Relative Albumin Clearance in

high-risk pregnant women for P.I.H." was performed at the Central Women's Hospital in 1990-91. Forty-eight cases of high-risk pregnant women for P.I.H. were studied for early prediction of this condition, the hypothesis being that there is an early derangement of nephelometric function long before the onset of clinical P.I.H./P.E. This study is aimed to detect the PIH/PE as early as possible by means of Relative Albumin Clearance using the spectrophotometer. The subjects were selected according to the criteria set down in the protocol. Urine and blood samples were taken; first sample at 24 weeks and subsequent ones at 2 weekly intervals from 28 weeks till the third day of puerperium. For the nephelometric function, the performed biochemical studies were serum and urine albumin (Bromocresol Green method) and serum and urine creatinine (Alkaline Picrate method) using the spectrophotometer. These biochemical tests were performed at the Biochemistry laboratory, Department of Medical Research. Out of 48 cases, 26 cases developed PIH/PE and the rest were normal. The biochemical parameters from the normal group and those who developed PIH/PE were analysed and compared. The outcome of the delivery was also analysed.

**The approaches to learning of final year medical students.** *WIN-MAY & KHIN-THET-WAI.* Department of Health Manpower.

The way in which students approach their learning seems to depend on a number of factors. These include the teaching methods, curriculum density and assessment methods. A study was under taking on final year medical students at the Institute of Medicine I, Yangon to identify their learning approaches, using the Adelaide Diagnostic Learning Style Inventory for Medical Students, (version 2). The results showed that the medical students had higher scores on the surface and strategic approaches than on the more desirable deep approach. Further longitudinal studies will be required to determined whether there is a change in learning approaches as the students progress through medical school to find out the relationship between context and environment on their learning approaches.

**Orientation of medical education towards community needs in the Union of Myanmar.** *\*WIN-MAY, \*\*KHIN-THET-WAI & \*\*\*KHIN-SAN-NAING.* \*Department of Health Manpower, \*\*Institute of Medicine (I) & \*\*\*Institute of Medicine (II).

Orientation of medical education towards community needs was first initiated in Myanmar in 1972. This was practiced by introducing community medicine into the under-graduate medical curriculum. Since then it has evolved and developed into what will be described henceforth. Community orientation commences in the preclinical

years mainly through the collection of certain physiological parameters with regards to nutrition in the local community. Deficiencies detected are corrected by means of supplementation as well as advise to the community. In the para clinical years there are visits to health centres and participation in occupational and environmental health programmes. The students also take part in family health care within the community, usually a suburban one by means of family attachments. There are residential programmes in small towns within both urban and rural settings for a three-week period. In the internship year, two weeks residential training in a field practice area under supervision is mandatory. In all these programmes, students are involved in data collection for community diagnosis, data

analysis and priority setting with start from the Township Health Department which is responsible for the health care for both urban and rural community. During the residential training periods, they work with health personnel taking part in any health care activity being carried out in that community. In the internship residential training, community health surveys are contacted; the data analyzed and programmes are planned with the health staff for the further implementation. During vacations, students can volunteer to take part in any of the ongoing National Health Programme activities, such as immunization programme and latrine construction programmes. In this way the medical schools involved themselves in the improvement of the health status of the community.

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