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The relationship between the intensity of malaria infections and different types of G-6-PD deficiency in children living in malaria endemic areas of Burma

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The sample consisted of 88 males and 159 females from Taikkyi and Thantaung townships. Subjects were school children under 14 years of age. Using WHO (1969) standard methods and recently developed cytochemical method (Van Noorden & Vogels 1985) 14 (15.9%) G-6-PD deficient males (hemizygous) , 7(4.4%) homozygous females and 17(10.7%) heterozygous females were detected. Out of 14 hemizygous males, 5 were positive with P. falciparum and 3 were positive with P. vivax. In 7 homozygous females, one was infected with P. falciparum and two with P. vivax. Out of 17 heterozygous females, 6 were infected with P. falciparum and two with P. vivax . P. falciparum parasitaemia in normal male was 6.0% (mean of 19 subjects), male G-6-PD hemizygote was 1.0% (mean of 5 subjects) , female G-6-PD normal was 8.6% (mean of 34 subjects), female G-6-PD homozygote was 1.2% (one subject only) , and female heterozygote was 1.0% (mean of 6 subjects). P. vivax parasitaemia in male G-6-PD hemizygote was 1.0% (mean of 3 subjects), female G-6-PD normal was 6.2% (mean of 9 subjects), female G-6-PD heterozygote was 1.0% (mean of 2 subjects). The cytochemical staining method was also used to distinguish the intensity of malarial parasites in individual cells of heterozygotes. It was observed that the malaria parasites infect both G-6-PD deficient and normal cells but parasitaemia was significantly and uniformly lower in deficient cells (normal cells = $0.8 \pm SE \ 0.118$; deficient cells = $0.4 \pm SE \ 0.052$) ($P < 0.01$) .

INTRODUCTION

Glucose - 6 - phosphate dehydrogenase deficiency has attained a high frequency especially in many areas where endemic malaria due to P. falciparum existed. The study of this enzyme in man has a fascinating history which was reviewed more than twenty

years ago by Tarlov et al. (1), Beutler (2 , 3) and Motulsky (4). It has been postulated that the X-linked G-6-PD deficiency may protect against fulminant P. falciparum in some unknown fashion , Friedman (5,6,7). The suggestion that G-6-PD deficiency may confer a selective advantage in the presence of P. falciparum (8)

has been supported by some population studies by Luzzatto et al., (9) , Siniscalco et al (10), Luzzatto (11) and Guggenmoos-Holzmann et al (12), though questioned by others like Martin et al., (13) and Bernstein et al. (14).

To date, conclusive evidence for protection by G-6-PD deficiency remains to be found when the malaria parasite may not depend on either the host cell enzyme directly or on its products. In Burma G-6-PD deficiency (as measured by GSH stability test) was reported by Aung-Than-Batu et al (15) in 4-14 % of healthy populations of different ethnic groups (15). Hla-Pe et al. (16) and Kyaw-San-Hla (17) also carried out studies on the correlation of G-6-PD deficiency and malaria but the method used to test the G-6-PD was not very sensitive.

The present study was done in two malaria hyperendemic areas of Burma to investigate further protection of G-6-PD deficiency to falciparum malaria. The relationship of the parasite density and different types of G-6-PD deficiency was also studied to find out which type of G-6-PD deficiency in particular has selective advantage against malarial infection.

MATERIALS AND METHODS

SUBJECTS AND METHODS

Subjects

Two malaria endemic areas were studied:

1. Chingone village tract, Taikkyi township (32m above

sea levels) 50 miles north of Rangoon: A random sample of 114 secondary school children (age between 10-14 years, 49 males and 65 females) were selected.

2. Thantaung township (178. 3m above sea levels) 195 miles north-east of Rangoon , a random sample of 133 secondary children (age between 10 to 14 years: 39 males and 94 females) were selected.

Collection of sample

5-7 ml of venous blood in Acid-citrate-dextrose (ACD) (4:1) was collected from each subject and transported on ice to the mobile laboratory where, (1) Brewer methemoglobin reduction test, (2) Elution test, (3) Blood film and (4) MTT-linked spot test were performed. Cytochemical staining technique was performed only at DMR , Rangoon. Blood samples were preserved either in ACD and NADP at 4.0 or in the forms of haemolysates.

The cytochemical staining method was done according to Van Noorden & Vogels (18).

The MTT-linked spot test was done according to WHO (19).

The Methaemoglobin Reduction test was done according to WHO (19).

Methaemoglobin elution test for G-6-PD heterozygotes was done according to Luzzatto et al. (9)

Parasites were counted from Giemsa stained thin blood smears, and expressed in terms of 10⁴ erythrocytes .

RESULTS

Male hemizygote from Taikkyi had higher parasite prevalence rate than those from Thantaung township (Table 1a). By looking at the female groups, the prevalence rates of malaria

infection followed the same pattern as in the case of males (Table 1b). Females in Taikkyi township had higher incidence rate of malaria than those from Thantaung township.

Table 1a. The relationship between malarial infections and various types of G-6-PD deficiency

Types of G-6-PD Deficiency	T A I K K Y I					T H A N T A U N G				
	P.f ¹ + ³	P.v ² Posi- tive	Mixed infec- tion	No mala- ria	Total	P.f ¹ + ³	P.v ² + ³	Mixed infec- tion	No mala- ria	Total
Male Normal	12 (28.6%)	5 (11.9%)	1 (2.4%)	24 (57.1%)	42 (100%)	7 (21.9%)	4 (12.5%)	1 (3.1%)	20 (62.5%)	32 (100%)
Male Hemizygous	3 (42.9%)	2 (28.6%)	0 (0%)	2 (28.6%)	7 (100%)	2 (28.6%)	1 (14.3%)	0 (0%)	4 (57.1%)	7 (100%)
Total	15 (30.6%)	7 (14.3%)	1 (2.0%)	26 (53.1%)	49 (100%)	9 (23.1%)	5 (12.8%)	1 (2.6%)	24 (61.5%)	39 (100%)

P.f¹ = Plasmodium falciparum

P.v² = Plasmodium vivax

+³ = Positive cases

Table 1b. The relationship between malarial infections and various types of G-6-PD deficiency

Types of G-6-PD Deficiency	T A I K K Y I					T H A N T A U N G				
	P.f ¹ + ³	P.v ² Posi- tive	Mixed infec- tion	No mala- ria	Total	P.f ¹ + ³	P.v ² + ³	Mixed infec- tion	No mala- ria	Total
Female Normal	17 (30.9%)	5 (9.1%)	2 (3.6%)	31 (56.4%)	55 (100%)	17 (21.3%)	4 (5.0%)	0 (0%)	59 (73.7%)	80 (100%)
Female Hetero- zygous	3 (37.5%)	2 (25.0%)	0 (0%)	3 (37.5%)	8 (100%)	3 (33.3%)	0 (0%)	0 (0%)	6 (66.7%)	9 (100%)
Female Homo- zygous	0 (0%)	1 (50%)	0 (0%)	1 (50%)	2 (100%)	1 (20%)	1 (20.0%)	0 (0%)	3 (60.0%)	5 (100%)
Total	20 (30.8%)	8 (12.3%)	2 (3.1%)	35 (53.8%)	65 (100%)	20 (21.3%)	5 (5.3%)	0 (0%)	69 (73.4%)	94 (100%)

P.f¹ = Plasmodium falciparum

P.v² = Plasmodium vivax

+³ = Positive cases

In both the areas, Taikkyi and Thantaung, malaria infection had no selection between the different types of G-6-PD deficiency.

All G-6-PD deficient groups (hemizygotes, homozygotes and heterozygotes) were found to be susceptible to both falciparum and vivax malaria (Fig. 1). All groups from Taikkyi township had

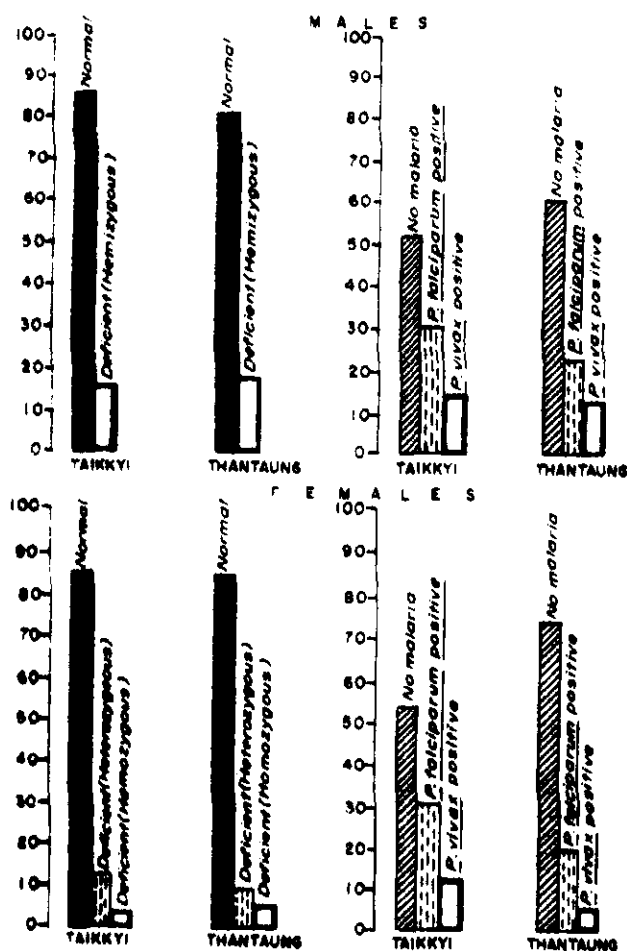


Fig. 1 - Relationship between various types of G-6-PD deficiency and malarial infections in Taikkyi and Thantaung townships

higher prevalence rates of malarial infections than those from Thantaung township. This could be due to the difference in geographical variations i.e. altitude above sea levels. Table (2) shows parasitaemia

densities in various types of G-6-PD deficiency.

Although the malarial infection had no selection between the G-6-PD normal, G-6-PD hemizygosity and G-6-PD homozygosity, the parasite density between these groups were remarkably different. Mean parasitaemia in male G-6-PD normal was 6.0%, male G-6-PD deficient hemizygous is 3.0%, female G-6-PD normal is 8.6%, female G-6-PD deficient homozygous was 1.2%. The falciparum parasitaemia levels were significantly higher in both male G-6-PD normal and female G-6-PD normal groups, whereas mean parasite density in male hemizygote was higher than those of female heterozygote and female homozygote.

Mean vivax parasitaemia in males normals and female normals were significantly higher than those of male hemizygotes, female heterozygotes and female homozygotes.

Table (3) shows the parasite density in G-6-PD normal and deficient cells of female heterozygotes.

The cytochemical staining method has been able to demonstrate the parasite densities in individual cells of eight female heterozygotes reported in this study. The mean parasitaemia in G-6-PD normal cells was $0.791 \pm SE 0.118$ and the mean parasitaemia in G-6-PD deficient red cells was $0.354 \pm SE 0.052$.

Parasite densities in G-6-PD deficient cells were uniformly and significantly lower than those of red cells with normal G-6-PD enzyme activity ($P < 0.01$).

Table 2. Parasitaemia densities in various types of G-6-PD deficiency

Types of G-6-PD deficiency	Positive with <u>P. falciparum</u>	Mean parasitaemia	Positive with <u>P. vivax</u>	Mean parasitaemia
Male Normal	0.3 - 22.3%	6.0% (19)	0.7 - 3.0%	1.8% (9)
Male Hemizygous	0.7 - 4.6%	3.0% (5)	0.3 - 1.7%	1.0% (3)
Female Normal	1.0 - 16.7%	8.6% (34)	1.0 - 11.0%	6.2% (9)
Female Heterozygous	0.3 - 1.7%	1.0% (6)	0.7 - 1.3%	1.0% (2)
Female Homozygous	1.2%	1.2% (1)	0.7 - 1.3%	1.0% (2)

Table 3. Parasite density in G-6-PD normal and deficient cells of female heterozygotes

Code number	Plasmodium species	Parasites/10 ⁴ erythrocytes		
		Normal cells	Deficient cells	Mean parasitaemia
TK-010	<u>falciparum</u>	121 (1.21%)	51 (0.51%)	1.72%
TK-044	<u>falciparum</u>	23 (0.23%)	8 (0.08%)	0.31%
TK-075	<u>falciparum</u>	74 (0.74%)	26 (0.26%)	1.02%
TK-038	<u>falciparum</u>	80 (0.80%)	42 (0.42%)	1.22%
TK-113	<u>falciparum</u>	121 (1.21%)	51 (0.51%)	1.72%
TK-126	<u>falciparum</u>	73 (0.73%)	40 (0.40%)	1.13%
TK-002	<u>vivax</u>	92 (0.92%)	39 (0.39%)	1.31%
TK-045	<u>vivax</u>	49 (0.49%)	24 (0.24%)	0.73%
Mean parasitaemia		0.791±SE 0.118	0.354±SE 0.052	1.14%

* Figures in parentheses represent parasitaemia levels

SE = Standard Error

DISCUSSION

Glucose-6-phosphate dehydrogenase deficiency (G-6-PD deficiency) is a genetic trait with much circumstantial evidence but little clinical evidence to support its role in malaria resistance, Motulsky (4). As related by Motulsky (4), he and others located high frequencies

of G-6-PD deficiency in malarial regions and simultaneously looked for protection against malaria. The first observation has been repeated many times, whereas the second effort has, with some dramatic exceptions, yielded negative results Livingstone (20, 21).

The worldwide distribution of G-6-PD deficiency is primarily associated with malaria, and the most striking examples of this are of microgeographical mapping (8). Mountain people, free of exposure to infection, carry a much lower proportion of G-6-PD deficient genes (Gd) than related lowland people (10, 22, 23). In this study, the distribution of G-6-PD deficiency showed no significant difference between children from Taikkyi (32m) and Thantaung township (178.3m), although there is a difference in altitude above sea level. On the other hand, it could be emphasized that Karens had higher G-6-PD deficiency

rates than Burmese, Aung-Tha-Batu & Hla-Pe (15). If the comparison is just between Karens males from Pa-an and Karens males from Thantaung, the difference was about 7.9% (14% and 21.87%), whereas the difference between Burmese from Rangoon and Burmese from Taikkyi is about 7.7% (9% and 16.67%). Therefore, it could be concluded that, apart from geographic variation, people living in malaria endemic areas had higher incidence of G-6-PD deficiency than people living in malaria free areas.

The female heterozygote (Gd^{+}/Gd^{-}) has about 25-75% of normal G-6-PD activity in the blood. About one-half the red cells produced normal levels expressing the normal gene; the other were deficient.

Luzzatto, Usanga, Reddy (9) used this situation to show a differential host competence for malaria between normal and G-6-PD deficient cells. They took blood samples from malaria-infected (Gd^{+}/Gd^{-}) females in Africa. When the cells were selectively stained for G-6-PD activity, there was a paucity of parasites in the deficient red cells compared with the normal cells.

However, the smears stained with the elution method could not give a definite differentiation of the malarial parasites. The differentiation of parasites in the red cells stained with elution technique was somewhat faint in colour. The cytochemical staining method of Van Noorden & Vogels (18) used

in this study gave better results than the elution technique previously described. Although the normal G-6-PD activity was carried between individuals, the parasitaemia was uniformly lowered in the portion of G-6-PD deficient cells. Cytochemical staining method was more tedious than the elution technique as regards to time and chemicals, but the results obtained by cytochemical staining method was far better than that of elution technique. The colour intensities between G-6-PD deficient and G-6-PD normal cells were distinguishable and the malarial parasite stained with cytochemical reagent were clearly recognizable.

It was surprising to see that the malarial parasite infect both Gd^{-} and Gd^{+} cells but the parasitaemia was significantly and uniformly lower in deficient cells. Although the malarial parasites were susceptible to Gd^{-} cells in vivo, their survival could be impaired at certain parasitaemia levels because of the insufficient amount of host G-6-PD enzyme. It was also reported that G-6-PD deficient cells are found to be never totally lacking the enzyme, Luzzatto & Testa (24), Beutler (25) but it may not be sufficient enough to support malarial parasites to reproduce and multiply intra-erythrocytically.

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REFERENCES

1. Tarlov, A.R., R.W. Kellermeyer., G.J. Brewer, P.E. Carson and A.S. Alving. Hemolytic effect of therapeutic drugs. Clinical considerations of the primaquine-type Hemolysis. Journal of American Medical Association 1962; 180:388.
2. Beutlwe, E., M. Yeh and V.F. Fairbanks. The normal human as a mosaic of X-chromosome activity: Studies using the gene for G-6-PD deficiency as a marker. Proceedings of the National Academy of Sciences, U.S.A 1962; 48:9.
3. Beutler, E., C.K. Mathai and J.E. Smith. Biochemical variants of glucose-6-phosphate-dehydrogenase give rise to congenital nonspherocytic hemolytic disease. Blood 1968; 31:131.
4. Motulsky, A.G. In "The Role of Natural Selection in Human Evolution". F.M. Salzano ed. North Holland Pub., Amsterdam 1965; 31:131.
5. Friedman, M.J. Oxidant damage mediates variant red cell resistance to malaria. Nature (Lon.) 1979; 280:245-247.
6. Pasvol, G., D.J. Weatherall and R.J.M. Wilson. Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum* Nature 1972; 270:171-173.
7. Pasvol, G., R.J.M. Wilson, B.J. Smalley and J. Brown. Separation of viable schizont-infected red cell of *Plasmodium falciparum* from human blood. Annals of Tropical Medicine and Parasitology 1987; 72:87-88.
8. Allison, A.C. Population genetics of abnormal haemoglobins and glucose-6-phosphate-dehydrogenase deficiency. In: Jonxis, J.H.P. ed. Abnormal haemoglobins in Africa, Oxford, Blackwell 1965:365.
9. Luzzatto, L., E.A. Usanga and S. Reddy (1969). Glucose-6-phosphate-dehydrogenase deficient red cells: Resistance to infection by malarial parasite. Science 1969; 164: 839-942.
10. Siniscalco, M., L. Bernini, G. Filippi et al. Population genetics of haemoglobin variants, thalassaemia and G-6-PD deficiency, with particular reference to the malaria hypothesis. Bulletin of World Health Organization, 1966; 34:379-393.
11. Luzzatto, L. and U. Bienzle. Genetics of Red Cells and susceptibility to malaria. Blood 1979; 54(5):961-976.
12. Guggenmoos-Holzmann, I., U. Bienzle and L. Luzzatto. *Plasmodium falciparum* malaria and human red cells. II Red cell genetic traits and resistance against malaria. International Journal of Epidemiology 1982; 10:16-22.
13. Martin, S.K., L.H. Miller, D. Alling et al. Severe malaria

- glucose-6-phosphate-dehydrogenase deficiency a reappraisal of the malaria G-6-PD hypothesis. Lancet 1979;1:524-526.
14. Bernstein S.C., J.E. Bowman and L.K. Kaptue-Noche. Population studies in Cameroon. Haemoglobin S, glucose-6-phosphate deficiency and falciparum malaria. Human Heredity 1980;30:251-258.
 15. Aung-Than-Batu and Hla-Pe. Glucose-6-phosphate-dehydrogenase deficiency in Burma. Union of Burma Journal Life Sciences 1969;2:251-258.
 16. Hla-Pe, Tin-Win and Phyu Phyu. Studies on protective action of glucose-6-phosphate-dehydrogenase deficiency against malaria. Abstract, Research Paper Reading session of Medical Sciences Division 1982.
 17. Kyaw-San-Hla (1984). The influence of erythrocyte Glucose-6-phosphate-dehydrogenase status on the clinical course of Plasmodium falciparum infection. M.Med.Sc. (Thesis), Institute of Medicine, Mandalay, 1984.
 18. Van Noorden, C.J.F. and I.M.C. Vogels. A sensitive cytochemical staining method for glucose-6-phosphate-dehydrogenase activity in individual erythrocytes. British Journal of Haematology 1985;60:57-63.
 19. W.H.O. Scientific group on the standardization of procedures for the study of G-6-PD. Technical Report Series, Technical Report Series, No.366, 1967.
 20. Livingstone, F.B. "Abnormal Haemoglobins in Human Populations" Aldine, Chicago, Illinois, 1967.
 21. Livingstone, F.B. Malaria and human polymorphisms. Annual review of Genetics 1971;5:33.
 22. Stamatoyannopoulos, G., G.R. Fraser, A.G. Motulsky, P. Fessas, A. Akrivakia and T. Papayannopoulou. On the familial predisposition to favism. American Journal of Human Genetics 1966;18:2553.
 23. Flatz, G. and S. Sringam. Lancet, 1963; 2: 1248-1250. Cited in Friedman, M.J. In: Biochemistry and Physiology of protozoa. Second Edition, 1981, vol. (Edited by Levandawsky, M. & Hunter, S.H.) New York, London. Academic Press.
 24. Luzzato, L. and Testa. Human erythrocyte glucose-6-phosphate-dehydrogenase: Structure and function in normal and mutant subjects. Current Tropical Haematology 1978;1: 1-70.
 25. Beutler, E. Glucose-6-phosphate dehydrogenase deficiency. The Metabolic Basis of Inheritance Edited by J.B. Stanbury, J.B. Wijngarden and D.S. Frederickson, 4th ed., 1978 pp. 1430-1451. McGraw-Hill, New York.

The growth stimulating factor in parabiotic rats

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Littermate Wistar rats were parabiosed at 30 days and daily individual food intake and individual weight gain of fifteen parabiotic pairs were recorded as control parabiotic pairs. Another fifteen pairs were used for the experiment in which one partner of parabiotic pair was completely fasted for 4 days and then refed ad libitum till body weight returned to the prefasting level and throughout the experiment, individual body weight changes and individual food intake of both partners of test parabiotic pairs were recorded. During fasting period, daily food intake and weight gain of non-fasted partner of test parabiotic pair increased significantly over that of the individual rat in control parabiotic pair. Moreover, weight loss of fasted partner is same as that of individual fasted rat. The above findings indicated the presence of factor/factors in plasma of fasted partner stimulating food intake and growth of non-fasted partner of the test parabiotic pair. The present study also reveals that there is greater body weight increase per. g. food consumed (food conversion efficiency) of fasted partner during refeeding period whereas that of non-fasted partner decreased significantly during that period. Food intake and factors controlling it have been extensively studied by Physiologists and Nutritionists interested in growth and its regulation. Various external and endogenous stimuli initiate and terminate feeding. Of the endogenous stimuli humoral factors and their mode of action are the subjects of several recent studies (1,3,4,6). The present study is undertaken in order to further clarify and assess the role of humoral factor in the regulation of food intake and growth.

MATERIALS AND METHODS

Parabiotic procedure

Littermate Wistar rats were parabiosed at 30 days. The operative procedure was essentially that described by Bunster and Meyer (2).

Determination of individual food intake and individual weight changes

10 days after operation, parabiotic pairs were fully active and feeding normally. There were no signs of skin rejection between partners and the existence of

plasma exchange was demonstrated in some of the pairs by using radio-iodinated serum albumin.

The parabionts were trained to consume their entire daily ration of food in a 6:00 hour period. Subsequently, the pairs were conditioned to eat in the specially designed partitioned cages, which permitted some freedom of movement but prevented access to food or water in the partner's compartment. The diet was that of the Department of Medical Research, Burma, Rat chow with an energy value of 3.25 Kcal/g. Water from individual bottle was available at all times. Environmental temperature was maintained between 22° and 25°C and was recorded daily.

An adjustable stage was specially designed and used in conjunction with a balance in order to weigh the individual partner of the parabiotic pair under light anaesthesia separately. The balance used was Top-Pan, Top E.500 balance with the accuracy of 0.01 g. This method of separate weighing of the individual partner of the parabiotic pair was demonstrated to be accurate within ± 0.05 g.

Design of experiment

Control parabiotic pairs: Fifteen parabiotic pairs were kept as controls and their daily individual food intake and weight gain were recorded.

Test parabiotic pairs: Fifteen pairs of rats were used for the experiment in which one partner of parabiotic pair was completely fasted for four days and then refed ad libitum till body weight returned to the prefast-

ing level. Individual body weight changes and individual food intakes of each partner (fasted partner as well as non-fasted partners) were recorded throughout the prefasting, fasting, and refeeding periods.

RESULTS

The mean daily individual food intake of each partner of control parabiotic pairs was determined and shown in Fig. 1. The average daily food intake of each partner of control parabiotic pair for the entire period of experiment was found to be $11.41 \pm \text{S.D. } 0.15$ g.

The average food intake of non-fasted partner of the test parabiotic pairs during prefasting period, fasting period and refeeding period are shown in Figure 1. During the fasting period the food intake of non-fasted partner increased significantly (5% probability level) over that of the individual rat of the control parabiotic pair. It was highest on the first day then gradually decreased till it reached the prefasting level during refeeding period.

As shown in Figure 1, the daily food intake of the fasted partner during refeeding period was highest on the first day of refeeding and then it declined slowly and reached the prefasting level on twelveth day after refeeding.

The mean individual weight gains of each partner of the control parabiotic pairs were shown in Figure 2. The average daily individual weight gain in control parabiotic pair is

1.54 $\pm$ S.D 0.91 g.

The effect of complete fasting and refeeding of one partner on the weight of each partner of the test parabiotic pairs was shown in Figure 2. During the

after refeeding. It is surprising to see that there was marked weight increase of the non-fasted partner on the first day of refeeding period. The weight of the non-fasted partner then fell gradually and reached the

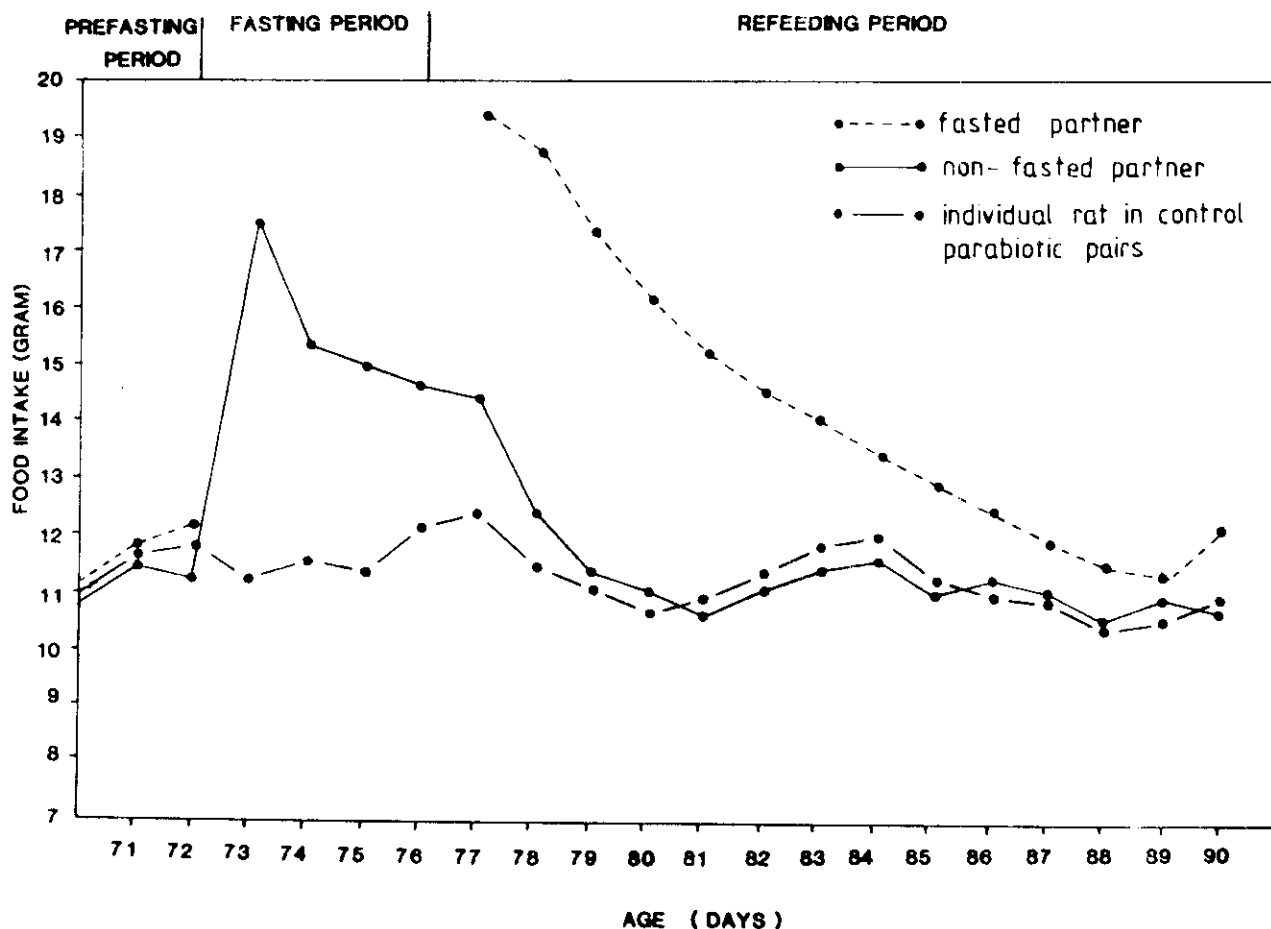


Figure.1- The effect of complete fasting and refeeding of one partner on changes in food intake of other partner in test parabiotic pairs.

fasting period the mean body weight of the non-fasted partner increased significantly over that of individual rat in control parabiotic pairs. On refeeding, weight of fasted partner increased abruptly on the first day and prefasting weight is gained on twelveth day

prefasting level on the sixth day of the refeeding period. As expected, the weight of the fasted partner fell rapidly the during fasting period and on refeeding increased rapidly to prefasting level within twelve days.

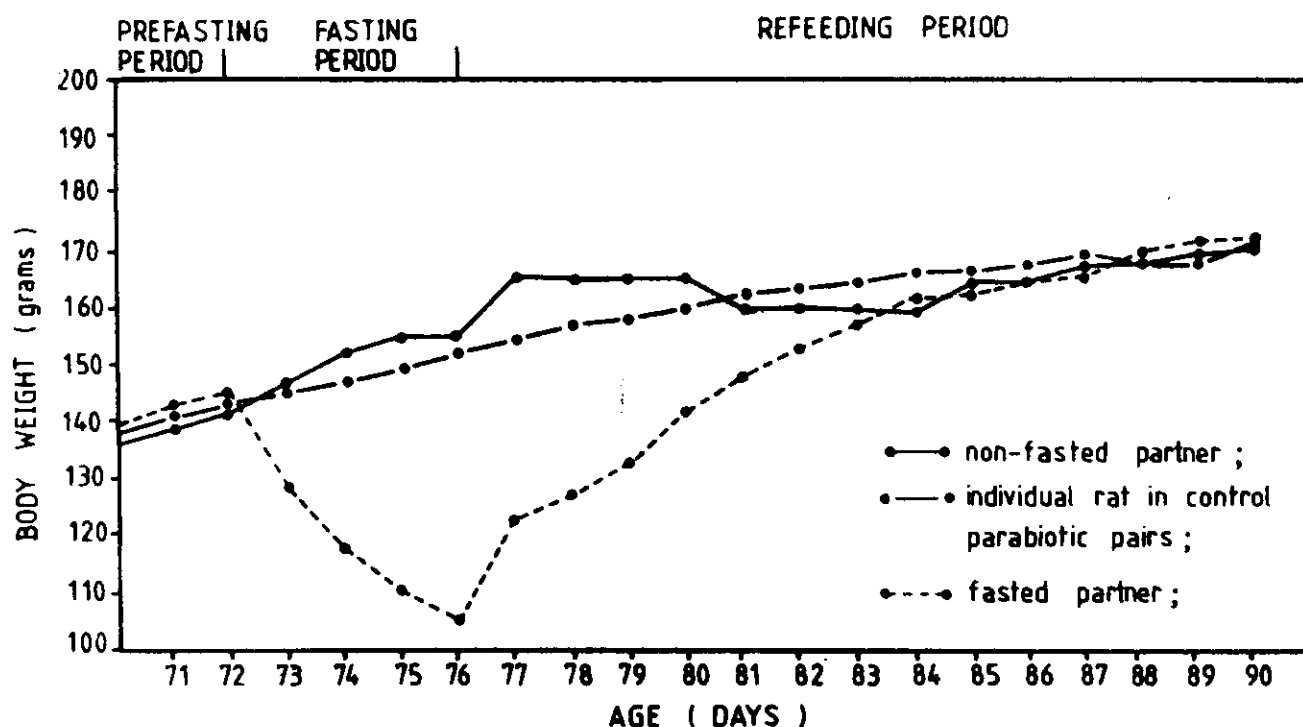


Figure.2- The effect of fasting and refeeding of one partner on changes in growth(weight) of both partners in test parabiotic pairs.

Food conversion efficiency (i.e., body weight increase per g. of food consumed) was calculated for different periods of experiment in both fasting and non-fasting partner and shown in Table 1. The table portrayed that the food conversion efficiency of fasted and non-fasted partner did not differ significantly from one another during prefasting period. There was no difference in food conversion efficiency of non-fasted partner between the prefasting and fasting period. However, the food conversion efficiency of fasted partner was significantly increased during refeeding period whereas that of non-fasted partner decreased

significantly (significant at 5% probability level).

Table 1. Food conversion efficiency (percent) of fasted and non-fasted partner of test parabiotic pairs

Parabiotic Pairs	Prefasting period	Fasting period	Refeeding period
Fasted partner	20.42	-	32.79
Non-fasted partner	21.39	20.95	9.23

DISCUSSION

The marked increase in food intake of the non-fasted partner of the test pair during the fasting period indicates that the fasting state has generated some factor/factors in the fasted partner which was transmitted through plasma to

the non-fasted partner and has stimulated the latter to increase its food intake.

It appears that this factor can be generated and act rapidly. Its stimulatory effect is highest on the first day and then gradually declines but continues to be active throughout the fasting period. On resumption of feeding the stimulatory effect decreases rapidly to prefastion level indicating that the generation and transmission of the humoral factor has rapidly declined. The mechanism of action of this humoral factor may be either through inhibition of satiety centre or stimulation of feeding centre.

The observation of a gradual decline in stimulatory effect of the humoral factor even during the fasting period may be explained as due to decreased generation and transmission of the humoral factor or due to decrease in response of the satiety or feeding centre. Parameswaren et al.(5) described the existence and circulation of humoral factor inhibiting food intake in parabiotic rats where one partner was electrically stimulated in the hypothalamus. Our two studies therefore complement and reinforce each other.

As expected, the increase food intake of the non-fasted partner of the test pair was accompanied by a greater weight gain when compared with that of the individual rat of control parabiotic pair. Moreover, this increased weight gain continued into the refeeding period up to five days even though food

intake had declined to prefasting levels.

The great increase in food intake and rapid weight gain in the fasted partner when refeeding commences is noteworthy. It is probable that the humoral factor in the circulation at the beginning of the refeeding period may have stimulated this urgent increase in food intake and rapid weight gain. With the commencement of refeeding the generation of the humoral factor probably gradually declined with consequent decline in food intake and in rate of weight gain.

A consideration of the changes in food conversion efficiency shows that this may also play a role in the weight changes observed. The greater food conversion efficiency in the fasted partner during refeeding may have contributed toward the rapid regain of body weights to prefasting levels. Also the lowered food conversion efficiency in the non-fasted partner during refeeding may also have contributed toward the shedding of excess weight gained during fasting period and the return to prefasting level. This observation complement those of Perbjornstrop, Edstrom, Kral, Lundholm, Presta, Walk and Yang (6) who observed that greater food efficiency is responsible for inability of the obese subjects to lose weight to a level commensurate with decrease in food intake. These changes in food conversion efficiency during refeeding in fasted and non-fasted partners suggest that the humoral factor

may not only influence the satiety/feeding centre but may also influence on metabolism in some manner which is still not clearly understood.

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REFERENCES

1. Booth, D.A. The physiology of appetite. British Medical Bulletin 1981;37:135-140.
2. Bunster, E. & R.K. Meyer. An improved method of parabiosis. Anatomy Records 1933; 57:339-343.
3. Jequier, E. Thermogenesis and human obesity. Nestle Research News 1982/83.
4. Langhans, W., U. Ziegler, E. Scharrer & N. Geary. Stimulation of feeding in rats by intraperitoneal injection of antibodies to glucagon. Science 1982;218:894-96.
5. Parameswaren, S.V., A.B. Steffen, G.R. Hervey & L.D. Ruiter. Involvement of humoral factor in regulation of body weight in parabiotic rats. American Journal of Physiology 1977 ; 232 (5) : 150 - 157.
6. Per-Bjorntorp, S. Edstrom, K. G. Kral, K. Lundholm, E. Resta, D. Walks & M.U. Yang. Refeeding after fasting in the rat: effects on body composition and food efficiency. American Journal of Clinical Nutrition; 36(3).

Patterns of acute renal failure in Russell's viper bite patients

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Fifty-two patients with acute renal failure due to Russell's viper bite were studied. 34 patients had oliguric and 18 had non-oliguric types of acute renal failure. There was a higher incidence of complications such as gastrointestinal bleeding, renal angle tenderness and facial oedema in patients with oliguria. These patients also had a higher serum creatinine, fractional excretion of sodium and renal failure index when compared to polyuric patients. Mortality rate was about 32% in patients with oliguria in spite of peritoneal dialysis in 8 patients. No patients in the polyuric group needed dialysis and none died. The difference may be due to more severe renal lesions in patients with oliguric acute renal failure.

INTRODUCTION

Acute renal failure (ARF) is a syndrome of rapid onset of impaired renal function characterised by progressive azotemia and usually oliguria. Consequently oliguria had come to be regarded as a cardinal feature of acute renal failure. But as early as 1943 Shen(1) recognised patients with azotemia in the absence of oliguria. The emphasis since then had been on the quality rather than the quantity of urine passed.

Oliguria is defined as passing of less than 400 ml of urine per 24 hours. Patients who passed more than 400 ml per 24 hours throughout the azotemic period are therefore regarded as having nonoliguric acute renal failure. Anderson et al. (2) in 1977 recognised 54 non-oliguric ARF patients out of 92 patients

who met the criteria for acute tubular necrosis. More recently retrospective studies have suggested that nonoliguric ARF may account for 20 to 30% of all cases of ARF (3,4).

Postulations had been made to explain the mechanisms of higher urinary flow. They include (1) interference with renal concentrating ability; (2) maintenance of a high rate of flow through the distal nephrons thereby preventing equilibration of tubular with a hypertonic medullary interstitium and; (3) interference with the formation of an hypertonic medullary interstitium. Toxin related nephropathy appears to be more frequent in nonoliguric ARF. Schrier and associates (3) suggested that decrease in morbidity and mortality in non-

oliguric ARF is due to higher glomerular filtration rate and relative preservation of tubular function. But the degree of renal damage appears to be the essential difference between oliguric and nonoliguric ARF.

Acute renal failure is the most devastating effect of Russell's viper bite in Burma and oliguria had been stressed as the hallmark of ARF in these patients. We therefore undertook a study to establish the clinical and biochemical pattern of ARF in patients with Russell's viper bite. The study period was from 1984 to 1988 during the rice harvesting seasons from October to January each year.

PATIENTS AND METHODS

All patients with Russell's viper envenomation admitted into Tharawaddy Township Hospital during the study period were clinically and biochemically assessed for evidence of renal disturbances. All patients with serum creatinine more than 1.3 mgm and/or oliguria (urine volume less than 400 ml per 24 hours) without obvious pre-renal cause were then studied. In oliguric patients dehydration was corrected, if necessary, after measuring central venous pressure. Patients responding by diuresis were excluded from the study. Twelve apparently healthy subjects were also studied as controls. Urine volume of 400 ml per 24 hours was taken as a dividing line for classifying ARF into oliguric and nonoliguric ARF.

All patients included in the study were thoroughly examined and kept under close observation. Fluid balance charts and other relevant data were accurately recorded. Daily urine and blood samples were collected for biochemical measurements. Venom antigen levels were measured on admission for confirmation of biting snakes. All samples were kept frozen until tested at the Department of Medical Research.

Treatment: All patients received lyophilised monospecific refined freeze dried antivenom for Russell's viper (BPI) 40 ml (intravenous injection within 10 minutes) was given initially and repeated 6 hours later if blood is still non-cottable.

General measures for acute renal failure were instituted for all patients. If oliguria persists after rehydration, if necessary, intravenous frusemide 40-250 mgm and dopamine 2.5ug/kg/hour in 500/ml of isotonic saline infusion was given. None of the patients with nonoliguric ARF received diuretics or any medication known to alter the antidiuretic release and renal response to antidiuretic hormone.

All patients were kept in hospital for a minimum of 5 days. Patients considered for dialysis were referred to the Rangoon General Hospital.

Laboratory Methods: Venom antigen was measured by enzyme linked immunosorbent assay (ELISA). Biochemical analysis was done by standard methods.

RESULTS

A total of 52 patients was studied. 34 patients (41%) had oliguric ARF. In these patients oliguria set in within first 24 to 48 hours after admission into hospital. 18 patients (22%) passed more than 400 ml of urine per 24 hours throughout the azotemic phase of the illness. The mean daily urine volume was 2204 ml in these patients.

Table(1) summarises the clinical features of 34 oliguric ARF and 18 nonoliguric ARF patients. In patients with oliguric ARF there was a significantly higher incidence of gastrointestinal bleeding (21% compared to nil in nonoliguric ARF), renal angle tenderness (32% compared to 6% in nonoliguric ARF), and facial and conjunctival oedema (47% compared to nil in nonoliguric ARF). The incidence of other clinical signs were very similar in the two groups.

Table 1. Clinical features in patients with oliguric and nonoliguric acute renal failure.

Clinical Parameters	Oliguric acute renal failure n = 34	nonoliguric acute renal failure n = 18	P value
Age (years): mean±SD	36±3	36±9	
Sex (M/F)	23/11	16/2	
Time interval between bite and anti-venom (hours):	6±1.2	6±1.1	
Venom antigen (mg/ml): mean±SD	72±5	85±6	NS
Local swelling (no. of cases)	33(97%)	16(89%)	NS
Blisters (no. of cases)	4(12%)	Nil	
Necrosis (no. of cases)	2(6%)	Nil	
Regional lymphadenitis (no. of cases)	27(79%)	11(61%)	NS
Systemic bleeding (no. of cases)			
- Gums	9(26%)	3(17%)	NS
- Gastrointestinal	7(21%)	Nil	<0.05
- Genitourinary	13(38%)	4(22%)	NS
Hypotension (no. of cases)	17(50%)	5(28%)	NS
Renal angle tenderness (no. of cases)	11(32%)	1(6%)	<0.05
Facial & conjunctival oedema (no. of cases)	16(47%)	Nil	<0.01

The mean±SEM of serum creatinine and blood urea nitrogen in control subjects were 1.3mgm/dl and 20.4mg/dl respectively. The biochemical data from the two groups of patients are shown in Table 2. There was a significantly higher blood urea nitrogen and serum creatinine in patients with nonoliguric ARF. Fractional excretion of sodium was 25 ± 16 and 3 ± 1 in oliguric ARF and nonoliguric ARF respectively. Renal failure index (as expressed as the ratio of urinary sodium (mmol/L) and urine/plasma creatinine ratio) was very high, i.e. 42 ± 9 in oliguric ARF and comparatively low i.e. 4 ± 1 in nonoliguric ARF.

Table 2. Severity of renal failure in 34 oliguric and 18 spontaneous nonoliguric patients (mean±SEM)

Index	Oliguric patients n = 34	nonoliguric patients n = 18	p value
Blood urea nitrogen (mg/dl)	136±19	57±6	<0.001
Serum creatinine (mg/dl)	6±0.8	2±0.2	<0.001
Urine sodium	106±10	78±9	<0.05
Urine/Plasma Creatine	5±1.2	23±3	<0.001
Fractional excretion of sodium (%)	25±16	3±0.8	<0.001
Renal failure index	42±9	2±0.4	<0.001

Response to treatment

Eleven patients with oliguric ARF responded to diuretics and dopamine. 8 patients were dialysed. 11 patients died from this group. Mortality rate was about 32%. None of the patients with nonoliguric ARF required dialysis and none died.

DISCUSSION

The incidence of nonoliguric acute renal failure in our series was about 34%. This is a little higher than Meyers' et al.(4) and Singh et al.(5) series (30%) but much lesser than Shen et al.(1) study. Anderson et al (2) reported that toxin related nephropathy is more frequent in nonoliguric acute renal failure. Although the exact mechanism or cause of nonoliguric acute renal failure in Russell's viper bite victims was not known, we assume that direct renal toxicity due to snake venom might be contributing to this type of acute renal failure. In our series, patients with oliguric acute renal failure showed evidence of more severe renal impairment. Patients were ill and serious complications like gastrointestinal bleeding were more frequent. Biochemical picture also indicated more severe degree of renal failure in these patients. The low FENA in non-oliguric patients indicates that lesser degree of damage done to functional integrity of the tubules. Miller and associates (6) also found that 10% of patients with non-oliguria had FENA of 1%. Renal failure index values of 4 to 10% was reported both in oliguric and non oliguric acute renal failure (7). The mortality rate was 32% in oliguric ARF in spite of peritoneal dialysis done in 8 patients. None needed dialyses or die from non-oliguric ARF. These findings signifies a more severe renal lesion in oliguric ARF and, in a group of patients with ARF resulting from an identical etiology and receiving similar management, supports the view that the essential dif-

ference between the two groups is the degree of renal damage. Urine output during the azotemic phase is therefore, an important prognostic factor.

In conclusion, we would like to stress the existence of high output renal failure in patients with Russell's viper bite, and mere absence of oliguria should not exclude renal damage.

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REFERENCES

1. Shen,S.C. et al. Studies on the destruction of red blood cells.1. Mechanism and complications of haemoglobinuria in patients with thermal burns, spherocytosis and increased osmotic fragility of red blood cells. The New England Journal of Medicine 1943; 229: 701-013.
2. Anderson,R.J.et al. Nonoliguric acute renal failure. The New England Journal of Medicine.1977; 296:1134-1138.
3. Schrier, R.W. et al . Acute renal failure : pathogenesis, diagnosis and treatment.

Advances in Nephrology.
1981;10:213-40.

4. Meyers.C, et al. The clinical course of nonoliguric acute tubular necrosis. Proceedings of the American Society of Nephrology. 1974; 7: 62.

5. Singh,R. et al. Nonoliguric acute tubular necrosis. Proceedings of the American

Society of Nephrology. 1874; 7:85 .

6. Miller et al. Polyuric pre-renal failure. Archives of Internal Medicine. 1980; 140:907-909.

7. Handa,S.P. et al. Diagnostic indices in acute renal failure. Canadian Medical Association Journal. 1967; 96:78.

Clinical trial of indigenous antipyretics : Testing of antipyretic efficacy of five traditional medicine formulations on TAB-induced pyrexial volunteers

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Two grams each of five traditional medicine formulations (TMF) - TMF-06, TMF-24, TMF-25, TMF-35A and TMF-35B- orally administered with 150 ml of betel leaf preparation, had underwent a clinical trial of antipyretic efficacy on a total of 315 TAB-induced pyrexial Burmese volunteers. Both positive control of acetyl salicylate 600 mg orally and negative control of no drug treatment were included. The trial design was performed on a single (observer) blind basis. Effectiveness of the agents at the peak of induced pyrexia were observed for three hours. Only TMF-06 possessed the antipyretic efficacy (78-88%) as could be observed in 87-96% of responders, in comparison to those of the acetyl salicylate. Nevertheless, TMF-24 and TMF-35B showed a lesser antipyretic activity. Therefore, TMF-06 was found to be the most recommendable antipyretics to be used at indigenous medicine centres.

INTRODUCTION

Fever is a very common affliction and many indigenous drugs are used extensively as antipyretics at the traditional medicine outpatient departments (1). However, none of them have ever undergone scientific scrutiny. The aim of this trial is to evaluate scientifically the antipyretic efficacy of traditional medicine formulations (TMF) namely TMF-06, TMF-24, TMF-25,

TMF-35A and TMF-35B using acetyl salicylate as positive control on a single blind fashion.

MATERIALS AND METHODS

The formulations were supplied by the authorities of the Indigenous Medicine Council of the Department of Health Services. A total of 315 subjects both male and female of

ages between 12 to 50 years participated. The trial was conducted in three parts.

(1) Determination of method of fever induction

The temperature of each healthy subject was initially determined by taking three successive oral temperature with an interval of five minutes in between recordings. Their means were taken as basal temperature. Based on the fact that Salmonella typhosa endotoxin could induce pyrogenic reaction (2), five subjects were given 0.5 ml of TAB vaccine subcutaneously, another five were given 0.5 ml intramuscularly and the last five subjects were given 1.0 ml intramuscularly. Their changes in temperature were recorded. Only the group administered with 1.0 ml of TAB vaccine intramuscularly showed satisfactory fever induction and the induced pyrexia was found to be raised about 1.0°C lasting for five more hours.

(2) Investigation of own control applicability

The fever pattern was not reproducible in the same individual by the same method and therefore same subject serving as its own control could not be instituted.

(3) Main trial

The main trial was conducted on a total of 290 subjects, both male and female of ages between 12 to 50 years. The basal

temperature of the subjects were first taken, then they were given 1.0 ml of TAB vaccine intramuscularly. The temperature was recorded at every thirty minutes for a total duration of eight hours. The subjects with satisfactory fever induction at the fifth hour were classified into high pyrexial responders (those with induced pyrexia of 1.0°C or more above basal level) and low pyrexial responders (those with induced pyrexia of 0.5°C to 0.9°C above basal level). Then the subjects were randomly allocated into two control groups and six test groups. The subjects in negative control group were not given any drug. The subjects in positive control group were given a single oral dose of 600 mg of acetyl salicylate together with 150 ml of betel leaf preparation which was prepared by boiling 0.5 g of betel leaves in 150 ml of boiled water for 5 mins. The subjects in the 5 test groups were given a single oral dose of 2 g of one of the test drugs with 150 ml of betel leaf preparation. The subjects in the last test group were given 150 ml of betel leaf preparation only.

The temperature in the six half-hourly observations following the oral administration of the drugs were noted and changes of temperature from that of the fifth hour were calculated. The mean temperature profile was illustrated graphically and the antipyretic efficacy was presented in a tabulated form.

Measures were taken to note any side effects during the trial and prompt and adequate treatment given as required.

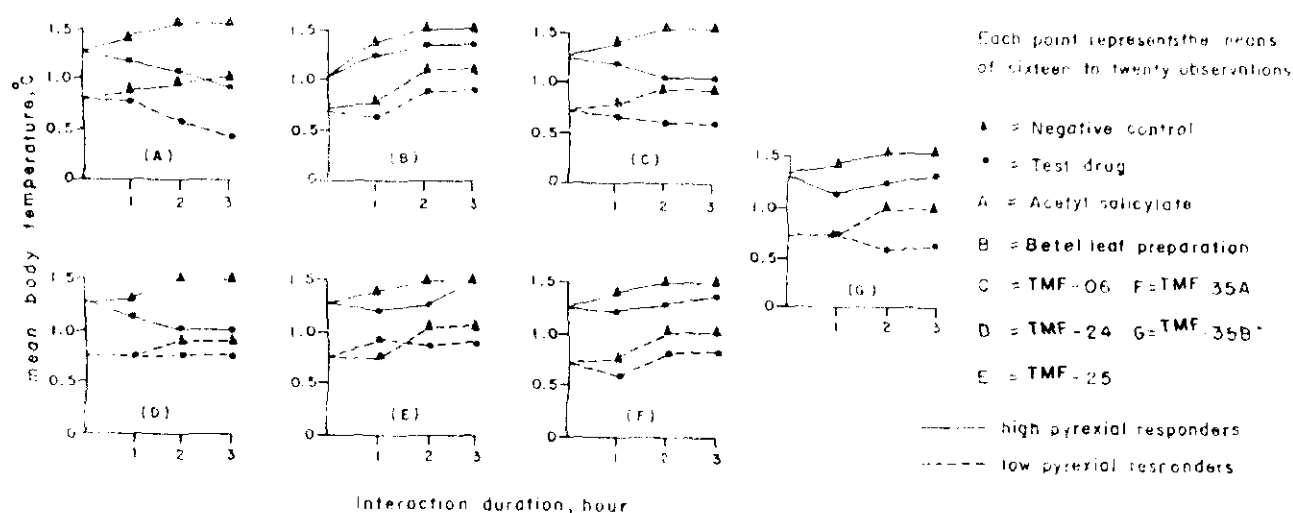


Fig. 1- Mean temperature profiles of acetyl salicylate and test drugs after drug intervention at the fifth hour in high and low pyrexial responders

RESULTS

The antipyretic efficacy of the six test drugs and acetyl salicylate in terms of temperature lowering capability are shown in Figure 1. Betel leaf preparation, TMF-25 and TMF-35A showed no significant antipyretic activity. Acetyl salicylate and TMF-06 showed significant antipyretic activity in 53% and 50% of subjects respectively in high pyrexial responders. In low pyrexial responders, acetylsalicylate, TMF-06, TMF-24, and TMF-35B respectively showed significant antipyretic activity in 61%, 53%, 40% and 37% of the subjects.

Compared to acetyl salicylate, the antipyretic efficacies of the various test drugs in order of merit were found to be as follows: TMF-06, TMF-24 and TMF-35B (Table 1).

Table 1- Temperature reducing capabilities of drugs with marked antipyretic activity						
No.	Drug	Temperature reducing capability				
		High pyrexial responders		Low pyrexial responders		
		Heat temperature reduction°C	Significance efficacy Student's t test	Heat temperature reduction°C	Significance efficacy Student's t test	Significance efficacy Student's t test
1	Acetyl salicylate	0.50	100	0.05	100	0.05
2	TMF-06	0.16	88	0.05	25	100
3	TMF-24	0.20	48	0.05	17	100
4	TMF-35B	0.16	40	0.05	33	100

DISCUSSION

This trial established the method of fever induction in testing drugs with reputed antipyretic effects. It also showed that the subjects cannot be used

as its own control. In this trial all six test drugs exhibited antipyretic activity. However, only TMF-06, TMF-24 and TMF-35B exhibited a statistically significant antipyretic activity, out of which TMF-06 was most efficacious either in terms of percentile response or temperature lowering capability.

REFERENCES

1. Indigenous practitioners

society, Drugs Used by the Burmese Traditional Medicine, Indigenous Medical Education, Ministry of Health, Burma. (Burmese version) 1982;21-67.

2. Greisman, S.E and R.B. Hornick
Comparative pyrogenic reactivity of rabbit and man to bacterial endotoxin. Proceedings of the Society for Experimental Biology 1969; 131 (1) : 1154-1158.

Application of modified Enzyme-Linked Immunosorbent Assay (ELISA) for detecting virulent strains of enteroinvasive *E.coli* and *Shigella*

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A virulent isolate of *Shigella dysenteriae* 1 strain, HW 829 was used for the production of specific antibody. Three rabbits (Japanese White strain) were immunized and the serum was obtained. These sera were absorbed with a virulent derivative of the same strain (i.e. HW 829). Tube agglutination titer of immune sera was varied from 1:1,280 to 1:5,120 with living and 1:5,120 to 1:20,480 with boiled cells of the homologous strain. The absorbed sera were utilized as an antibody in ELISA assay using BIOTIN kit. The investigation of 33 *Shigella* and 22 enteroinvasive *E. coli* (EIEC) strains indicated complete agreement between ELISA results and those of the other virulence tests (Sereny test and HEP-2 tissue culture assay).

INTRODUCTION

The invasive potential of *Shigella* and enteroinvasive *E. coli* (EIEC) is increasingly recognized as an important pathogenic factor (1). Three methods for testing the invasive potential of enteroinvasive microbes are commonly used nowadays. These are production of dysentery in primates, Sereny test (2) and in vitro tissue culture test (3) using sensitive cell lines. As these methods for the study of invasive capabilities are applicable to individual isolates only, a rapid and economical test is needed for the routine use and

epidemiological studies. In this paper, how a modified ELISA test has been introduced to identify virulent *Shigella* and IEC strains as a routine test was reported.

MATERIALS AND METHODS

Clinical isolates of 54 *E. coli* strains, 33 *Shigella* and 14 strains of *Salmonella* were included in the study. Among the *E. coli* strains, 22 were enteroinvasive, 9 were enterotoxigenic and 23 were enteropathogenic according to biochemical and serological identification methods of Edward

and Ewing (4). Kerato conjunctivitis test (Sereny test) was performed according to the method of Sereny. And HEP-2 tissue culture assay was done in order to check the invasive property as mentioned by Day et al. (3).

Production of antibodies - A virulent isolate of Shigella dysenteriae 1 named HW 829 was used for the production of specific antibodies. This strain was kindly provided by National Institute of Health, Tokyo, Japan. Three rabbits were immunized intravenously at 4-day interval with 0.2, 0.5, 1.0 and 2.0 ml respectively, of a suspension containing 10 living bacteria per ml. Blood was obtained on day 4 after the last injection. Tube agglutination titers of the three immune sera varied from 1:1,280 to 1:5,120 with living and 1:5,120 to 1:20,480 with boiled cells of the homologous strains.

These sera were absorbed with nonvirulent, Sereny-negative derivative of HW 829 strain, which was obtained by serial passages on nutrient agar at 42° C. The resultant sera did not show any agglutination with both live or boiled cells of HW 829 strain. Sera were membrane-filtered and stored at -80° C without preservation.

ELISA Assay - Wells of Dynatech Micro-Elisa plates were sensitised overnight at 4° C with 10 bacteria suspended in 100 ul of coating buffer (Na_2CO_3 1.59g, NaHCO_3 2.93 g in 1000 ml of distilled water, pH 9.6). 100 ul of rabbit immune sera diluted in PBS-Tween 20 was added, followed

by 1 h incubation at 30° C. Then 100 ul of conjugate (anti-rabbit IgG labelled with horse raddish peroxidase (Vestestain, ABC kit, Vector laboratory, Inc) diluted 1:2,000 in PBS-Tween containing 0.5% BSA was added.

After further incubation for 1 h at 30° C, the reaction was developed by adding 150 ul of the substrate (O-phenylenediamine-dihydrochloride in citric acid buffer, pH 6.0). The reaction was stopped after 10 minutes by adding 50 ul of strong sulphuric acid.

In each experiment, strains were tested in three parallels. Optical density (OD) was measured at 492 nm with Dynatech ELISA reader. Wells without bacteria served as blanks. Additional controls were wells with normal rabbits serum and wells without rabbit immune serum. The blanks were used to set the photometer at 0.

RESULTS

Optimization of the ELISA test - ELISA reactivity of one of the three antisera was tested against six pairs of Sereny-positive and -negative Shigella and E. coli (enteroinvasive strains). Different dilutions of antisera were used in the ELISA test. OD values are shown in Table 1. Antisera reacted with the Sereny-positive strains at a remarkably higher dilution than did with the negative strains regardless of serogroups. In further studies, antisera were used in a dilution of 1:1,000.

Table 1. Correlation between virulence and reactivity of antisera at different dilutions in ELISA test.

Strains	Sereny test	1:200	OD values in dilutions 1:500	1:1,000	OD range
HK 829 virulent	+	1.03	0.97	0.93	0.93
HK 829 non-virulent	-	0.04	0.00	0.00	0.00
<i>E.coli</i> 0 124	+	1.33	0.98	0.90	0.90
	-	0.00	0.00	0.00	0.00
<i>E.coli</i> 0 116	+	0.98	0.92	0.86	0.76
	-	0.00	0.00	0.00	0.00
<i>E.coli</i> 0 145	+	1.06	0.99	0.92	0.84
	-	0.00	0.00	0.00	0.00
<i>E.coli</i> 0	+	1.50	1.22	1.12	1.00
	-	0.02	0.03	0.00	0.00
<i>E.coli</i> 0 154	+	1.02	0.92	0.92	0.80
	-	0.00	0.00	0.00	0.00

Reactivity of antisera with strains of different origin - Thirty-three *Shigella* and 22 *E. coli* strains were examined by this method. The strains that were positive by Sereny test reacted strongly with the antisera (OD range 0.36 to 1.5) (Table 2). For these strains the mean OD value was 0.96 ± 0.28 standard deviation. Contrary to this, Sereny-negative strains had a low OD value (range 0.00 to 0.3) with a mean of 0.7 ± 0.005 standard deviation.

According to these values a strain was considered as positive when the OD value exceeded 0.32 (the mean -2 SE for the Sereny-positive strains). The difference between the OD values of the two groups was found to be significant at $p=0.0001$ by the Student's 't' test. At this dilution antisera gave a clearcut differentiation between

virulent and non-virulent isolates and the antisera did not react with the other enteric pathogens like ETEC, EPEC or *Salmonella* spp. This feature was so pronounced that positive strains could be identified at the wells by the naked eyes.

Table 2. Comparison between virulence and antibody reactivity.

Strains	No. of strains virulence tested	ELISA positive	ELISA negative	OD range
<i>Shigella dysenteriae</i>	+	27	6	0.36-1.16
	-	1	0	0.14-0.18
<i>S. flexneri</i>	+	1	0	0.71
<i>S. sonnei</i>	+	2	2	0.14-0.2
<i>E. coli</i> 0 28	+	3	0	0.44-0.72
0 112	-	1	0	0.20
0 124	+	3	0	0.67-1.46
0 136	+	4	0	0.38-1.24
	-	4	0	0.00-0.60
0 145	+	1	1	1.50
0 152	+	2	0	1.10-1.19
	-	1	0	0.02
0 164	+	3	0	0.56-0.80
Total positive	+	44	6	0.36-1.50
Total negative	-	11	11	0.00-0.18

Virulence was estimated by the method of Sereny and Hegazi (1958) using culture tests.
Range of OD means values as estimated by three parallel wells ± 2 SE.

DISCUSSION

The determination of virulence of invasive *E. coli* and *Shigella* strains has both clinical and epidemiological importance. The recent methods for the study of invasive properties are applicable only to individual isolates. These methods are not suitable for identification of a large number of isolates. A rapid and economical test is needed for epidemiological purposes.

On the basis of results for 44 sereny-positive and 11 sereny-negative strains of different

serogroups, it appeared that specific antisera reacts with a unique antigenic moiety expressed by the enteroinvasive strains.

When the virulency of the strains was checked by using in vitro tissue culture test, all the sereny-positive strains show invasion into the HEP-2 cells.

These results suggested that virulent strains of EIEC and Shigella had similar or identical antigens which is absent in the virulent strains (Table 3) and other enteric pathogens like EPEC, ETEC and Salmonella spp. (Figure 1). Therefore, the present ELISA technique is suitable to test efficiently large numbers of strains or colonies within 24 hours. Further improvement of the test to detect antibody directly from fecal samples is necessary.

Table 3. Comparison of ELISA to other virulence tests

Strains	No. tested	ELISA	Sereny	HEP-2 cell invasion
<u>E.coli</u>	16	+	+	+
	6	-	-	-
<u>Shigella</u>	28	+	+	+
	5	-	-	-

REFERENCES

1. Labrec, E.H., Schneidec, H., and Formal, S.B. et al. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. Journal of bacteriology 1964;88:1503-1518.

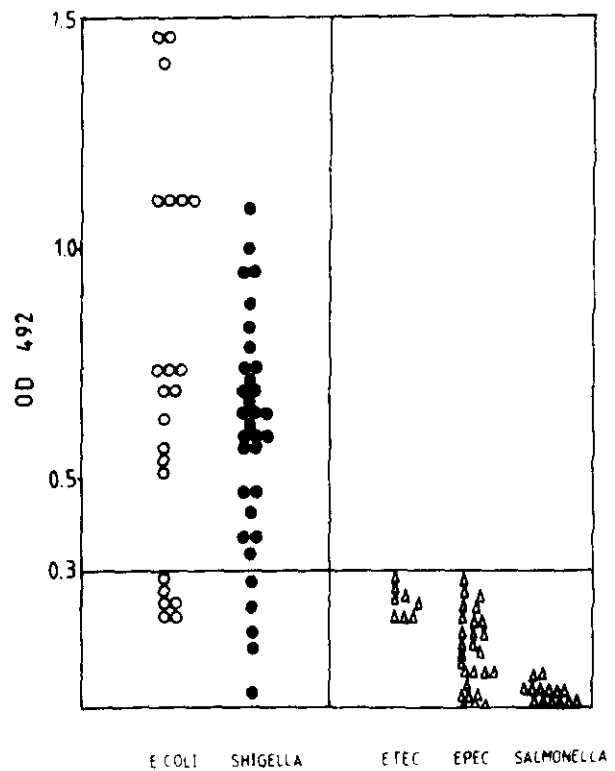


Figure 1. Reaction of antisera to virulent and avirulent Shigella, E.coli (EPEC, ETEC and EIEC) and Salmonella spp.

2. Sereny, B. Experimental kerato conjunctivitis shigellosa. Acta Microbiologica Academic Scientia (Hungary) 1956;4:367-377.
3. Day, N.P., Scotland, S.M., and Rowe, B. Comparison of an HEP-2 tissue culture test

with the Sereny test for detection of Enteroinvasiveness in Shigella spp. and Escherichia coli. Journal of clinical Micrology 1981; 13 (3):569-597.

4. Edward, P.R. and Ewing, W.H. (Eds). Identification of Enterobacteriaceae. Burgess, Minneapolis, 67-142.

Some observations on the effect of berberine against trichomoniasis in Burmese women

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Vaginal swabs taken from 401 women with various kinds of discharge were collected in Pavlova's (1) transporting medium and examined for Trichomonas vaginalis microscopically and by 24-hr of cultivation at 37° C. 66(16.4%) were positive for T. vaginalis, 52(13.0%) for Entamoeba histolytica and 18 (4.5%) for both T. vaginalis and E. histolytica. In vitro sensitivity drug testing of berberine chloride were done prior to therapy using metronidazole as control. Metronidazole and berberine chloride were found to be trichomonadocidal at the concentrations of 5 and 50 mcg/ml respectively. Five patients were treated with metronidazole orally, 8 locally with trichomycin vaginal tablets, 17 with berberine chloride orally, 4 by intramuscular emetine hydrochloride injection and 19 by local and systemic treatments. Out of 17 patients, clinical cure was achieved by berberine chloride in 5 patients with negative cultures, and 8 patients with positive cultures.

INTRODUCTION

Trichomonas vaginalis is a primary pathogenic parasite, described by Donne in 1836 (2) and has long been regarded as a harmless commensal of the human vagina. In 1916 Hohne (3) described a clinical unit the so-called "trichomoniasis" of women whose vaginae contained many of these flagellates. The vaginitis produced by this flagellate was recognised as a sexually transmitted disease. Although there are drugs that have been found to remove T. vaginalis from vagina, it has not been possible to remove the organism from the urethra, Skene's ducts and greater

vestibular gland causing relapses especially in patients with lowered defence mechanisms. Another drawback has been the length of time which most of these therapeutic methods required. To overcome the above situations, the search for new potent drugs is desirable. In this paper, we report the testing of berberine chloride both in vitro and clinically, in parallel with metronidazole and trichomycin as control drugs. It also treats the more recent knowledge of the clinical aspects and treatment of trichomoniasis, as also the laboratory diagnosis.

MATERIALS AND METHODS

A. **Samples:** Samples of vaginal discharge were obtained from randomly selected patients attending ante- and post-natal outpatient departments of the Central Women's Hospital, Rangoon. The specimens were collected from the posterior fornix of the vagina with a sterile glass syringe or cotton swabs and inoculated into a culture tube containing Pavlova's (1) medium. The inoculated media were maintained at 37°C prior to further investigation which commenced within 2 hours.

B. **Examination for trichomonads:** Within 2 hr after the collection of sample, 0.05 ml aliquots of the inoculated medium were examined microscopically at the magnifications of 100X and 400X. Cultures containing trichomonads were retained for drug testing in vitro. Negative cultures were re-incubated and examined again after 24 hrs. Of 66 pure trichomonad-positive cases, only 53 were completed for therapeutic trial.

C. **In vitro drug testing:** Drug sensitivity experiments were performed in vitro before any treatment was commenced. The samples were subcultured in Pavlova's (1) medium containing heat-inactivated horse serum (Burroughs Wellcome No.2) in a proportion of 1 part of horse serum to 4 parts of medium. Sodium benzyl penicillin (160 units/ml), streptomycin sulphate (100 mcg/ml), chloramphenicol (100 mcg/ml) and nystatin (50 mcg/ml) were added to prevent bacterial and fungal growth (4).

The test drugs berberine chloride and metronidazole were dissolved in serial dilutions in 5ml of the culture medium and approximately 100,000 active trichomonads were inoculated into each tube. The tubes were incubated at 37°C and readings taken at 24 hr intervals. No differences were noted at these two intervals.

D. **Treatment:** All treatments were administered under the supervision of a physician to ensure that the full dosage of each drug was taken by the respective patients. The condition of the cervix and vaginal wall and the colour of vaginal discharge were noted at pre- and post-therapy. The patients were instructed to avoid vaginal douching and sexual intercourse during therapy.

E. **Drugs:** Drugs used for the treatment were emetine hydrochloride (Burma Pharmaceutical Industry, Rangoon, Burma) by intramuscular injection, metronidazole tablets (Burma Pharmaceutical Industry, Rangoon, Burma) orally, Trichomycin vaginal tablets (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) locally and berberine chloride extracted from Coptis teeta Wall "golden thread" was given orally. The extraction of berberine was done according to the method of Tin-Tin-Ohn & Maung-Maung-Galay (5).

The following dosage regimens were used: (i) emetine hydrochloride by intramuscular injection, 1 gr. for 6 nights; (ii) metronidazole tablets, 200 mg thrice daily for 7 days given orally; (iii) trichomycin vaginal tablets, 50,000 IU inserted

locally at night and in the morning for 10 days; and (iv) berberine chloride, 100 mg capsules three times daily for 7 days orally. In the case of failure of the first treatment with any drug, trichomycin was the drug of choice for the second treatment.

F. Assessment of cure: Assessment of cure was based preliminarily on the microscopic disappearance of T. vaginalis in the first post-therapy culture obtained on the day following the last day of therapy. In some instances, with patients whose initial post-therapy cultures were negative, it was possible to culture serial specimens at weekly intervals. This allowed for a determination of the duration of the suppressive effect following cessation of therapy. The clinical evaluation involved interviews with the patients regarding their symptoms before therapy, after therapy, and at each follow-up visit. For practical reasons, the symptoms adjudged were limited to: (a) presence of abnormal vaginal discharge, (b) pruritus of the vulva and/or the vagina, and (c) the combination of both discharge and pruritus. Clinical improvements in the conditions of the cervix and vaginal wall served as the second criterion for the assessment of cure.

RESULTS

The prevalence of trichomonal and amoebic vaginitis among 401 women with various kinds of discharge was found to be 16.4 per cent (66) for pure T. vaginalis infection, 13.0 per

cent (52) for E. histolytica infection, 4.5 per cent (18) for mixed infection of T. vaginalis and E. histolytica and the remainder 66.1 per cent (265) showed no protozoal infection. The rate of T. vaginalis infection for the women with various types of discharge can therefore be estimated as 21.0 per cent.

The clinical condition of the cervix in the 84 proven cases of trichomoniasis, 50.0% (42) had cervical erosions, 2.4% (2) with cervicitis and 47.6% (40) with no cervical erosion or other visible lesion.

The distribution of T. vaginalis infection by age group in women visiting gynaecological ambulatories from other countries and in the present study are compared in Table 1. Kucera and Kral (6) and Asmera and Linhart (7) in Czechoslovakia found that the highest prevalence rate of T. vaginalis infection occurred in the 20 to 29 yr and 30 to 39 yr age groups. In the present study the highest infection rates were also found in these two age groups.

Table 1. Distribution of Trichomonas vaginalis infection in women with respect to various age groups

AGE GROUP (yr)	JIVOREC et al.		KUCERA & KRAL		ASMERA & LINHART		PRESENT STUDY	
	Czechoslovakia		Czechoslovakia		Czechoslovakia		BRNA	
	No.	%	No.	%	No.	%	No.	%
15-19	15	7.8	12	4.8	44	26.6	11	13.1
20-29	18	9.5	136	48.8	71	42.1	27	33.3
30-39	12	17.9	185	66.2	171	111.7	26	31.9
40-49	54	28.4	11	3.9	111	66.4	71	86.9
50	11	6.1	99	34.7	41	24.7	8	9.8
Total	140		363		337		84	

The relationship between the colour of discharge and T. vaginalis infection is shown in

Figure 1. It was observed that the colour of discharge in all infected individuals were either white, yellow, reddish-brown or yellowish-green discharge. However, *T. vaginalis* infection was associated most frequently with white discharge.

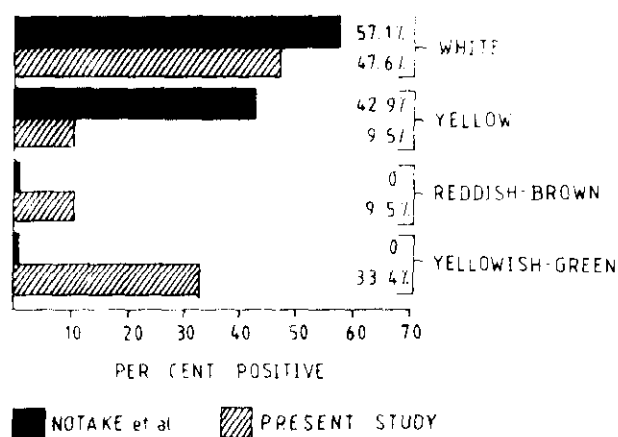


Figure 1. Relation between *Trichomonas vaginalis* infection and colour of discharge

The follow-up results of treatment with various trichomocidal drugs and the effectiveness of the various treatment regimens in the elimination of *T. vaginalis* from the vulvo-vaginal flora are shown in Table 2. The groups treated locally with trichomycin vaginal tablets and with this treatment combined with metronidazole orally gave the highest elimination rates accompanied by negative cultures and showed relief of subjective symptoms coincident with infection. It was startling to see that even cases of

pronounced vaginitis were completely cured after less than one week's treatment with trichomycin. The group having metronidazole oral therapy gave the second highest elimination rate, 4 out of 5 patients yielding negative cultures with clinical cure. Unexpectedly, 2 out of 4 patients were cured by intramuscular injection of emetine. It was also found that one patient was completely cured by a combined regimen of emetine and metronidazole. The group taking berberine chloride gave the lowest elimination rate; 5 (29.4%) clinically cured patients yielding negative cultures and 8 (47.1%) clinically cured but with positive cultures, out of 17 cases.

Table 2. Follow-up results of treatments with various drugs

Drug	Total number treated	Clinically cure with negative culture	Clinically cure with positive culture	Clinically not cured with positive culture
Emetine	4	2	0	0
Emetine-Metronidazole	1	1	0	0
Metronidazole	5	4	1	0
Metronidazole-Trichomycin	19	19	0	0
Trichomycin	7	7	0	0
Berberine chloride	17	5	8	4

DISCUSSION

Mascall (8) found that the maximum rate of *T. vaginalis* infection occur in 30 to 40 year-old subjects who may be highly active sexually. The factor of transmission by sexual intercourse also becomes evident when the social situation is examined. Jirovec and Petru (9) in a review stated that the prevalence rate of *T. vaginalis*

is higher in divorced women having 48.2 % whereas the total positive rate is only 23.7%. They reported that the higher percentage in divorced women could in all probability be attributed to a more frequent sexual promiscuity.

Notake, Taya and Sasaki (10) found in their study that most discharges having trichomonads are either white or yellow. In our study, it was also found that T. vaginalis infection was associated most commonly with white discharges.

Although berberine chloride gave a comparatively low parasite elimination rate in clinical trial (Table 2), it had a potent trichomonadocidal effect in vitro at a concentration of 50 mcg/ml. It was also reported that there is no sign of toxicity of berberine hydrochloride observed in children with acute diarrhoea (11).

It has been reported that berberine can be used in acute diarrhoeas (11;12) and is effective against Shigella, Pseudomonas, E. coli and Proteus (13) infections, intestinal parasites (14), protozoal infection as E. histolytica (15; 16; 17) and in non-specific gastroenteritis (18). No toxic or allergic side reactions were encountered during berberine therapy in all the studies. The low efficacy of this drug in the treatment of trichomoniasis could possibly be increased if it were administered locally or as a more tolerated compound.

REFERENCES

1. Pavlova, E. A. Medical Parasitology i. Parasit. Bolezni. 1938; 7,224-227. Cited by Jirovac and Petru. Advances in Parasitology 1968;6:117-188.
2. Donne, A. Animacules observes dans les matieres purulentes et le product des secretinns des organes genitaux de l'homme et de la femme. Czechoslovakia Royal Academy of Sciences 1936;3:385. Cited by Jirovec and Petru. Advances in Parasitology 1968; 6:168.
3. Honne, O. Zentbl. Gynaecology 1916;40:4-5. Cited by Jirovec and Peru. Advances in Parasitology 1968;6:117-188.
4. Parades, F.R. & Hawkins, D.F. Sensitivity of Trichomonas vaginalis to chemotherapeutic agents. Journal of Obstetrics and Gynaecology of British Common Wealth 1973;80:86-88.
5. Tin-Tin-Ohn & Maung-Maung-Galay. Alkaloids from Coptis teeta Wall. Union of Burma Journal of Science and Technology 1968;1:613-621.
6. Kucera,K. & Kral,V. Cas. Lek. cesk. 1944;83:263-299. Cited by Jirovec and Peru. Advances in Parasitology 1968;6:172.
7. Asmera, J. & Linhart, J. Countribution to the distribution of Trichomonas vaginalis in the Ostrava region. Czechoslovakia Journal of Epidemiology, Microbiology and Immunology 1962;11:203-205.

8. Mascall, M. Some reflections in Trichomonas vaginalis. British Journal of Venereal Diseases 1954;30:156-162.
9. Jirovec, O. & Petru, M. Trichomonas vaginalis and trichomoniasis. Advances in Parasitology 1968;6:117-188.
10. Notake, Y., Taya, M. & Sasaki, H. Candidiasis in obstetrics and gynaecology. Journal of Antibiotics 1958;6:9-13.
11. Kamath, S.A. Clinical trials with berberine hydrochloride for the control of diarrhoea in acute gastroenteritis. Journal of the Association of Physicians of India 1967;15:55-59.
12. Sharda, D.O. Berberine in the treatment of diarrhoea in infancy and childhood. Journal of Indian Medical Association 1970;54:22-24.
13. Purohit, K.R. & Rao, Subhadra. Neomycin compound with berberine : Trial in infantile diarrhoea. Antiseptic 1969;66:855-863.
14. Deshpande, P.R. Trial with berberine tannate for diarrhoea in children. Bharat Medical Journal 1969;1:28-32.
15. Dutta, N.K. & Iyer, S.N. Antiamoebic value of berberine and kurchi alkaloids. Journal of Indian Medical Association 1968;54:22-24.
16. Myint-Oo, Tu, M. & Sein-Gwan. In vitro effects of some indigenous plant extracts on Entamoeba histolytica. Seventh Burma Research Congress (Abstract) 1972;117.
17. Subbiah, T.V. & Amin, A.H. Effect of berberine sulphate on Entamoeba histolytica. Nature (London) 1967;215:527-528.
18. Bhakat, M.P., Nandi, N., Pal, H.K. & Sandhu Khan, B. Therapeutic trial of berberine sulphate in non-specific gastroenteritis. Indian Medical Journal 1974;68:19-23.

Intestinal secretory response to
enterotoxigenic *E. coli* heat-stable(ST)
toxin by different strains of
suckling mice and rat

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INTRODUCTION

The heat-stable (ST) toxin of enterotoxigenic *E. coli* (ETEC) can be detected by the infant mice assay (1,2). Although ³²P-labelled gene-probe techniques are being developed (Sack, Wachsmuth and Tin-Aye, personal communication), these may not be available for some time in the developing countries. Thus, when large samples of *E. coli* isolates from stools need to be tested for the presence of ETEC ST-toxin, the availability of infant mice (DDY strain now being usually used) becomes the work-limiting factor. The purpose of this study is to determine whether other strains of mice and rats available in the Department of Medical Research (DMR) are equally capable of producing a fluid accumulation response when challenged with the same ETEC ST-toxin.

MATERIALS AND METHODS

Breeding colonies of Wistar rats and 10 strains of mice (DDY; ICR; Nu (Albino); Balb/c; C57 BL/6J; YEL/DMR; DBA2; Nu(Black); C3 H/He; CHO/DMR(57) were maintained by the Animal Services Division of DMR (3). Suckling animals (2-4 days old) were used for assay of ST-toxin of ETEC. A known strain (C175/83 W12-3) of ST-producing ETEC isolate was obtained from the Bacteriology Research Division; crude ST-toxin was obtained by inoculating this strain in 1.5 ml trypticase soy broth kept under vigorous agitation at 37 degrees centigrade over-night on a New Brunswick Model G2 roller shaker, then centrifuge at 10,000 rpm (12,000-17,000 g) for 30 minutes, and the supernatant fluid was used for intragastric injection. Uninoculated medium was used as control. Also, suckling animals not given any injections at all were used as additional controls. About 0.08% Evans Blue dye was added to the toxin and control medium to serve as a marker. ST-toxin or control medium was injected using a tuberculin syringe. The milk-filled stomach was identified below the lungs between the liver and the spleen as a white patch. The needle was introduced transabdominally into the stomach, and 0.1 ml of ST-toxin or control medium was injected. About 3 and one-half hours after injection, the suckling animals were sacrificed by a sharp blow on the head instead of anaesthesia by chloroform since physiological changes in the gut could occur in response to chloroform. The stomach was

examined to confirm existence of dye in the stomach. The entire intestines from the gastroduodenal junction to the recto-anal junction was removed in toto, and weighed on an analytical balance sensitive to +0.001 gramme. The remaining body carcass without the gut was also weighed using the same balance. For each mice or rat, the ratio of the gut-weight was calculated, and this ratio was used as a measure of fluid accumulation response to ST-toxin or control medium.

RESULTS

Table shows the intestinal fluid accumulation response to ST-toxin of ETEC by different strains of suckling mice and Wistar rats. All suckling animals models were found to respond to the ST-toxin by producing gut-to-carcass ratios of more than 0.085.

Table: Fluid accumulation response to ST-toxin of ETEC.

Strain of mice	ST-toxin	control medium	uninoculated
DDY	0.1432(0.00996)	0.0651(0.00323)	0.0757(0.00669)
ICR	0.1145(0.00568)	0.0614(0.00294)	0.0613(0.00311)
Nu(AIn)no	0.1145(0.00389)	0.0644(0.00223)	0.0647(0.00171)
Balb/C	0.1081(0.00473)	0.0650(0.00318)	0.0616(0.00256)
C BL/6J 57	0.1135(0.00292)	0.0585(0.00219)	0.0604(0.00263)
YEL/DMR	0.1148(0.0110)	0.0643(0.00161)	0.0627(0.00302)
CHO	0.0907(0.00472)	0.0580(0.00311)	0.0587(0.00196)
DBA 2	0.0956(0.00408)	0.0485(0.00256)	0.0633(0.00600)
Nu (Black)	0.0979(0.00530)	0.0603(0.00205)	0.0685(0.00720)
C H/He 1	0.1082(0.00569)	0.0619(0.00410)	0.0728(0.00312)
Wistar rats	0.0967(0.00320)	0.0584(0.00165)	0.0599(0.00159)

DISCUSSION

In our study, intestines and body carcasses were weighed individually for each animal, and the gut-to-carcass ratio for each animal was calculated. In Dean's first study (1), guts and body carcasses were weighed in groups of three or four animals. The advantage of weighing and calculating gut-to-carcass ratios individually rather than in groups of 3 or 4 animals was that reproducible and statistically reliable results were obtained with even five animals only (3).

Differences in the calculation of gut-to-carcass weight ratios exist. In a study by Baselski, both the stomach and the entire intestines were weighted; in the original experiment by Dean et al (1), and in a later study reported by Takeda et al (4), only the entire intestines was weighed. The latter method was employed in our experiments. Oral administration of ST-toxin has been recommended as even more precise than intra-gastric injection since in some cases direct injection of the toxin could cause leakage (4). However, such introduction of ST-toxin orally using a feeding needle could also cause oesophageal rupture and inadvertent injection of toxin into the thoracic or peritoneal cavity.

This study demonstrates that all strains of mice and Wistar rats are equally capable of exhibiting a fluid accumulation response to ETEC ST-toxin.

ACKNOWLEDGEMENT

We thank Daw Mar Mar Nyein for provision of ETEC ST-toxin.

REFERENCES

1. Dean, AG, Ching Y-C, Williams, RQ, Harden, LB. Test for Escherichia coli enterotoxin using infant mice: application in a study of diarrhoea in children in Honolulu. Journal of Infectious Diseases 1972; 125: 407-411.
2. Morris, GGK, Merson, MH, Sack, DA, et al. Laboratory investigation of diarrhoea in travellers to Mexico: evaluation of methods for detecting enterotoxigenic Escherichia coli. Journal of Clinical Microbiology, 1976; 3: 486-495.
3. Baselki, V, Briggs, R, Parker, C. Intestinal fluid accumulation induced by oral challenge with Vibrio cholerae or cholera toxin in infant mice. Infection and Immunity 1977; 15; 704-712.
4. Takeda, T, Takeda, Y, Miwatani, Ohtomo, N. Detection of cholera enterotoxin activity by suckling hamsters. Infection and Immunity 1978; 19; 752-754.

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## Effect of fasting and *Escherichia coli* heat-stable (ST) toxin on intestinal epithelial cell counts in rats

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### INTRODUCTION

Because of the intimate relationship between the intestinal cells and ingested foods, it is expected that food may play a role in regulating intestinal epithelial cell renewal (1). This study aims to compare the crypt and villous epithelial cell counts in upper small intestines of healthy suckling Wistar rats, during fasting states, and during a state of toxic secretory response to enterotoxigenic *E. coli* (ETEC) heat-stable (ST) toxin.

### MATERIALS AND METHODS

Two- to four-day old suckling Wistar rats weighing four to six grammes were obtained from the Animal Services Division. That suckling Wistar rats were responsive to ST-toxin of ETEC had been demonstrated in a previous study (2). A known strain (Cl75/83 W12-3) of ST-producing ETEC isolate was obtained from the Bacteriology Research Division; crude ST-toxin was obtained by inoculating this strain in 1.5 ml trypticase soy broth kept under vigorous agitation at 37

degree centigrade overnight on a New Brunswick Model G2 roller shaker, then centrifuge at 10,000 rpm (12,000-17,000 g) for 30 minutes, and the supernatant fluid was used for intragastric injection in rats. In experiment 1, rats were given intragastric injection of either ST-toxin containing medium or control (not inoculated with *E. coli*.) medium. In experiment 2, two control rats were injected intragastrically with control medium, twelve rats were fasted (food and water was not given) at room temperature for 6 hours after which 6 rats were injected with control medium and 6 rats with medium containing ST-toxin of ETEC. Experiment 3 was similar to experiment 2 except that the fasting time was prolonged to 24 hours.

The response to toxin or control medium was assessed using the method described by Giannella (3), with the modification that rats were killed with a sharp blow on the head instead of anaesthesia by chloroform since physiological changes in the gut may occur in response to chloroform. An analytical balance sensitive to  $\pm 0.001$  gramme was used. Gut-to-carass weight ratios  $>0.085$  were taken as a positive secretory response (3).

From each rat, small intestinal sections were taken and slides made. Three slides (each containing 9 to 10 transverse sections) were selected for cell counting using an Olympus microscope (Model P220.240); only those containing axially-sectioned crypts and villi, (i.e., base, middle and tip of villi

and crypt were all in the plane of the sections) were selected for counting. The left-hand column cells were counted 3 times, and only when 2 counts or more tallied were the counts recorded for each villi or crypt columns. This counting process was repeated for 3 slides per rat.

## RESULTS

With 6-hour fasting, the number of crypt cells were reduced from 5.83 (SE  $\pm 0.39$ ) to 4.33 (SE  $\pm 0.31$ ) which was statistically significant ( $P < 0.01$ ); this was also accompanied by a significantly ( $P < 0.01$ ) reduced crypt-villous ratios from 0.14 (SE  $\pm 0.01$ ) in control rats to 0.10 (SE  $\pm 0.01$ ) in fasted rats. No significant changes were observed with 24 hour fasting alone or during toxic secretory state following injection of ST-toxin of ETEC. Although cell counts were not significantly altered during toxic secretory state in response to ST-toxin after 6-hour fasting, crypt-villous ratio was significantly ( $P < 0.01$ ) increased to 0.18 (SE  $\pm 0.01$ ). During toxic secretory response to ST-toxin after 24-hour fasting, crypt cell counts were increased significantly ( $P < 0.01$ ) to 7.33 (SE  $\pm 0.42$ ), villi counts were reduced significantly ( $P < 0.02$ ) from 41.83 (SE  $\pm 2.24$ ) in control rats to 35.0 (SE  $\pm 1.36$ ) and crypt-villous ratio was significantly ( $P < 0.01$ ) increased to 18 (SE  $\pm 0.01$ ).

## DISCUSSION

Starvation in rats was shown to be associated with a decrease in proliferating cells and prolongation of the cell renewal cycle (4). This may be due to a specific effect of lack of food or due to a general response to negative nitrogen balance produced by starvation (4). Upon refeeding, these changes reversed (4). So far, epithelial cell counts in crypts and villi of suckling Wistar rats during ST-toxin induced secretory response and during ST-toxin induced secretory response after starvation have not yet been documented. It is possible that ST-toxin which produces cGMP was acting in a manner similar to alpha-adrenergic stimulation (norepinephrine which also releases cAMP and can cause stimulation of crypt and villi proliferation by itself or through inhibition of locally active chalone which regulate cell proliferation) (5). With 24-hour fasting, the effect of fasting and that of ST-toxin complemented each other: the actively proliferating crypt cell count was increased (possibly due to stimulation by ST-toxin) whereas the villi count was reduced (possibly due to fasting).

## ACKNOWLEDGEMENT

We thank Daw-Mar-Mar-Nyein for provision of ETEC ST-toxin.

## REFERENCES

1. Cheng, H, and Leblond, CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. American Journal of Anatomy 1974; 141; 461-80.
2. Khin-Maung-U, Nilar-Khin, Swe-Mar-Kyi, Aye-Myint-Swe, Kathi-Aye, Khin-Maung-Zaw. Intestinal secretory response to enterotoxigenic E. coli heat-stable (ST) toxin by different strains of suckling mice and rats. Burma Health Sciences Research Journal 1989; 1(1);
3. Gianella, RA. Suckling mouse model for detection of heat stable E. coli. enterotoxin: characteristics of the model. Infection and Immunity 1976; 14; 95-9.
4. Aldewachi, HS, Wright, NA, and Appleton DR. The effect of starvation and refeeding on cell population kinetics in the rat small bowel mucosa. Journal of Anatomy 1975; 119; 105-21.
5. Eastwood, GL. Gastrointestinal epithelial renewal. Gastroenterology 1977; 72; 962-75.



## Monitoring the efficacy of mefloquine quinine and amodiaquine by in vivo and in vitro studies in uncomplicated falciparum malaria

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### INTRODUCTION

Resistance of Plasmodium falciparum strains to quinine, four aminoquinolines and sulphadoxine pyrimethamine has been reported from various parts of Burma (1,2,3). Mefloquine, a highly effective new antimalarial drug, has been used successfully in multidrug resistant falciparum malaria. Its usefulness has been reported by many investigators (4,5). Mefloquine has been used in a dosage of 500 mg combined with sulphadoxine 1000 mg and pyrimethamine 50 mg (as a standard dose regimen).

Combination of mefloquine with sulphadoxine and pyrimethamine with an aim to delay or prevent the onset of drug resistance has become debatable because of their poorly matched pharmacokinetics.

It has been shown that after an oral dose low blood concentrations of mefloquine persist for long periods unprotected by sulphadoxine pyrimethamine and the basic

rationale for the MSP combination appears flawed (6).

In addition, there have been reports of standard dose MSP failure cases appearing now and then. Therefore the usage of higher dosage regimens of mefloquine as a single agent need to be seriously considered in order to find out if the efficacy of this drug is dose-related. Also, the efficacy of quinine, the most important life saving antimalarial drug in use, need to be periodically reevaluated. At the same time, amodiaquine is included in this study in order to assess if any reliance upon this drug is justifiable or not.

In this study, efficacy of mefloquine, quinine and amodiaquine has been assessed both in vivo and in vitro and the results analyzed and compared.

### MATERIALS AND METHODS

Area of the study (in vivo study) - The study was carried out in patients suffering from acute uncomplicated falciparum malaria who were admitted to the Malaria Research Unit of No. (2) Military Hospital from December '86 to December '87. The patients are military personnel from various malaria endemic areas throughout Burma, where chloroquine resistance rate is 80 to 90 % and sulphadoxine pyrimethamine resistance rate well over 60 %.

**Case selection and exclusion -**  
Patients above the age of 12 years with the following criteria were included in this trial.

(1) A positive peripheral blood smear with Plasmodium falciparum asexual parasite count of more than 1000/microlitre

(2) Patients must have no severe manifestation of malaria.

(3) Patients must not have been treated with antimalarial drugs in the past 14 days.

(4) Female patients who are pregnant were excluded from this trial.

**Investigations -** Pretreatment thick and thin films were made to determine the parasite species and density. Thereafter thick films were made 6 hourly until 3 consecutive films become negative. Then parasite count was monitored daily up to day 7 and weekly on day 14, 21 and 28.

The asexual parasite density per microlitre of blood was calculated by counting the number of asexual falciparum parasites per 300 wbc. A white blood cell count of 6000/microlitre was assumed to be control for the study.

**Drug Administration -** The following drug regimens are employed.

Regimen 1. single oral dose of mefloquine 1000mg

2. intravenous quinine 600 mg in 250 ml of 5% D/W infused over 2 hours given 8 hourly from 48 hours then followed by oral quinine 600 mg tds for 5 days.

3. amodiaquine 1500 mg over a period of 3 days. (amodiaquine alone, amodiaquine + erythromycin, amodiaquine + tetracycline)

All drugs were given under supervision of the clinical staff and strict precautions were taken to prevent reinfection during the 28-day study period.

## RESULTS

**Regimen 1. Mefloquine 1000 mg -**  
A total of 45 patients were treated and all were cured. The average parasite clearance time was 47.03 hrs.

**Regimen 2. Intravenous Quinine -**  
Seventy cases were included. Sixty-four cases were cured. A cure rate of 91.42 % was achieved.

**Regimen 3. Amodiaquine -** Eighty cases were included. (amodiaquine alone 23 cases amodiaquine+erythromycin 31cases amodiaquine+tetracycline 26cases)

Responsiveness to above 3 regimens was not significantly different and average cure rate achieved only 72.5 %.

**In vitro sensitivity of Plasmodium Falciparum to mefloquine, amodiaquine and quinine on military personnel.**

**Area of the study -**

(1) Rangoon (No.2 Military Hospital, cases are from regions of malaria endemic areas of Burma.

(2) Moulmein (535 MB)

**Case of selection -** Patients with following criteria are included.

(1) Patients with asexual parasitaemia of 1000/microlitre to 90,000/microlitre

(2) Patients who have not taken any antimalarial drugs within the past one week

## RESULTS (IN-VITRO) AND DISCUSSION

The in vitro sensitivity test using different concentrations of quinine yielded a 100% sensitivity at the internationally accepted level in all the above mentioned areas of Burma. According to the results of this study, carried out from 5/8/87 to 17/8/87, Minimal Inhibitory Concentration (MIC) of quinine turned out to be 17.3 microgram/ml in Rangoon in 1985 and 4.3 microgram/ml in Moulmein.

The in vitro sensitivity rate with mefloquine gave an equally good results with 100% sensitivity rate in all areas tested. MIC of mefloquine according to this study came out as 0.28 microgram/ml in Moulmein and 0.31 microgram/ml in Rangoon.

The results of in vitro sensitivity test with amodiaquine turned out to be much poorer than the results of the above 2 drugs.

The test carried out in Rangoon showed the resistance rate of amodiaquine to be 25%. Therefore both the in vivo and in vitro studies clearly show that drug resistance to mefloquine and quinine were not significant while resistance to amodiaquine was found to be highly significant.

In conclusion, our data suggest that among uncomplicated cases

of *falciparum malaria* mefloquine and quinine if properly administered are still highly effective in standard dosage. But given the plasticity of *Plasmodium falciparum* this concentration may not hold true in the few years to come.

## ACKNOWLEDGEMENT

1. This study was carried out under the auspices of the Directorate of Medical Services, Ministry of Defence and the Department of Medical Research.

2. Mefloquine was supplied by Mepha pharmaceutical specialities, Switzerland.

## REFERENCES

1. Kyaw-Win and Aye Aung. Resistance of *P. falciparum* malaria to sulphonamide compounds. Burma Medical Journal June 1983; 29(2).

2. Kyaw-Win et al., Alternative drug regimens for multidrug - resistant *falciparum malaria* Burma Medical Journal Jan-Mar. 1985; 31(1)(5).

3. Kyaw-Win et al., The use of quinine in malaria, Second Medical Speciality Conference.

4. Jose-Maria de Souza. A phase II Clinical Trial of Mefloquine in Brazilian male subjects. Bulletin of the World Health Organization 1983; 61(5).

5. Advances in Malaria  
Chemotherapy. Technical Report  
Series (711), World Health  
Organization, Geneva 1984.

6. Combination treatment for

falciparum prophylaxis. The  
Lancet, March 21, 1987.

7. Chemotherapy of Malaria  
revised 2nd ed. World Health  
Organization. 1986.

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Serum haptoglobin in falciparum malaria patients during the acute phase of infection and following treatment

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INTRODUCTOIN

Fall of packed cell volume (PCV) with accompanied anaemia is one of the important complications in patients with high rate of *P. falciparum* infection. In our series on 76 patients admitted to the hospital with severe falciparum malaria, 12 % of the patients had PCV less than 20% and the timely correction of PCV had saved lives of these patients (1). Although the fall in PCV was assumed to be due to haemolysis (2) haemoglobinuria and pink colouration of plasma were absent in our patients. The present study was undertaken to measure the degree of haemolysis occurring in the pateents heavily infected with falciparum malaria by following the serum haptoglobin levels serially during the full clinical course of treatment.

PATIENTS AND METHODS

A total of 20 patients with *P. falciparum* hyparasitaemia in blood (more than 5% of RBCs

parasitised) at the time of admission to the hospital was studied. Quinine was given to 10 patients at a dosage of 10 mg/kg body weight at three times daily for 7 days. Amodiaquine 1500mg (divided dose in 3 days) was given to another 5 patients and MSP (mefloquine 750 mg, sulfadoxine 1500mg, pyrimethamine 75mg, in single dose) was given to remaining 5 patients. Blood samples were collected on days 0,1,2,4,5,6,9,11,14,21, and 28. In 3 patients blood sample were collected till day 42.

Haptoglobin measurement- The colourimetric method based on the peroxidase activity of the haptoglobin-methemoglobin complex with the results as expression of haemoglobin binding capacity as described by Owen, Better and Hoban (1960), was employed.

G6PD enzyme activity - All subjects were screened for G6PD deficiency by the Spott Brewer's test and only patients who were free from G6PD deficiency were chosen for the study.

Parasite count and temperature were recorded daily on all patients until the parasites totally disappeared from the circulation. Serum haptoglobin levels in 14 apparently healthy subjects from the same area were also measured as controls.

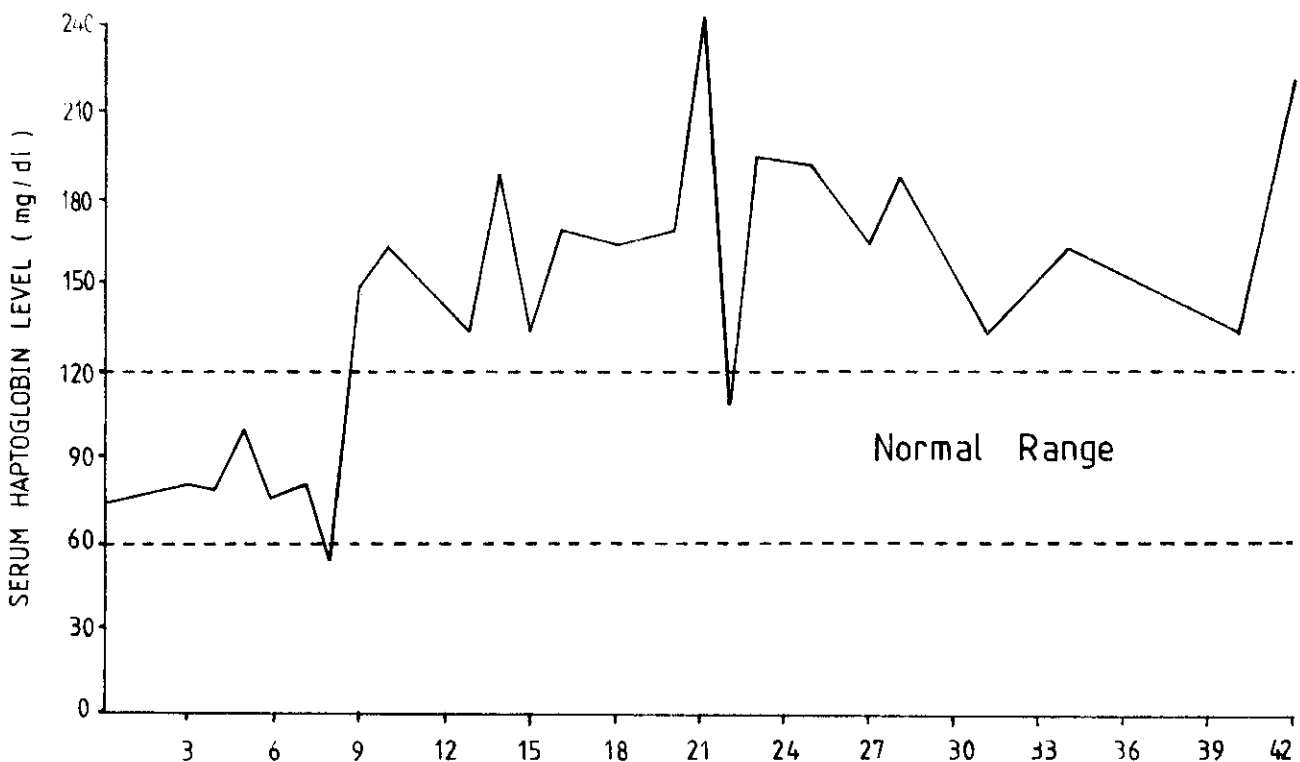
RESULTS

Serum haptoglobin level in 14 healthy subjects was 115 ± 37 mg/dl and among malaria patients at day 0 the mean haptoglobin level was 67 ± 32 mg/dl. The difference between the two groups was not statistically

significant. But 25 % of the patients had haptoglobin levels less than 38 mg/dl which is regarded as low value. There was also no difference in the trend of the mean haptoglobin levels of the patients treated with three drugs.

One patient treated with quinine and one who was treated with MSP had persistent low serum haptoglobin up to second and fourth days respectively. Another patient treated with MSP have serum haptoglobin level within normal control values on admission but showed low levels on fourth and seventh day of treatment. All other patients in

all treatment regime had serum haptoglobin levels within control range and some in the upper level of normal control values. The PCV values in all patients on admission had greater than 20 % in this series except one who had PCV 14 %. None of the patients studied went down to PCV less than 20% during the clinical course of any treatment. The low value of serum haptoglobin did not go parallel with the low PCV found in some patients. The mean serum haptoglobin among patients treated with quinine is shown in figure 1.



MEAN SERUM HAPTOGLOBIN LEVEL IN MALARIA PATIENTS TREATED WITH QUININE

The mean parasite clearance times were 78 hours after quinine, 106.87 hours after

amodiaquine and 56 hours after MSP treatment.

DISCUSSION

In falciparum malaria Phillips et al. (1) reported ahaptoglobin in 10 out of 11 patients with cerebral malaria and in 19 out of 23 patients with uncomplicated malaria. Patients with low level of haptoglobin is recorded in the rest of the patients. Hill et al. (4) also report ahaptoglobinaemia in malaria patients from Melanesia.

But in our series ahaptoglobin-aemia was not recorded. Only the low level of haptoglobin was recorded. In our another study consisting of 5 patients with low PCV and 3 patients with cerebral malaria and hyperparasitaemia also had only reduced level of haptoglobin. Ahaptoglobinaemia was not recorded.

Nyo Nyo (3) does not find malaria patients with ahaptoglobinaemia; only low levels of haptoglobin is recorded in her series. Moreover, people with ahaptoglobinaemia which can also be a genetic disorder was not also observed in Burmese people. Thus we wish to conclude that malaria can cause low level of haptoglobin but not to very low levels. Patients with ahaptoglobinaemia presented in previous reports may be probably associated with genetic disorder instead of malaria.

Our finding is in agreement with Monjour et al. (5). They also find malaria patients with low level of haptoglobin. But since the haptoglobin level of the general population is also low they concluded that the contribution of malaria to haptoglobin level is not crucial.

In our study the level of haptoglobin was not significantly reduced in all patients treated with quinine for 7 days. Hence it is not likely that quinine can cause severe haemolysis in malaria patients.

REFERENCE

1. Pe-Than-Myint and Tin-Shwe. The importance of frequent monitoring and timely fresh blood transfusion in the management of severe falciparum malaria. Research Paper Reading Session, Medical Science Division 1986-87; P. 17.
2. Phillips, R.E. The importance of anaemia in cerebral and complicated falciparum malaria: The role of complication, dyserythropoiesis and iron sequestration. Quarterly Journal of Medicine. New Series. 1986; 58: 305 - 323.
3. Nyo-Nyo, Mya-Maung and Chit-Maung. Serum haptoglobin level in diseases. Paper Reading Session, Medical Science Division 1982; P. 8.
4. Hill, A.V.S., Whitehouse D.B., Bowden, D.K., et al. Ahaptoglobinaemia in Malanaesia: DNA and malaria antibody studies. Transection of Royal Society of Tropical Medicine and Hygiene 1987; 81 : 573 - 577.
5. Monjour, L., Trape J.F., Druilhe P., et al. Malaria and haptoglobin content of serum in rural population in upper Volta. Annals of Tropical Medicine and Parasitology 1982; 76 : 105-107.

Studies on reddish-brown stained urine excreted in rats after *Vipera russelli* (Russell's viper) venom administration

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INTRODUCTION

The kidney is, among the organs, frequently involved in the Russell's viper bites. The spectrum of renal manifestation in human Russell's viper bite victims are renal failure, proteinuria, gross haematuria as reported from Burma and Thailand (1,2), haemoglobinuria from Thailand (3,4) and myoglobinuria from Sri Lanka (5). In the present study the nature of the reddish-brown pigment excreted is identified and the possible role of coagulant protein in producing the reddish-brown urine in experimental rats is shown.

MATERIALS AND METHODS

Dessicated Russell's viper venom commercially available from the Burma Pharmaceutical Industry, Rangoon, Burma was used. It was fractionated on Sephadex G-75 column as described in Fig 1. where 5 fractions were obtained. Protein was measured by the method of Lowry et al (6) or by UV absorption at 280 nm. Disc electrophoresis at pH 8.5 was carried out in cylindrical glass tubes containing polyacrylamide (15%) with 0.1% sodium dodecylsulphate for 180 mins at 5 mA per tube (7). High

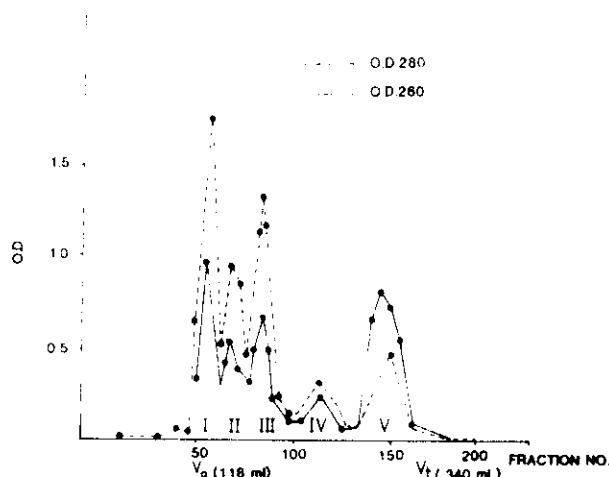


Fig 1. Fractionation of *Vipera russelli* (Russell's viper) venom. Gel-filtration of 600 mg Russell's viper venom on a Sephadex G-75 column (90X1.5cm) eluted with 0.1M ammonium acetate buffer, pH 6.8. Flow rate 30ml/hr; 3ml fractions were collected.

molecular weight markers kit from Pharmacia (Sweden) was included in each run to estimate the molecular size. Coagulant activity was measured from the ability to shorten the coagulation time of human citrated plasma. One unit of coagulant activity was arbitrarily defined as the activity possessed by 1 ug of the crude venom. Phospholipase enzyme activity was determined according to the method of Boquet et al (9). One unit of the phospholipase enzyme activity is defined as the amount of enzyme which produces haemolysis in the system corresponding to an optical density (O.D) of 0.5 at 540 nm.

RESULTS

In Table I the enzymatic

Table 1. Properties of crude *Viper russelli* venom and its fractions

	Coagulant activity U/mg*	Phospholipase activity U/mg*	No. of animals excreting reddish-brown stained urine	Statistical test for haemoglobinuria (crude vs fraction)
Crude venom	1000.0	2500.0	16 (10)†	-
Fraction I	34,890.0	66.7	10 (12)	p>0.8
I (heat treated)	20.0	61.2	0 (10)	p<0.0003
I (heparin treated)	40.0	58.0	1 (10)	p<0.0003
I (PBP treated)	32,980.0	0	9 (10)	p<0.8
II	3,837.0	1533.0	7 (10)	p>0.6
III	193.0	5999.5	0 (7)	p<0.0004
III (PBP treated)	137.0	0	0 (7)	p<0.0004
IV	0	93.0	0 (6)	p<0.0002
V	0	0	(-)	(-)
Control (Saline)	(-)	(-)	0 (16)	(-)

† : Figures in parenthesis is the total number of animals tested.

* : p value was calculated by the Fischer test.

* : One unit is the activity possessed by 1-ug of crude venom.

* : One unit is the amount of enzyme which caused haemolysis in the test system corresponding to an O.D. of 0.5 at 540 nm.

PBP : p - bromophenylacetyl bromide.

(-) : Not tested.

activities of the crude Russell's viper venom and its gel-filtrated fractions are summarised. Coagulant activity was found to be highest in fraction I and phospholipase activity in fraction III. Fraction V was rich in nucleotides which showed highest UV absorption at 260 nm. The fraction I to V contained 20%, 10%, 35%, 10%, and 1% of the total protein in the crude venom respectively. SDS-PAGE disc electrophoresis of the fractions I, II, III, IV, and V showed 3, 4, 4, 2 and 1 major protein bands respectively, while the crude venom showed 10 major protein bands.

The toxicity tests were carried out on Wistar rats at a dose of 100 ug venom protein per 100g body weight by intraperitoneal injection. The control group received saline only. 24hr urine samples were collected from the animals after venom injection. 70 to 80% of the rats, receiving crude venom or its gel-filtered

fraction containing high activity of coagulant protein excreted reddish-brown stained urine but none in the control or other groups (Table 1). When reddish-brown stained urine solubility in 80% ammonium sulphate solution was tested, the red pigment in the urine was found to be insoluble haemoglobin precipitate rather than myoglobin. Relatively very few red blood cells were seen microscopically in the sediment of the freshly collected reddish-brown stained urine, indicating that the venom was producing haemoglobinuria in these rats. However, the plasma of the blood collected from the animals at 6 hr after venom injection showed very faint pink colouration. Incubation of washed human or rat red blood cell suspension with crude venom, up to 200 ug, at 37 degree centigrade for 1 hr did not cause haemolysis.

Reddish-brown stained urine was not excreted in rats injected with heparin treated crude or gel-filtrated fraction I. Venom was incubated with heparin (Sigma, sodium salt, Grade I) at 37 degree centigrade for 30 min. in the ratio of venom protein to heparin, 1:6 (w/w). This heparin treatment could have inhibited the coagulant activity and at the same time neutralised the basic property of phospholipase enzyme by the acidic property of the heparin. Hence fraction I was heated at 70 degree centigrade for 30 min. to inactivate the coagulase protein without affecting phospholipase activity (10). This heated fraction I also failed to produce reddish-brown stained urine in rats. We also

found that the treatment of fraction I with p-bromophenylacetyl bromide, the specific inhibitor of phospholipase activity did not prevent reddish-brown stained urine excretion in the rats.

DISCUSSION

These results indicate that haemoglobinuria was not due to direct intravascular haemolysis but possible by mechanical traumatisation of red blood cells during their trapping in microthrombin due to very severe disseminated intravascular clotting occurred in the rats. However, Mebs(11) shows that both coagulant factor and phospholipase enzyme are required for producing haemoglobinuria in rats by the Tropidechis carinatus venom. Our study strongly suggested that the coagulant protein in the Russell's viper venom was mainly responsible for producing haemoglobinuria in rats and the phospholipase enzyme activity in the venom was not required.

REFERENCE

1. Myint-Lwin, Warrell,D.A., Phillips,R.E., Tin-Nu-Swe, Tun-Pe and Maung-Maung-lay, Bites by Russell's viper (Vipera Russellii) in Burma:Haemostatic, Vascular and Renal disturbances and response to treatment. Lancet 1985; 11:1259-1264.
2. Sitprija,V.and Boonpucknavig V., The kidney in tropical snake bite. Clinical Nephrology 1977; 8:377-383.
3. Sitprija,V., Renal diseases in snake-bite. Natural Toxins (Eaker,D.and Wadstrom,T.,Eds) Pergamon, England ; 43-48.
4. Sitprija, V., Renal pathogenesis of snake-bite. A review. Abst: 0138, First Asia-Pacific Congress on Animal, Plant and Microbial Toxins Singapore. 24-27 June 1987;0138.
5. Jayarajah, R., Russell's viper bite in Sri Lanka. American Journal of Tropical Medicine and Hygiene 1984; 33: 506-510.
6. Lowry,O.H.,Rosebrough,N.J., Farr,A.L. and Randall,R.J., Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 193: 265-275.
7. Davis, B. J., Disc electrophoresis-II. Method and application to human serum proteins. Annals of the New York Academy of Sciences 1964; 121: 404-427.
8. Esnouf,M.P& Williams,W.J., The fractionation of Russell's viper (Vipera russelli) venom with special reference to the coagulant protein. Biochemical Journal 1962; 84: 52-62.
9. Boquet,P.,Izard,Y,M and Meaume,J., Biochemical and Immunological studies on Naja nigricollis venom. Annals of Institute of Pasteur 1967; 112: 233-240.
10. Haung,H.C & Lee,C.Y., Isolation and pharmacological properties of phospholipase A2 from Vipera russelli (Russell's viper) snake venom. Toxicon 1984;22: 207-217.

11. Mebs, D., Studies on Tropidechis carinatus (Rough-scaled snake). Toxicon 1982;20: 1085-1087.
- haemoglobinuria produced by venom from the Australian snake



Malaria situation in a forested foot-hill area of Pegu Division

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INTRODUCTION

Exploratory surveys were carried out by the Medical Entomology Research Division, Department of Medical Research to select suitable areas for anopheline ecology studies. During July 1987, entomological and parasitological survey was conducted at Tha-bye-wa village, Oktwin Township, Pegu Division. This village is situated 17 miles away from Oktwin on the Toungoo-Prome road. It is about 700 feet above sea level and is built in a valley among the foot-hills of Pegu Yoma at the confluence of Ka-baing and Tha-bye-chaungs. There are about 40 houses with a population of about 180 persons. Most houses have only 3 walls and is easily accessible to mosquitoes. Many perennial streams are present near the village which is surrounded by deep forest on all sides.

MATERIALS AND METHODS

Entomological survey-
Mosquito catches were done by the following methods:

(1) Human bait biting catches where the collector catches all mosquitoes that bite him with a WHO Sucking Tube.

(2) Big bed-net (11'x11'x6') catches with two adult human baits sleeping in the net.

(3) Big bed-net (11'x11'x6') catches with a cow as bait inside the net.

Blood examination- Thick and thin films were taken from all persons present in the village during the survey period. All blood films were stained with Giemsa and examined in the DMR laboratory.

Spleen examination and investigations- After taking blood films, history of recent illness and febrile attacks was recorded. All children between 2-9 years of age were examined for spleen enlargement according to Bruce-Chwatt (1980) (1). Occupation and travelling habits of all adults were also recorded.

RESULTS

Entomological results

(a) Fauna

Table 1: Fauna of Anophiles (Cellia) mosquitoes recorded

SPECIES	Number caught from		Man biting rate (mbz)
	Human	Cattle	
An. dirus	46	4	4.6
An. minimus	7	9	0.7
An. vagus	96	450	9.6
An. tessellatus	26	61	2.6
An. pacificus	6	4	0.6
An. rivipes	1	27	0.1
An. Jamesi	2	2	0.2
An. annularis	-	-	-
An. splendens	-	2	-

Other species of mosquitoes recorded were:

1. ANOPHELES -
Anopheles (Anopheles) sinensis,
Anopheles (Anopheles) umbrosus,
Anopheles (Anopheles)
barbirostris.
2. AEDES -
Aedes (Scutellaris) albopictus,
Aedes (Aedimorphus) vittatus,
Aedes (Stegomyia) craggi
3. CULEX -
Culex (Culex) tritaeniorhynchus,
Culex (Culex) gelidus, Culex
(Culex) vishnui
4. ARMIGERES -
Armigeres (Armigeres) theobaldi,
Armigeres (Leicesteria) flavus.
5. HEIZMANNIA -
Heizmannia (Heizmannia) communis

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

Harrison & Scanlon (2), Reid (3), Sirivanakarn (4), Yiau-Min-Huang (5).

(b) Dissection Results

An. dirus were all brought back alive to the DMR laboratory for further taxonomy, polytene and mitotic chromosome studies. Out of 16 An. minimus dissected, one caught biting man outdoors was found to have oocysts. Out of the total An. vagus, 76 caught from human baits were dissected and one was found with sporozoites in the salivary glands. Elisa identification of sporozoites was not carried out. An. tessalatus, An. jamesi and An. maculatus were found to be uninfected.

(c) New records of mosquitoes from Burma

(1) All the female mosquitoes identified as An. philippinensis were given blood meal and

brought back alive to the laboratory to raise iso-female lines. Using the method of Reid (1967) (3), detail morphological studies were carried out on larval & pupal skins and Fl adult males. The results indicated that these mosquitoes which were previously identified as An. philippinensis were actually Anopheles (Cellia) nivipes Theobald (1903). This is the first time that An. nivipes has been recorded from Burma. Larval & pupal skins and adult specimens are now preserved in DMR.

(2) During daytime, mosquitoes of Aedes (Stegomyia) annandalei subgroup were caught biting man in the forest. After rearing in the laboratory, detail examination of pupal skins, adult morphology and male genitalia was carried out according to Yiau-Min-Huang (1977) (5). The specimens were found to be Aedes (Stegomyia) craggi Barraud, which was also recorded for the first time from Burma. Specimens of male & female adults, pupal skins and male genitalia are preserved at DMR.

Blood Examination Results

Table 2. Parasitaemia rate according to age group

Age group (in years)	No. exam'd	No. positive	% positive
1	4	2	50.0
1 - 4	12	9	75.0
5 - 9	8	5	62.5
10 - 14	11	7	63.6
15 - 19	10	8	80.0
20	28	10	35.7
Total all age groups	71	41	56.2
Under 10 years	24	16	66.7

out of 41 malaria parasite positive blood films, 36 was identified to be P. falciparum and 5 films was found to have mixed infection with P.falciparum & P.vivax. Five P.falciparum gametocytes carriers were also detected.

Spleen rate determined in 2-9 years age group according to Bruce-Chwatt (1980) (1) was observed to be 88.2% (15/17). Out of 15 children with splenomegaly, the parasite rate was 73.3% (11/15) and fever rate was 60% (9/15). The average enlarged spleen (AES) according to Hackett's method as quoted by Bruce Chwatt (1980)(1) was calculated to be 1.4.

The high man vector contact rate of a very efficient malaria vector, An.dirus, the high parasitaemia & spleen rates and high infant parasite rate indicated that this is a highly endemic area. With the end of the rainy season, An.minimus and An.maculatus, generally associated with slow-running clear streams will increase as many perennial streams were observed in the area. The ecology of the area, with its dense teak forests & banana plantations and with high relative humidity, is ideal for vector survival throughout the year. Hence, it can be assumed that malaria transmission will occur throughout the year. Presumably, the local residents are taking some antimalarial drugs because we detected 15 blood films with drug-treated parasites which were not included in calculating the positive rate. There is no health service personnel in the village. The nearest health centre is at

Pauk-taw about 11 miles away and there is no regular transportation. For such areas, Community Health Workers (CHW) should be made available. Anti-malarial drugs should be made available to the community through the village elders or anyone in the village who is trusted by the villagers. Easy availability of drugs will prevent mortality and keep morbidity low.

Prevention of man-vector contact by use of bednets, mosquito coils and use of cattle as zooprophylaxis should be some control methods for demonstration.

REFERENCE

1. Bruce-Chwatt, J.L.: Essential Malariology, William Heinemann Medical Books Ltd., London 1980.
2. Harrison, B.A. & Scanlon, J.E.: Contribution of the American Entomological Institute. The subgenus Anopheles of Thailand 1975; 12:1.
3. Reid, J.A.: Two forms of An.philippinensis in Malaysia, Journal of Medical Entomology 1967; 4(2):175-179.
4. Sirivanakarn, S.: Contribution of American Entomological Institute. A review of Culux in the oriental region 1976; 12:2.
5. Yiau-Min-Huang: Contribution of the American Entomological Institute. The subgenus Stegomyia of Aedes in South East Asia. III, The W-albus group of species 1977; 14:1.



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Original Articles

These should be headed with the title, the name(s) of the author(s), and the address(es) where the work was done. They should be accompanied by an abstract of not more than 150 words which will precede the main text of the paper and should convey its scope. The articles are usually divided into Introduction, Materials and Methods, Results, Discussion, Acknowledgement and References. In principle, an original article should not exceed 3500 words (i.e., 5 printed pages) including the abstract, tables, figures and references.

Short Reports

These should be similarly headed, but do not need an abstract nor divisions into sections. They should not be longer than one printed page or 1000 words, including references (not more than 5) and one illustration or figure if necessary.

Correspondence

Letters to the Editor on general topics of interest related to health, comments on papers published in this Journal, and, if appropriate, replies to comments are welcome. Letters should generally not exceed 350 words; tables and figures are not accepted. References (not more than 2) may be given only if essential.

Submission of Manuscripts

Manuscripts and figures should be sent in duplicate (i.e., the original with one good copy) to: The Editor, The Burma Health Sciences Research Journal, Department of Medical Research, No.5, Zafar Shah Road, Dagon P.O., Rangoon, Burma.

Manuscripts should be typewritten on one side only, with double-spacing throughout (including references) and liberal margins. Manuscripts must be accompanied by a letter, signed by the Principal Author that it is submitted solely to this Journal. Contributions must be written in English, clearly and concisely.

Illustrations (photographs, drawings, graphs, tables) should not be more than 4. Details of results presented in this way should not be repeated in the text. Photographs should be well-contrasted black-and-white glossy prints, and approximate final size. They should be accompanied by a lettering guide on a separate sheet.

Line drawings, maps and graphs should be in Indian Ink on white paper. All should be of a size suitable for reduction to single column or full-page width.

Tables and legends to figures should be typewritten.

References

The number of references should be kept to a minimum; only those that are indispensable to substantiate a statement or as sources of further information should be included. Number the references consecutively in the order in which they are first mentioned in the text. The following forms may be used:

- Aung Than Batu & Hla Pe (12) showed . . .
- Khin Maung Tin *et al* (3) reported . . .
- Several authors (7-12) suggested . . .

All published work referred to should be listed in numerical order at the end of the text on a separate sheet.

References to articles should contain the following: name(s) and initial(s) of author(s), title of the article, full name of the journal, year of publication, volume no., issue number (in parenthesis), first and last pages. Examples:-

(a) One author: Khin-Maung-U. In vitro determination of intestinal amino acid (14-C-L-Glycine) absorption during cholera. American Journal of Gastroenterology 1986;81(7):536-538.

(b) More than 6 authors: Pe-Than-Myint, Mg-Mg-Lay, Aung-Shwe-Saw *et al*. A comparative study of Artemether (Qinghaosu derivative) and standard anti-malarial drugs in the treatment of falciparum malaria in Burma. Burma Medical Journal 1985; 31(1): 36-43.

(c) Book: Thein-Hlaing. Acaris lumbricoides infections in Burma. In: Ascariasis and its Public Health Importance. Crompton, DWT, *et al* (Eds) Taylor and Francis, London, 83-112.

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