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Review–type articles and case reports are accepted for publication by the Bulletin of Chiang Mai Medical Technology. All manuscripts must be original and should have preferably not been previously submitted to any other publication. Preference is given to material which is of general interest to medical practitioners and research workers in clinical medicine.

Manuscripts must be as concise as possible and should be typed in English with double line spacing. They should be forwarded to the editor, Bulletin of Chiang Mai Medical Technology, Faculty of Medicine, Chiang Mai University. The title should be limited to a maximum of 10 words and the article broken up with suitable subtitles. Black and White photographs may also be submitted and under special circumstances, colour may be accepted

All accepted manuscripts are subject to coppy editing, 20 reprints are return to the author.

Manuscripts should be arranged in this form :–

An abstract of not more than 100 words containing a brief outline of the paper must accompany the manuscript.

Introduction.

Materials and Methods.

Results of Experiment.

Discussion.

References.

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ที่.....

วันที่.....

ถึง บรรณาธิการ วารสารเทคนิคการแพทย์ เชียงใหม่

ข้าพเจ้ายินดีบอกรับเป็นสมาชิก วารสารเทคนิคการแพทย์ เชียงใหม่ ขอได้ส่งวารสารถึงข้าพเจ้า ดังนี้

นาม... .....

สำนักงาน.....

บ้านเลขที่ .....ถนน.....

ตำบล..... อำเภอ .....

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ลงชื่อ.....

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# HYPERSEGMENTED NEUTROPHILS AND GIANT MYELOCYTES IN PROTEIN CALORIE MALNUTRITION

By

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## Abstract

The qualitative leukocyte changes suggestive of erythroid maturation factor (folates, vitamin $B_{12}$) deficiency as evidenced by the presence of giant neutrophilic bands and metamyelocytes, a high lobe average, higher percent of the hypersegmented neutrophils are frequently observed in PCM children. Their significance and relationship with the deficiency state of iron, follates, vitamins $B_{12}$ and E were investigated. It is concluded that although those leukocyte changes are the well known signs of megaloblastic anemias their presence in children with PCM are among the most common findings and apparently have no relationship with the vitamin $B_{12}$, vitamin E or folate deficiency. The results suggested that it may related to the associated infections and iron deficiency.

## INTRODUCTION

Protein calorie malnutrition (PCM) is the most prevalent disease of nutritional deficiency in the world. (1) Although it affects primarily the young children, older children and adults are not spared. (2,3) From the earlist clinical description of PCM, pallor and anemia have been among the chief signs described. (4,5,9) Since then many descriptions have appeared in the literature. (4,5,6,7) In the majority of uncomplicated PCM the anemia observed are of normochromic normocytic type. However, the peripheral smear very often shows anisocytosis, poikilocytosis, occasional target cells with normal white cells and platelet counts. (8-21) Many

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investigators also observed PCM children with hypochromic microcytic anemia. (14-16, 18, 19, 22, 23) Macrocytic anemia is a prominent part of PCM in certain parts of the world (3, 11,12,14,15,18,21,24-27) such as in India, (14,21) Egypt, (19) South America, (26) and Africa. (27)

PCM is still one of the major pediatric problem in Northern Thailand. (28) Our previous studies (23) indicated that the etiologies of anemia in these children are protein and iron deficiencies although the striking leukocytic changes known to be associated with the erythroid maturation factor (EMF) deficiencies (esp. folic acid, vitamin $B_{12}$) were noted, This included the presence of hypersegmented neutrophilic leukocytes in blood smear, the observation of large proerythroblasts and giant metamyelocytes in bone marrow smears.

This is the analysis of the investigation of the significance of the above-mentioned leukocytic changes in relation to the abnormalities in the folic acid, vitamin $B_{12}$, vitamin E and iron status observed in children with PCM.

## MATERIALS AND METHODS

Twenty two children between 1 - 3 year of age with clinical evidence of severe PCM according to Gomez's criteria (29) and a hemoglobin of less than 10.2 gm/100 ml. were admitted to the Pediatric Ward of Chiang Mai St. Louis Research Center. They were divided into 4 study group. The twelve week protocol was consisting of 2 six-week periods as follow.

SCHEDULE FOR SUPPLEMENT HEMATINICS

| | FIRST 6 WEEKS | SECOND 6 WEEKS |
|---|---|---|
| GROUP I | None | None |
| GROUP II | Iron + Vit. E | Iron + Vit. E |
| GROUP III | Vit. E | Iron + Vit. E |
| GROUP IV | Iron | Iron + Vit. E |

Whole blood hemoglobin levels were determined by the method of crosby, (30) microhematocrit was done by the method of McGovern et al. (31) White cell count and platelet count were carried out by the standard technics. ( 32,33 ) Reticulocyte counts were done with the New methylene blue method. (34) Bone marrow aspiration from the spinous proceses were carried out at interval. Defferential cell counts were done on the bone marrow and peripheral blood smears stained with Wright's stain. The stained peripheral blood smears were examined carefully for the petcentage of hypersegmented neutrophils, lope counts and lope averages. ( 35 ) Bone marrow smears prepared by squash technic were stained for hemosiderin and stainable liron with the Prussian Blue stain ( 36 ) then scored according to the criteria of Rath and Finch (37) Agar gel electrophoresis of hemoglobins was done by the method of Yakulis et al. (38) Heinz body preparations were made utilizing methyl violet in saline. (39) Glucose-6-phosphate dehydrogenase levels were also estimated. ( 40 ) Blood volume, red cell mass and 51 Cr. red cell survival were measured at interval. ( 41 ) Serum folates, ( 42 ) vitamin $B_{12}$, (43) vitamin E, (44) serum iron and total iron binding capacity ( 45 ) were measured at intervals.

TABLE I: LABORATORY FINDINGS ON ADMISSION

| No. | Diagnosis | Hb. gm/100 ml | Serum Fe | TIBC | Folate | $B_{12}$ | Vit. E | Hyper seg. % | Lope average | B.M. giant myeloid | B.M. Stainable Iron |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MK | 9.9 | 40 | 208 | – | 720 | 0.40 | 8 | 3.3 | + | + |
| 2 | K | 7.4 | 28 | 70 | 3.8 | 1860 | 0.10 | 2 | 2.3 | + | 0 |
| 3 | K | 7.9 | 46 | 63 | 3.4 | – | 0.10 | 1 | 2.4 | + | ± |
| 4 | K | 5.7 | 46 | 51 | 2.7 | 658 | 0.10 | 1 | 2.9 | + | + |
| 5 | M | 9.7 | 62 | 305 | 5.6 | – | 0.10 | 0 | 2.6 | 0 | 0 |
| 6 | MK | 5.9 | 30 | 165 | 8.1 | – | 0.24 | 2 | 2.9 | + | +++ |
| 7 | M | 9.8 | 38 | 232 | 7.8 | 825 | 0.30 | 0 | 2.3 | + | + |
| 8 | M | 9.6 | 26 | 135 | 2.0 | 2620 | 0.35 | 0 | 2.1 | ++ | +++ |
| 9 | K | 8.6 | 100 | 102 | 5.5 | 346 | 0.10 | 0 | 2.6 | 0 | + |
| 10 | MK | 8.0 | 52 | 268 | 12.5 | 3430 | 0.10 | 4 | 2.9 | ++ | 0 |
| 11 | M | 9.7 | 74 | 125 | 14.0 | 1030 | 0.10 | 6 | 2.8 | + | ++ |
| 12 | M | 10.1 | 46 | 380 | 16.7 | – | 0.43 | 0 | 2.8 | 0 | ± |
| 13 | MK | 7.5 | 46 | 136 | 3.4 | 1290 | 0.22 | 2 | 2.9 | ++ | +++ |
| 14 | M | 10.0 | 44 | 196 | 5.0 | 570 | 0.10 | 0 | 2.3 | ++ | + |
| 15 | K | 9.2 | 66 | 72 | 4.7 | 1121 | 0.10 | 3 | 3.2 | + | ++ |
| 16 | K | 9.8 | 112 | 186 | 5.5 | 1582 | 0.10 | 5 | 2.8 | + | ± |
| 17 | MK | 8.9 | 95 | 213 | 1.8 | 238 | 0.10 | 5 | 2.6 | ++ | ± |
| 18 | MK | 9.0 | 58 | 98 | 3.0 | – | 0.27 | 0 | 2.5 | 0 | ± |
| 19 | K | 9.8 | 96 | 115 | 3.5 | 331 | 0.15 | 2 | 2.7 | + | 0 |
| 20 | MK | 9.6 | 34 | 187 | 2.0 | 2075 | 0.30 | 1 | 2.5 | ++ | + |
| 21 | M | 10.0 | 52 | 227 | 5.5 | 743 | 0.20 | 8 | 3.0 | + | ± |
| 22 | K | 9.6 | 100 | 176 | 3.0 | 480 | 0.15 | 6 | 3.3 | + | + |

**NOTE**

– Serum Fe, TIBC and vitamin E levels are reported in mg/100 ml.

– Serum folate levels are reported in ng/ml.

– Serum vitamin $B_{12}$ levels are reported in pg/ml.

TABLE II: LABORATORY FINDINGS AFTER RECOVERY FROM PCM

| No: | Hb. | Serum Fe | TIBC | Folate | Vit. $B_{12}$ | Vit. E | Hyperseg % | Lobe average | B.M. giant myeloid | B. M. Stainable iron |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11.0 | 58 | 484 | 27.5 | 537 | 0.40 | 6 | 2.9 | 0 | 0 |
| 2 | 11.3 | 94 | 419 | 15.6 | 733 | 0.70 | 2 | 3.1 | 0 | 0 |
| 3 | 8.9 | 24 | 420 | 32.0 | 762 | 0.50 | 5 | 2.9 | 0 | 0 |
| 4 | 10.7 | 54 | 238 | 42.0 | 447 | 0.40 | 5 | 3.3 | + | 0 |
| 5 | 7.9 | 37 | 450 | 24.0 | – | 0.37 | 7 | 3.2 | 0 | 0 |
| 6 | 10.1 | 56 | 420 | 28.0 | – | 0.52 | 2 | 2.8 | + | 0 |
| 7 | 11.8 | 100 | 410 | 12.0 | 492 | 1.40 | 1 | 2.4 | 0 | 0 |
| 8 | 10.8 | 94 | 342 | 14.1 | 630 | 1.94 | 2 | 2.9 | 0 | 0 |
| 9 | 10.6 | 100 | 320 | 28.0 | 483 | 1.48 | 3 | 3.2 | 0 | 0 |
| 10 | 10.6 | 70 | 404 | 17.5 | 695 | 1.15 | 4 | 3.1 | 0 | 0 |
| 11 | 11.0 | 74 | 338 | 24.5 | – | 1.90 | 2 | 3.1 | 0 | 0 |
| 12 | 12.0 | 100 | 353 | 16.4 | – | 1.67 | 0 | 2.8 | 0 | 0 |
| 13 | 10.7 | 144 | 328 | 33.5 | 426 | 1.20 | 17 | 3.7 | 0 | ++ |
| 14 | 11.2 | 94 | 384 | 52.0 | 85 | 1.67 | 6 | 3.3 | 0 | 0 |
| 15 | 12.0 | 120 | 400 | 32.0 | 480 | 1.50 | 4 | 3.0 | 0 | 0 |
| 16 | 10.8 | 54 | 315 | 26.0 | 265 | 1.37 | 0 | 3.0 | 0 | ± |
| 17 | 11.6 | 66 | 398 | 19.0 | – | 1.30 | 2 | 3.0 | 0 | + |
| 18 | 12.3 | 84 | 331 | 25.5 | – | 1.48 | 3 | 3.3 | 0 | 0 |
| 19 | 10.5 | 68 | 420 | 12.0 | 50 | 1.12 | 3 | 2.9 | 0 | 0 |
| 20 | 12.4 | 80 | 365 | 31.0 | 1030 | 1.53 | 1 | 3.1 | 0 | ± |
| 21 | 12.4 | 90 | 375 | 36.8 | 820 | 1.40 | 6 | 3.1 | 0 | 0 |
| 22 | 11.7 | 58 | 350 | 32.0 | – | 0.90 | 15 | 3.7 | 0 | ± |

TABLE III : CORRELATION BETWEEN "LOW" SERUM FOLATE AND INCIDENCE OF HYPERSEGMENTED NEUTROPHILS (5% OR MORE)

| SERUM FOLATE LEVEL | INCIDENCE OF HYPERSEGMENTED NEUTROPHILS | |
|---|---|---|
| | ON ADMISSION | AFTER RECOVERY |
| LESS THAN 3 ng/ml. | 2/5 | 0 |
| MORE THAN 3 ng/ml. | 4/16 | 8/22 |

Note : There is no correlation between the presence of hypersegmented neutrophils (5% or more) and the "low" serum folate level.

TABLE IV : CORRELATION BETWEEN SERUM IRON SATURATION AND INCIDENCE OF HYPERSEGMENTED NEUTROPHILS (5% OR MORE) DURING RECOVER FROM PCM

| PATIENTS | SERUM IRON SATURATION | | |
|---|---|---|---|
| | LESS THAN 15% | 15–20% | MORE THAN 20% |
| WITH HYPERSEGMENTED NEUTROPHILS (8) | 3 | 1 | 4 |
| WITHOUT HYPERSEGMENTED NEUTROPHILS (14) | 1 | 4 | 9 |

## RESULTS

From the Tables I to IV as shows above it is evident that :

1. The presence of giant myeloid cells in the bone marrow and hypersegmented neutrophils (5% or more) is the common finding in children with severe protein calorie malnutrition especially before treatment.

2. There is no definite correlation between these findings and the blood levels of vitamin $B_{12}$, folates, vitamin E and iron. Thus, the interpretation of the presence of hypersegmented neutrophils and giant myeloid cells as the indication of megaloblastic dysplasia and or deficiency of above - mention hematinics in children with severe PCM may be misleading.

3. The patients who exhibit hypersegmented neutrophil more than 5% seem to have lower serum iron saturation than the others.

## COMMENTS

The most constant leukocytic abnormality in pernicious anemia and other megaloblastic anemias is the presence of hypers gmented neutrophilic granulocyte (46) or Cooke's macropolycyte. (47) They may have 8, 10 or more nuclear lobes. (47,48) Their abnormal dimensions have been explained on the basis of pleurinuclearity. (49) Such cells are rarely seen in health but are found in the blood in the folic acid deficiency or pernicious anemia.

The changes in the neutrophilic polymorphonuclear leukocytes may be assets by either noting the variation in average lobe index or lobe value. (50-53) (ie the total number of nuclear lobes in in 100 neutrophils divided by 100) and by the "rule of fives" ie. noting the proportion of neutrophilic leukocytes having 5 or more nuclear lobes. Three percent being suggested as the upper limit for such cells in normal subject. (53) Herbert has used $3.17 \pm 0.25$ as the normal value but has suggested that the normal lobe average must be separately determined by each lab. (52) It is noted that the earliest hematological evidence of folate deficiency is an increase in "lobe average" (54,55) This increase in the lobe average is apparent 7 weeks after the onset of folate deprivation. Such hypersegmentation may be noted 2 weeks earlier in the bone marrow aspirates. (56) Herbert (55) stated that the lobe everage is of special value as a dianostic feature since it is not masked by concomittant iron deficiency but the other workers who studied pregnant women with folic acid and and iron deficiencies found that it may not be always true. (57) Tasker (58) had demonstrated that when iron depletion is the limiting factor in hemopoiesis the morphologic change in marrow and peripheral blood will be those of iron deficiency. When adequate amount of iron are supplied the morphological change may be then reflect the presence of other coexisting deficiencies such as that of folic acid (53, 57, 59)

Recently, the occurrence of gaint metamyelocytes and increased segmentation of polymorphonuclear leukocytes have been noted patients with iron deficiency anemia. (46, 53, 60-64) An experiment in the rats has demonstrated that iron deficiency led to a reduced fromiminotransferase activity and an increase in FIGLU excretion (65) similar to those observed in the iron deficient patients. (62) Arakawa et al (60) also described the patient with congenital formiminotransferase deficiency associated with neutrophilic hypersegmentation. Other studies also suggested the interrelation between the metabolism of iron and vitamin $B_{12}$ (59), and iron and folates 65,67) It is postulated, therefore, that the iron

deficiency impaired the activity of the formiminotransferase enzyme and that this produce both the changes seen in neutrophils and the increased FIGLU excretion.

The other striking qualitative changes in leukocytes of the patients with megaloblastic anemias is the presence of the extraordinarily large leukocyte in the bone marrow and peripheral blood. This abnormal cellular development may occur at any state in the myeloid series but it is perticularly common among the metamyelocytes. It was thought that the macropolycytes are probably derived from these cells. (47) The frequent appearance of gaint neutrophilic band from and metamyelocytes with loose chromatin which is not typical of the Ehrlich megaloblast in children with PCM is also noted by many investigators. (7, 14, 19, 68-73, 76) Our patients as well as the other had their admission folate levels in the lower normal range or very low. (21,23-26,74,75) but the bone marrow picture were normal with occasional giant metamyelocytes which did not seem to have any correlation with serum folates, vitamin $B_{12}$ or E. (23.76) As a matter of fact, we as well as the others found the elevated serum vitamin-$B_{12}$ levels in PCM ( 19, 21, 23, 24, 26, 68,72,74,75,77 ) These high value have been blamed on ( 1 ) failure of tissue generally to take up vitamin $B_{12}$ properly ( 2 ) failure of the liver specifically, because of injury, to retain vitamin $B_{12}$ (78) Low serum vitamin E levels in severe PCM were noted by us and many others. (5,19, 23, 73, 74, 79-81 ) but its role in the pathogenesis of megaloblastic dysplasia in these children is still unsettled. (82)

The question whether the hypersegmented neutrophils noted in our patients are actually the polycytes of Ponder but not the classic macropolycyte was raised. The former have been observe in cases of infections but not related to megaloblastic anemias as do the latter. The hypersegmented neutrophil observed in our children were larger with less compact nuclear chromatin and more acidophilic cytoplasm similar to the classic macropolycytes. They were also found even when there is no evidence of infection or vitamin deficiency.

On admission, most of our childen had serum iron levels below the normal range comparable to the previous experiences (23,76) and supported the conclusion made earlier that iron deficiency is a conspicuous problem in PCM in Northern Thailand. (84) Serum iron levels are almost always low but not as low as, transferrin levels. This alteration produces high transferrin saturation in inspite of low aerum iron. (23,76,84-89) Whether the transferrin level is the most sensitive plasma marker for PCM remains to be seen. (85,90) Over the 12 weeks course there was a gradual drop in the stainable iron in the bone marrow even with adequate iron supplement. It is probably due to the impaired absorption from GI tract and markedly increased iron utilization during the hemoglobin regeneration.

## REFERENCES


1. Scrimshaw, N.S., and Behar, M.: Fed. Proc. 18:82, 1959.
2. Holmes, E.G., and Trowell, H.C.: Lancet I: 395, 1948.
3. Viteri, F.E., and Galindo, R.: Fed. Proc. 23: 399, 1964.
4. Correa, J.P.: Rev. Med. Yucatan 3: 86, 1908.
5. Trowell, H.C., Davies, J.N.P., and Dean, R.F.A., "Kwashiorkor", Arnold, London, 1954.
6. Gomez, J., et al : in Advances in Pediatrics, vol. VII, Levine, S.Z., Ed., The Year Book Publishers, Inc., Chicago. 1955. pp. 131 - 169.
7. Viteri, F.E., et al : in International Symposium on Vitamin - related Anemias. Vitamins and Hormones, Vol. 26, Academic Press, New York, 1968.
8. Altmann, A., and Murray, J.F.: S. African J. Med. Sci. 13: 91, 1948.
9. Trowell, H.C., Davies, J.N.P., and Dean, R.F.A.: Brit. Med. Med. J. II: 798, 1952.
10. Demaeyer, E.M., and Vanderborght, H.: Ann. Soc. Belge Med. Trop. 34: 417, 1954.
11. Adams, E.B.: Brit. Med. J. I : 537 : 1954.
12. Woodruff, A.W.: Brit. Med. J. I.: 1297, 1955.
13. Scrimshaw, N.S., et al : Pediatrics 16: 378, 1955.
14. Mehta, G., and Gopalan, C.: Indian J. Med. Res. 44: 727, 1956.
15. Lien - Keng, K., and Tumbelaka, W. A.F.J.: Ann Paediat. 194 : 257, 1960.
16. Kondi, A., et al : Arch. Dis. Childh. 38: 267, 1963.
17. Sandozai, M.K., et al : Brit. Med. J. II : 93, 1963.
18. Adams, E.B., and Scraggs, J.N.: Brit, J. Haemat. 11 : 676, 1965.
19. Sandstead, H H., et al : Am. J. Clin. Nutr. 17 : 27, 1965.
20. Chopra, J.G., et al : J. Trop. Pediat. 11 : 18, 1965.
21. Pereira, S.M., and Baker, S.J., Am. J. Clin. Nutr. 18 : 413, 1966.
22. Cronje, R.E., Savage, D.J., and Theron, J.J. : Proc. Nutr. Soc. S. Africa 2 : 27, 1961.
23. Progress Report to NIH., Anemia and Malnutrition Research Center. Volume II, 1969.
24. Satoskar, R.S. et al : Arch. Dis. Childh. 37 : 9, 1962.
25. Matoth, Y. et al : Pediatrics 33 : 694, 1964.
26. Velez, H., et al : Am. J. Clin. Nutr. 12 : 54, 1963.
27. Allen. D.M., and Whitehead, R.G. : Blood 25 : 283, 1965.
28. Thanangkul, O., Whitaker, J.A., and Fort, E.G. : Am. J. Clin. Nutr. 18 : 379, 1966.

29. Gomez, F., et al. : in Advances in Pediatrics. Vol. VII. Levine, S.Z., Ed. The Year Book Publishers, Inc. Chicago. 1955, p. 134.

30. Crosby, W. : USAF. Med. J. 5 : 693, 1954.

31. McGovern, J.J., et al : New Eng. J. Med. 253 : 308, 1955.

32. Cartwright, G.E. : Diagnostic Laboratory, Hematology, 4 th Ed. Grune and Stralton, New York, 1968.

33. Brecher, G., and Cronkite, E.P. : J. Appl. Physiol. 3 : 365, 1950.

34. Brecher, G. : Amm. J. Clin Path. 19 : 895, 1949.

35. Herbert, V. : Vitamins and Hormones, Vol. 26. Academic Press. 1968. pp. 528.

36. Donglas, A.S., and Dacil, J.V. : J. Clin. Path. 6 : 307, 1953.

37. Herbert, V. : Trans. Ass. Amer. Physcns. 75 : 307. 1962.

38. Yakulis, et al : Am. J. Clin. Path. 34 : 28, 1960.

39. Bentler, E., Dern, R.J., and Alving, A.S. : J. Lab. Clin. Med. 45 : 40, 1955.

40. Berger, L. : SIGMA Technical Bulletin, 1965.

41. Lajtha, L.G. : J. Pediat. 95 : 903, 1961.

42. Herhert, V. : J. Clin. Invest. 40 : 81, 1961:

43. Rosenthal, and Sarett, : J. Biol. Chem. 199 : 439, 1952.

44. Quaife, M.R., Scrimshaw, N.S., and Lowry, O.H. : J. Biol. Chem. 180 : 1229, 1949.

45. Caraway, W.T. : Clin. Chem. 9 : 188, 1963.

46. Winthrobe, M.M. : Clinical Hematology, 6 th. Edition, Lea and Febiger, Phila., 1967.

47. Jones, O.P. : Arch. Int. Med. 60 : 1002, 1937.

48. Kennedy, W.P., and MacKay, I. : J. Path. & Bacteriol. 44 : 701, 1937.

49. Schwartz, E. : Am. J. Anat. 79 : 75, 1946.

50. Herbett. V. : The Megaloblastic Anemias, New York, 1959.

51. Herbert, V. : Trans. Ass. Amer. Physcns 75 : 307, 1962.

52. Herbert. V. : Trans. Roy. Soc. Med. 57 : 377, 1964.

53. Chanarin. I., Rothman, D., and Berry, V. : Brit. Med. J. 1 : 480, 1965.

54. Herbert, V. : The Megaloblastic Anemia, 1964.

55. Herbert. V. : in Vitamins & Hormones. Vol. 26, 1968.

56. Herbert, V. : in "Diseases-a-Month", August, Year Book Publishers, Inc., Chicago. 1965.

57. Scott, I.M., and Sommerville, J.W. : J. Clin. Path. 18 : 322. 1965.

58. Tasker, P.W.G. : Trans. Roy. Soc. Trop. Med. Hyg. 53 : 291, 1959.

59. Cox, E.V. et al : Lancet. II : 998, 1959.

60. Varadi, S., Abbott, D., and Elwis, A. : J. Clin. Path. 19 : 33, 1966.

61. Velez, H., et al : Am. J. Clin. Nutr. 19 : 27, 1966.

62. Chanarin, I., Bennett, M.C., and Berry, V. : J. Glin. Path. 15 : 269, 1962.

63. Beard, M.E.J., and Weintraub, L.R. : Brit. J. Haemat. 16 : 161, 1969.

64. Dine, M.E., and Snyder, L.M. : New Eng. J. Med. 282 : 691. 1970.

65. Vitale, J.J., et al : J. Nutr. 88, 315, 1966.

66. Arakawa, Ts., et al : Ann. Paediat. (Basel) 205 : 1, 1965.

67. Vitale, J.J., et al : Lancet II : 393, 1965.

68. MacDougall, L.G., and Ross, G.I.M. : J. Pediat. 57 : 589, 1960.

69. Maclver, J.E., and Back, E.H. : Back, E.H. : Arch. Dis. Childh. 35 : 134, 1960.

70. Ghitis, J!, et al : Am. J. Clin. Nutr. 12 : 445, 1963.

71. Naeme; P.B., and Simson, J.C. : S. African J. Lab. Clin. Med. 10 : 27, 1964.

72. Viteri, F.E., et al : in "Mammalian Protein Mettbolism". Munrol, H.N., and Allison, J.B., Eds. Academic Press, N.Y., Vol., II, 1964.

73. Whitaker, J.A., et al : Am. J. Clin. Nutr. 20 : 783, 1967.

74. Majaj, A.S. : Am. J. Clin. Nutr. 18 : 362, 1966.

75. Adams, E.B., et al : Brit. Med. J. 3 : 451, 1967.

76. Progress Report to N.I.H., Anemia and Malnutrition Research Center, Volume III, 1970.

77. Adams, E,B., and Scaggs, J.N. : J. Pediat. 60 : 580, 1962.

78. Ranialingaswami; V. : Nature 201 : 546, 1964.

79. Behar, M., et al : Rev. col, Med. Guatemala 7 : 221, 1956.

80. Majaj, A.S., et al : Am. J. Clin. Nutr. 12 : 374, 1963.

81. Narvin, H.N., and Audu, I.S. : W. African Med. J. 13 : 3, 1964.

82. Suskind, R', and Olson, R.E. : presented in the "Inter - regional course on epidemiology, Prevention and treatment of protein calorie malnutrition, Chiang Mai, Thiland, Dec. 1971.

83. Kulapongs, P., et al : The report on anemia screening study (unpublished) 1971.

84. Kulapongs, P., et al : Presented at the Second Meeting of the Asian-Pacific Division of the International Society of Haematology, Melbourne, Australia, 1971.

85. MeFarlane, H., et al : Lancet I : 392, 1969.

86. Lahey, M.E., et al : Pediatrics 22 : 72, 1958.

87. Ward, E.E. : W. Indian Med. J. II : 171, 1962.

88. Edozien, J.C., and Udeozo, I.O.K. : J. Trop. Pediat. 6 : 60, 1960.

89. Kondi, A., et al : Arch. Dis. Childh. 38 : 267, 1963.

90. Mikasena, P. : Personal Communication.

วารสารเทคนิคการแพทย์ เชียงใหม่
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# A SURVEY OF FLUORIDE CONTENT IN DRINKING WATER IN CHIANG MAI.

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## Abstract

**The fluoride content in the drinking water (wells) in Chiang Mai was studied during the period from March to December, 1969. 252 water samples were collected from shallow wells from different areas of the 4 Amphurs (Saraphi, Hod, Prao and Muang) in Chiang Mai. All tests were performed the same day as collection. The results ranged from 0.05 to 1.0 p.p.m. with an average of 0.48 p.p.m., which is still in the acceptable range. Only one sample, collected from a well near a flouride mining area; showed a flouride content as high as 1.66 p.p.m.**

Clinical manifestations of fluorosis have been suspected in Nakorn Chiang Mai Hospital. (1) The origin of the fluoride has been unknown. Geographically the Northern part of Thailand, especially Chiang Mai has fluoride bearing minerals widly distributed in this area. Most of the population in rural areas in Chiang Mai had drunk water from wells. The sources of fluoride might be contamination from foods and drinking water.

It has been suggested that 1.0 - 1.5 p.p.m. of fluoride ingestion does not cause any cosmetic defect other than slight flecking of the enamel. (2) A fluorine content in drinking water of more than 1.5 p.p.m might be a cause of Crippling fluorosis, Asymptomatic Osteosclerosis, and Enamel mottling.

**The purpose** It was the Purpose of this investigation to survey the fluoride content in drinking water (wells) in Chiang

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Mai from March to December, 1969.

**Materials and Method**

Water samples were collected from Ban Saraphi, Amphur Saraphi, Ban Gongloy, Amphur Hod, Ban Sansai, Amphur Prao and Amphur Muang in Chiang Mai, Samples were fetched in 1 liter stoppered bottles of neutral glass or clean plastic bags and fluoride estimations were made within 24 hours after collection.

Measure 50 ml of water into a 100 ml volumatric flask and add 2.5 ml of fluoride reagent. Mix and keep at room temperature for one hour. Pour into Nessler tube ; measure density by Aqua tester ; compare with standard color. Read within 5 minutes.

**Results**

Two hundred and fifty two samples were collected ; 120 samples at Amphur Saraphi ; 122 samples at Amphur Prao ; 5 sapmles at Amphur Hod ; and 5 samples at Amphur Muang. It was observed that fluorides were present in all samples. The fluorine content averaged, 0. 16 p.p.m (Saraphi), 0.46 p.p.m. (Prao), 0.66 p.p.m. (Hod) and 0.66 p.p.m (Muang). for the 252 samples values ranged from 0.05 to 1.0 p.p.m. (Presented in table). of those 252 samples pH ranged between 5 - 7. The depth of the wells ranged from 2 to 8 meters, and averaged 5 meters.

**Discussion**

A survey was made of fluoride content in drinking water in Chiang Mai, from 252 samples colleeted from 4 Amphurs. The fluoride content in drinking water in Chiang Mai is lower than the world Health Standard (1.0-1.5 p.p.m).

In India, Ramamohana Rao N.V. et al examed 302 samples and found That 77.2 percentage ranged from 0.-1.5 p.p.m. and 22.8 percentage were over 1.5 p.p.m. The highest was 6.0 p.p.m. Most of the water came from rocky strata which was rich in fluoride. In Chiang Mai water came from the water table ; The wells were shallow wells.

Comparison of dental health of children in Amphur Ban Honge, Lampoon and children in Amphur Muang, Chiang mai showed 34 %decayed and 70% decayed respectively. The examination of drinking water from Amphur Ban Honge showed 1.6 p.p.m of fluoride content.

Comparison of fluoride content in drinking water from 4 Amphurs did not differ and was rather low. The drinking water does not cause fluorosis, it may be contamination from foods. in India, fluorosis was first reported by Shortt et el., later, the evidence of endemic fluorosis was recorded in various parts of the country. Anand D. et el had also observed that fluorosis exists in endemic form in different parts of the country and found that the cases increased when fluoride content in increased (8 p.p.m.), The cases

decreased when fluoride content decreased. (1.5 p.p.m.). The Fluorine content varies with the seasons. 'Boiling and filtering can remove some fluoride.

It has been suggested that developing teeth on exposure to fluorenated water become resistant to dental decay. In Chiang Mai fluorine content averaged 0.48 p.p.m. rather low, and the incidences of dental decay were high.

Incidence of fluoride in drinking water in Chiang Mai during March December 1969.

| Item | Amphur | Samples | Average of fluoride content (p.p.m.) | Range of Fluoride content (p.p.m.) |
|---|---|---|---|---|
| 1 | Saraphi | 120 | 0.16 | 0.05 – 0.40 |
| 2 | Prao | 122 | 0.46 | 0.10 – 1.00 |
| 3 | Hod | 5 | 0.66 | 0.60 – 0.80 |
| 4 | Muang | 5 | 0.66 | 0.60 – 0.70 |

## REFERENCES


1) Record suspected fluorosis in Nakorn Chiang Mai Hospital. Dr. Muni Keoplang.

2) World Health Organization Technical Report Service No 146 "Expert Committee on water Fluoridation" 1958

3) EHLERS and STEEL, Municipal and Rural Sanitation 5th ed. McGraw-Hill p. 77

4) The Determination of Fluoride, Technical Information No 611-75, Hellige Incorporated Garden City, N.Y.

5) Ram amohana Rao NV, et al: Endemic Fluorosis study of Distribution of Fluoride in Water Sources in Kurnool District of Andhra Pradesh; India J. Med Res, 52: 180-6, 1964

6) Panyatrong B,et al: Fluoride and Its Influence to Dental Health, October, 1968

7) Shortt, H.E. et al; Endemic Fluorosis in Madras Presidency, Ind. J. Med. Res, 25: 553 - 558, 1937

8) Shortt H.E., et al, Endemic Fluorosis in the Nellore Dist of South India. Ind. Med. Gaz 72, 396 - 398, 1037

9) Anand D, et al: Endemic Fluorosis and Dental Decay Preliminary Study, Indian J. Med. Res, 52: 117-23, 1964

วารสารเทคนิคการแพทย์ เชียงใหม่
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# "SERUM CAROTENE AND VITAMIN A LEVELS IN THAIS"

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## Abstract

**One hundred and twenty samples, supplied by the blood bank unit and medical students in Nakorn Chiang Mai Hospital, were studied for serum carotene and vitamin A levels in Thais, The frozen serum was analyzed, in duplicate, by macromethod using TFA. The average serum vitamin A in 109 men was 63 mcg/100 ml serum (S.D. ±21) and in 11 woman was 55 mcg/100 ml serum (S.D. ±16) The average serum carotene was 85 mcg/100 ml serum (S.D. ±44) in man and 147 mcg/100 ml serum (S.D. ±30) in woman. The average serum vitamin A level in man was 13% higher than in woman. The average serum carotene level in woman was 42% higher than in man. None had serum vitamin A level less than 20 mcg/100 ml serum. The relationship between serum carotenoids and vitamin A levels was not good, The correlation coefficient value was + 0.493.**

## INTRODUCTION

Vitamin A has long been recognized as an important factor in maintenance of the integrity of epithelial tissue, as well as having a role in the physiologic mechanisms of vision (1). Vitamin A is also essential for body growth (2, 3), may play a role in protein synthesis (4), and may also participate in reactions which affect the stability of cell membranes and of the membranes of subcellular particles (5).

Deficiency of vitamin A is a health problem of children living in remote areas. Keratinization of secretary epitheliums due to vitamin A deficiency make them move susceptible to invasion of infection organiams. Keratinization of the vascular tis-

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sue results in xerophthalmia which may lead to blindness. Xerophthalmia is the major cause of blindness in childhood.

In adult population, manifestations of vitamin A deficiency are not as obvious. Thus less attention was paid to vitamin A in adults. So for the data available for Thailand is one of the ICNND Surveys. (6) It is our interest to find the level of vitamin A and Beta-carotene, one of its precursors, in the serum of normal Thais living in the northern area; and to see the correlation between these substances. This finding will serve as a base line for further study in deficiency patients, in those with malabsorption, and with liver diseases.

**MATERIALS AND METHODS** (7, 8)

The materials analyzed were gotten from the blood bank unit of Nakorn Chiang Mai Hospital and the medical students, Chiang Mai University. The separated sera were kept frozen until analysis.

**PROCEDURE :** Precipitate 1 ml of serum with 2 ml of 95 % ethanol. Then extract the carotenoids and vitamin A by shaking vigorously 10 minutes with 2 ml of petroleum ether (B.P. 40 - 60 °C). Centrifuge at low speed and pipette 1 ml of supernatant (petroleum ether layer) into a 10×75 mm cuvette: Read the optical density quickly against fresh petroleum ether at 440 mu in a Coleman Jr. spectrophotometer. The supernatant is then evaporated in a 55-60 °C water bath. Set the spectrophotometer, at 620 mu, to zero optical density with 1 ml TFA reagent (a mixture of one volume trifluoroacetic acid and two volumes reagent grade chloroform; must be prepared prior to use). Add 1 ml of TFA reagent to the residue in cuvette, Mix quickly, place it in the spectrophotometer and record the optical density exactly 30 seconds after addition of reagent.

**STANDARD CURVES AND CACULATIONS:**

Make a stock standard solution of Beta-carotene by dissolving 20 mg Beta-carotene with a few ml of chloroform and dilute to a final volume of 100ml with petroleum ether. Dilute the stock standard 1 : 100 with petroleum ether and again dilute this working standard with petroleum ether to give solutions contain 0.5, 1.0, 1.5 and 2.0 mcg of Beta-carotene/ml respectively. Read the optical densities of these solutions against petroleum ether at 440 mu. The standard Beta-carotene curve is plotted. (mcg of carotene/ml V.S OD)

Make a stock standard solution of vitamin A by dissolving 3.0 mg of retinol or 3.44 mg of retinol acetate in a few ml chloroform and diluting to a final volume of 50 ml with petroleum ether. Dilute the stock solution 1 : 100 with petroleum ether and pipette this solution into cuvettes in different volumes to give the amounts of 0.15, 0.30, 0.60 and 0.90 mcg vitamin A/tube respectively. When solutions are evaperated to dryness. Continue

the reaction with TFA reagent. The opical densities are read at 660 mu and the standard curve in plotted. ( mcg of vitamin A/ tube V.S OD ).

Because Beta-carotene can give a reaction with TFA reagent, a correction factor must be made. A new suitable working carotene solution is made and pipetted into a cuvette in different volumes to give the amount of 4.8 and 10 mcg of Beta-carotene per tube respectively. Evaporate these solutions and follow the TFA reaction, Read the optical densities at 620 mu and the amount of vitamin A equal to carotene in the cuvettes is read from the standard curve of vitamin A. The factors are calculated by deviding the mcg of vitamin A/ tube by mcg of carotene/tube. The average value of these three factors is used for the correction factor (F).

Beta-carotenoids and vitamin A in the serum are calculated as :

1. mcg carotene/100 ml serum
   = mcg/ml of carotene read from curve × 200
2. mcg vitamin A/100 ml serum
   = ( mcg/tube of vitamin A read from curve - ( mcg/ml of carotene in the same sample × F ) ) × 200

## RESULTS

The samples were analyzed in duplicate. The average serum carotene and vitamin A levels in 109 healtly Thai males were 85 ± 44 mcg % and 63 ± 21 mcg % respectively. The average serum carotene and vitamin A levels in 11 healhty Thai females were 147 ± 30 mcg % and 55 ± 16 mcg % respectively. From these results, Thai males have a normal serum vitamin A levels higher than Thai femals by about 13 % and Thai females have a normal serum carotene levels higher than Thai males by about 42 %. The normal serum levels of vitamin A and carotene in Thais were not high compared with same other countries, Maybe the reason is that Thai daily food does not contain much vitamin A and carotenoids. The relationship between normal serum carotenoids and vitamin A levels is determined from 15 random sample from the data. The results did not show good correlation coefficient calculated was + 0.493. (Fig. I).

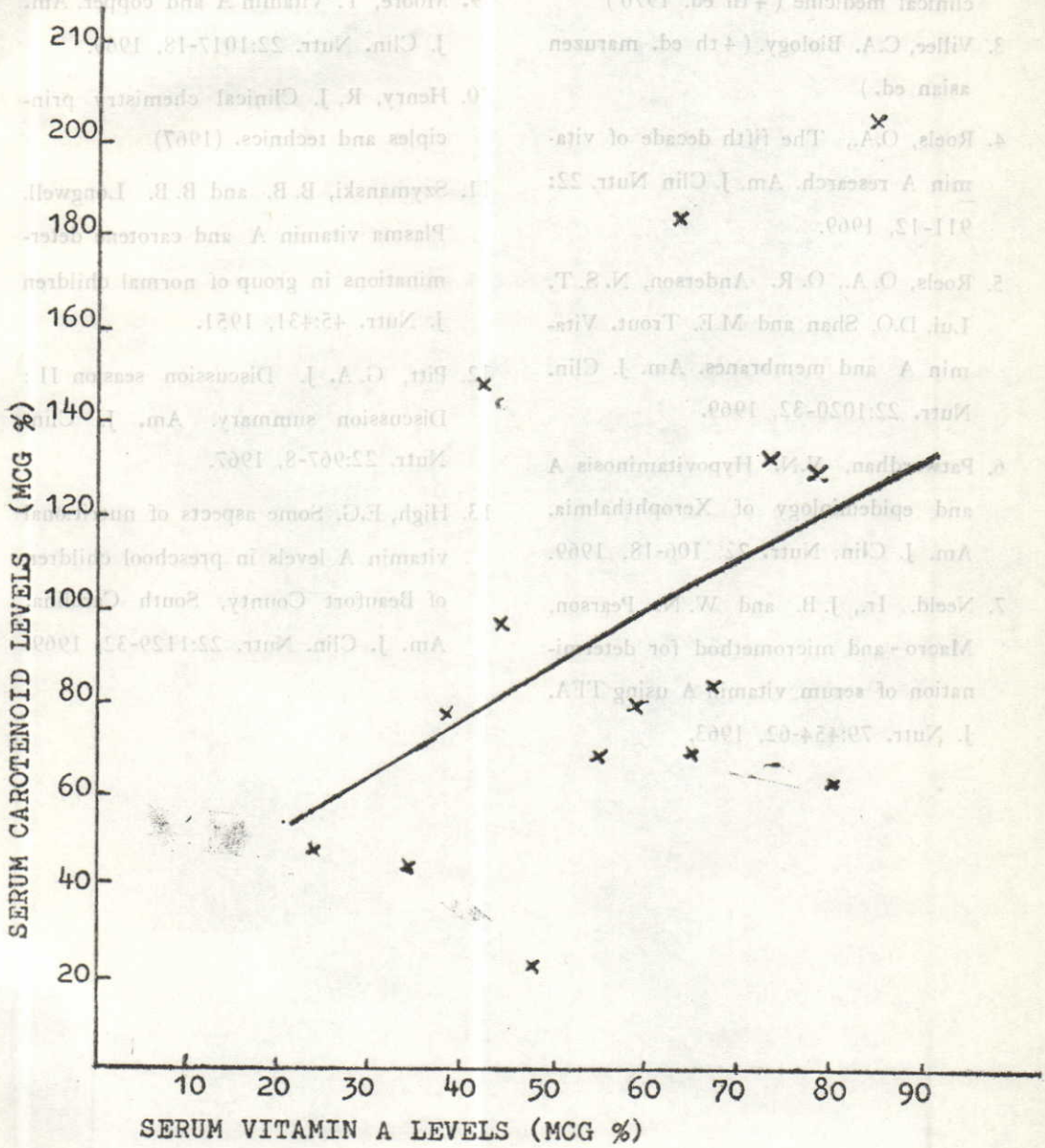
## DISCUSSION

There are vary wide variations in normal serum carotenoids and vitamin A levels. The values depend on race, sex and age. They have both a diurnal and daily variation. The carotenoids level also varies with seasons (6). The average serum vitamin A levels in differenc countries varies a great deal and is usually higher in men than in wemen (9) On the contrary, woman usually have a serum vitamin A and carotenoids level higher than boys (11). In The undeveloped countries, normal serum vitamin A levels'

are lower than the others, and have a high percentages of people who have levels below 20 mcg% i.e. in the low and deficient range. From the INND surveys, 18% of Thai men and 25% of Thai women had vitamin A levels below 20 mcg%. May be the difference between these mainly depends on the nutritional conditions of the subjects selected.

There are many reports showing the relationship between serum carotenoids and vitamin A levels. Some pailed to find a a relationship and some found a good relationship. Dr. Moodman (12) gave his advice that the relationship should depend on nutition. In a population that has carotenoids as the, main source of vitamin A, ; the relationship should be good, According to this, there must be a good relationship between serum carotenoids and vitamin A levels in Thais, because the main source of vitamin A in Thai food is vegetables that have plenty of carotenoids: But, from our results, we failed to find a good telationships. We agree with the reason professor Edward. G. High (13) gave before that a bad relationship was due to a great interference of non-provitamin A carotenoids in the serum.

Fig. 1 Relationship between vitamin A and carotenoid levels in serum of Thai people correlation coefficient = + 0.493.



## References


1. Harper, H.A. Review of physiological chemistry. ( 10 th ed. 1965 )
2. Hoffman, W. S. The biochemistry of clinical medicine ( 4 th ed. 1970 )
3. Villee, C.A. Biology. ( 4 th ed. maruzen asian ed. )
4. Roels, O.A., The fifth decade of vitamin A research. Am. J. Clin Nutr. 22: 911-12, 1969.
5. Roels, O. A., O. R. Anderson, N. S. T. Lui, D.O. Shan and M.E. Trout. Vitamin A and membranes. Am. J. Clin. Nutr. 22:1020-32, 1969.
6. Patwardhan, V.N. Hypovitaminosis A and epidemiology of Xerophthalmia. Am. J. Clin, Nutr. 22:1106-18, 1969.
7. Neeld. Ir., J. B. and W. N. Pearson. Macro - and micromethod for determination of serum vitamin A using TFA. J. Nutr. 79:454-62, 1963.
8. Davidsohn, I. and B.B. Wells. Clinical diagnosis by laboratory method. (13 th ed. 1966).
9. Moore, T. Vitamin A and copper. Am. J. Clin. Nutr. 22:1017-18, 1969.
10. Henry, R, J. Clinical chemistry principles and technics. (1967)
11. Szymanski, B. B. and B. B. Longwell. Plasma vitamin A and carotene determinations in group of normal children J. Nutr. 45:431, 1951.
12. Pitt, G. A. J. Discussion seasion II : Discussion summary. Am. J. Clin. Nutr. 22:967-8, 1967.
13. High, E.G. Some aspects of nutritional vitamin A levels in preschool children of Beaufort County, South Carolina. Am. J. Clin. Nutr. 22:1129-32, 1969.

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# Leptospirosis as a cause of Pyrexia of Unknown Origin in Chiang Mai Hospital

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## Abstract

**Agglutination-lysis test was employed in detecting leptospiral antibodies in 262 patients which had mostly been diagnosed as "pyrexia of unknown origin". Seventy cases or 26.7 % were positive and the predominant serotype was Leptospira wolffii, 30%, secondary was Leptospira icterohaemorrhagiae, 27.1 %. Most of the seropositive patients were in the 16 - 45 age group.**

## INTRODUCTION

Leptospirosis is one of the most important and cosmopolitan of the zoonoses, diseases transmitted among animals and from animal to man (1,2,3,4,5). It is a major economic and public health problem. For example, abortion in animals due to leptospiral infections is a serious economic problem. The etiologic agent is the organisms in the genus Leptospira. These organisms comprise two major groups, the so-called "saprophytic" and the "pathogenic" leptospires. The "saprophytic" or "water" leptospires are omnipresent in fresh surface waters, and are rarely associated with mammalian infection. Pathogenic leptospires are usually found in a wide veriety of wild and domesticated mammals, as carrier hosts, and cause acute, febrile, systemic disease of man and other animals (6,7,8)

Humans or animals may be infected by direct contact with urine or infected tissue of carriers, or by indirect contact. Water is important in the transmission of leptospires. Contact with water contaminated by carrier's urine is thought to be usual mode of transmission of the disease, the leptosires gaining entrance to the body

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through the alimentary or respiratory tracts or perhaps through abraded skin. General transmission from man to man is very rare, usually from animal to animal and from animal to man (5,9).

The disease in man is found most commonly among farmers, cane cutters, veterinarians and other people particularly exposed because of their occupation (10, 11). The disease exhibits many degrees of severity, varying from symptoms so mild as to be ignored by the patient to serious illness (7, 12, 13, 14, 15, 16). The clinical symptoms alone are not only variable but are non-specific for leptospirosis and definitive diagnosis must be based on demonstrating or isolating **Leptospira** and upon serological findings. Most patients who came to the hospital with fever are usually diagnosed as "pyrexia of unknown origin" (PUO) when a definite diagnosis is not obtained. PUO may be caused by many diseases and leptospirosis is one. So this paper tried to study the incidence of leptospirosis in the patients which had been diagnosed as pyrexia of unknown origin.

## MATERIALS AND METHODS

### I. Antigens

Twelve Leptospira serotypes originally isolated from human beings and animals in Thailand were used for microscopic agglutination - lysis tests. (Table I.) Stock cultures of the **Leptospira** were obtained rfom The Bangkok Leptospirosis Reference Laboratory, Faculty of Tropical Medicine, Mahidol University.

Stock cultures were maintained in Fletcher's semisolid medium (Difco*) containing 10% rabbit serum, and were incubated at 28-30°C aerobically. Subcultures were performed at three weeks intervals.

Living leptospiral antigens were used in microscopic agglutination - lysis tests. They were grown in liquid Stuart's medium, (Difco*) containing 10% rabbit serum and incubated at 28-30°C for 4-6 days. After incubation, the cultures were examined by dark-field microscopy for density, autoagglutination, and contamination. Only active, smooth, non-clumping cultures were used. Optimal density was 100-200 organisms per high power field, and if they were more concentrated they were diluted with Stuart's liquid medium.

To obtain optimal growth it was necessary to seed with large amounts of materials. For subculturing, nearly 0.5 ml. of the old culture was inoculated into the fresh medium. Rabbit sera were checked for the presence of leptospiral antibodies by the microscopic agglutination-lysis tests. Only seronegative sera were used, because leptospiral antibody may be the cause of poor growth or autoagglutination.

### II. Collection of Blood specimens

Blood specimens from the patients were collected and serum seperated from

the clot. All sera were stored at - 10 °C until used.

### III. Serological Method

Sera were examined for the presence of leptospiral agglutinins by the microscopic agglutination-lysis tests of Schuffner Mochtar (17), in which living 5 days old culturs were used as antigen. Twelve serotypes were employed.

By this method the sera were diluted 1 : 50 and then three drops of antigens were added to three drops of diluted serum. The final dilution was thus 1:100. Negative controls consisted of three drops of normal saline and three drops of antigen. Serum - antigen mixtures were mixed by rotation and incubated at room temperature for three hours. A loopful of each mixture was examined by low dry dark - field. Illumination without the use of a cover glass. Per cent of agglutination or lysis or both was read by comparing with the negative control. Less than 50% agglutination or lysis was recorded negative; greater than 50% positive.

Reaction at 1:100 dilution or above were considered significant. Sera positive at 1 : 100 dilution were retested at higher dilutions and the extent of antibody titer determined. The titers were expressed as the reciprocal of the highest serum dilution showing at least 50% agglutination or lysis or both of leptospires.

When the titers were the same with two or more serotypes, all were reported. But if the titers were not the same, lower titers were considered as cross rections.

### Results

Total 262 patients' sera were tested for leptospiral antibody and 70 or 26.7 % were positive. In these patients 196 cases were diagnosed as "pyrexia of unknown origin" and 47 cases or 23.9 % were positive ( Table II ). Most of the specimens were single collection; only in 10 cases was serum taken more than one time; and agglutination-lysis titers are shown in Table III.

Age distribution of 70 seropositive patients are shown in Table IV and most of them were in the 16 - 45 age group. Incidence of leptospiral serotypes among 70 seropositive patients are shown in Table V. The predominant serotype was **Leptospira wolffii,** 30% or 21 of 70, and secondarly was **Leptospira** icterohaemorrhagiae, 27.1% or 19 of 70.

Table I. Leptospiral Serotypes used for Agglutination-lysis Tests

| GROUP | DEROTYPE | STRAIN |
|---|---|---|
| Icterohemorrhagiae | L. icterohemorrhagiae | M 20 |
| Javanica | L. javanica | Veldrat Bataviae 46 |
| Canicola | L. canicola | Hond Utrect IV |
| Pyrogenes | L. pyrogenes | Salinem |
| Autumnalis | L. autumnalis | Akiyami A |
| Australis | L. australis | Ballico |
| Pomona | L. pomona | Pomona |
| Grippotyphosa | L. grippotyphosa | Moskva V |
| Hebdomadis | L. hebdomadis | Hebdomadis |
| | L. wolffii | 3705 |
| Hyos | L. hyos | Mitis Johnson |
| Bataviae | L. bataviae | Swart |

TABLE II

Provisional clinical diagnosis : Hospital patients *

| Provisional diagnosis | No. of patients | No. of seropositives ** |
|---|---|---|
| Pyrexia of unknown origin | 196 | 47 (23.9%) |
| Leptospirosis | 12 | 7 |
| Jaundice | 10 | 2 |
| Hepatomegaly | 2 | 1 |
| Septicemia | 4 | 2 |
| Enteric fever | 3 | 2 |
| Pneumonia | 7 | 2 |
| Anemia | 6 | 1 |
| Weakness | 1 | – |
| Other | 21 | 6 |
| Total | 262 | 70 (26.7%) |

* From serological request form.

** Minimal titer 1 : 100

TABLE III

Result of agglutination-lysis titers for the ten cases on which a paired sera were submitted.

| Patients | Agglutination - lysis titer at | | |
|---|---|---|---|
| | 1 st | 2 nd | 3 rd specimen |
| 1 | 1000 H | 1000 H | - |
| 2 | 300 I | 1000 I | - |
| 3 | 1000 I | 1000 I | - |
| 4 | Neg | Neg | 300 I |
| 5 | 1000 B | 1000 B | - |
| 6 | 100 I | 100 I | - |
| 7 | 100 G | 30,000 G | - |
| 8 | 3000 H | 10,000 H | 30,000 H |
| 9 | 300 H | 300 H | - |
| 10 | 1000 A | 3000 A | 1000 A |

H = Leptospira hebdomadis

I = Leptospira icterohemorrhagiae

B = Leptospira bataviae

G = Leptospira grippotyphosa

A = Leptospira akiyami A

TABLE IV

Age distribution in 70 seropositive * for leptospirosis from 262 hospital patients.

| Age (year) | No. |
|---|---|
| under 15 | 1 |
| 16 – 20 | 13 |
| 21 – 25 | 7 |
| 26 – 30 | 11 |
| 31 – 35 | 10 |
| 36 – 40 | 11 |
| 41 – 45 | 7 |
| 46 – 50 | 2 |
| 51 – 55 | 2 |
| 57 – 60 | 3 |
| over 60 | 3 |
| Total | 70 |

* Minimal titer 1 : 100

TABLE V

Serological distribution of Leptospira antigens giving the maximum titer in 70 seropositive* patients.

| Serotype | No | Per cent |
|---|---|---|
| L. wolffii | 21 | 30.0 |
| L. icterohemorrhagiae | 19 | 27.1 |
| L. javanica | 8 | 11.4 |
| L. bataviae | 7 | 10.0 |
| L. grippotyphosa | 7 | 10.0 |
| L. hebdomadis | 6 | 8.6 |
| L. akiyami A | 2 | 2.9 |
| Total | 70 | 100.0 |

* Minimal titer 1 : 100

## DISCUSSION

The serologic procedures for study of leptospirosis have been done using various methods ( 17,18,19,20,21,22,23,24 ). They are based on the fact that antibody is regular produced after infection. Leptospires are excellent antigens. It has been previously shown that leptospiral cells contain two major antigenic components, P antigen or peripheral antigen and S antigen or somatic antigen ( 25 ). P antigen is type-specific antigen, functioning as an agglutinogen, complement fixing antigen, and precipitinogen. S antigen is genus-specific functioning as complement fixing antigen and precipitinogen.

After intection specific antibodies are developed and may be detectable for a long time depending upon the nature of the antibodies. So detection of leptospiral antibodies depends upon the serological method and relationship to the time of infection.

The microscopic agglutination - lysis test of Schuffner and Mochtar (17) is the generally accepted test in leptospiral serology, and employs living leptospires as antigen. It is type-specific and seem to be the most effective in serological studies of leptospirosis, especially in surveys for past infection. Specific agglutinins can be detected an average of 8-12 days after infection, and reach a high peak in a short time. They are then constant for a period of about five days to two weeks. The persistence of high levels of homologous antibodies differs with the animal species. In general the level of titer gradually decreases, but usually persists as high as 1 : 100 - 1: 300 for a year, and possibly for life.

The difficalty with using the agglutination-lysis test is that employing multiple antigens is laborious and involves the possibility of infection, as well as the necessity of maintaining a large number of antigenically stable stock cultures to provide antigens. The routine application of this test in a diagnostic laboratory is therefore limited.

The incidence of leptospiral infection in patients was investigated and it was found that 26.7 % of patients were seropositive. Most of these seropositive cases were in the age range of 16 - 45 years. Sex distribution of suspect cases serologically positive was not differenct. The predominant serotype was **Leptospira wolffii,** similar to the result of Charuchinda (26), but the incidence in our studies was higher than previous study by about 10%. This may be due to the rate of hospitalization of infected patients, collection of specimens and other clinical factors. such as the clinical suspicion of the doctor. Symptoms may be quite varied, so the doctor must suspect the possibity in order to make the diagnosis. We can see from Table II that the provisional diagnosis was varied although the clinical picture was apparently consistant with leptospirosis.

In our studies usually single specimens were obtained and the agglutination - lysis method of Schuffner and Muchtor was employed. Unfortunate the test is often not diagnostic before the eighth to tenth day (16). So agglutination studies have little value during the acute phase of illness and could not differentiate from past infection especially with single specimens.

## REFERENCES


1. Diesch, S.L., et. al., Human leptospirosis acquired from squirreis, New Eng. J. Med, 276:838-842, 1967.
2. Galton, M.M., Menges, R.W., Shotts, E.B., Jr., Nahmias, A.J., and Heath, C.W., Jr. Leptospirosis : Epidemiology, Clinical manifestations in man and animals, and methods in laboratory diagnosis. Public Health Serv. Publication No. 951. and Washington, D.C.: U.S. Government Printing Office, 1962.
3. Hamdy, A.H., Brownlow, W.J., and Dedeaux, J.D., Leptospirosis in bovines and their human contacts in Egypt, Amer. J. Trop. Med., 11:98-101, 1962.
4. Van der Hoeden, J., Leptospirosis canicolaris in pigs and its probable transfer to human being, J. Infect. Dis., 98:33-38, 1956.
5. Van der Hoeded, J., Zoonoses : Elsevier Publishing Company, Amsterdam, 1964.
6. Alexander, A.D., Gleiser, C.A , Malnati, P. and Yoder, H., Observations on the prevalence of leptospirosis in canine populations of the United States, Amer. J. Hyg. 65 : 43-56, 1957:
7. Alston, J.M., and Broom, J.C., Leptospirosis in man and animals, Williams and Wilkins, Baltimore, 1958.
8. Babudieri, B., Animal reservoirs of leptospires, Ann. N.Y. Acad. Sci. 70: 393-412, 1958.
9. Van der Hoeden, J., The epidemiology and epizootiology of leptospirosis in Israel, J. Trop. Med. Hyg., 58 : 202-204, 1955.
10. Brewer, W.E., Alexander, A.D., Hakioglu. F., and Evans. L.B., Rice-field leptospirosis in Turkey : A serologic survey, Amer. J. Trop. Med., 9:229-239, 1960.
11. Nityananda, K., Leptospirosis-Serologic survey of occupational groups in Ceylon, J. Trop. Med. Hyg., 70: 250-254, 1967.
12. Coffey, J.H., Dravin, I., and Dine, W.C , Swineherd's disease (aseptic meningitis) due to Leptospira pomona, JAMA, 147:949-950, 1951.
13. Daniels, W. B., and Grennan, H.A., Pretibial fever : An obscure disease, JAMA, 122 : 361, 1943.
14. Edwards, G.A., Clinical Charecteristics of leptospirosis : Observations based on a study of twelve sporadic cases, Amer. J. Med., 27:4-17, 1959.
15. Edwards, G.A., Domm, B.M., Human leptospirosis, Medicine (Balt.), 39:117-156, 1960.
16. Edwards, G.A., Domm, B.M., Leptospirosis. Part I., Med. Times, 94:903-913, 1966.
17. Gouchenour, W.S., Jr., Yager, R.H., Wetmore, P.W., and Hightower, J.A., Laboratory diagnosis of leptospirosis. Amer. J. Pub. Health, 43:405-410, 1953.

18. Chang, R. S., and McComb, D. E., Erythrocyte sensitizing substances from five strains of leptospirae; Amer. J. Trop. Med.&Hyg., 3:481-489, 1954.

19. Chang, R.S., Smith, D.J.W., McComb, D. E., Sharp, C. F., and Tong, J. I., The use of Erythrocyte sensitizing substance in the diagnosis of leptospirosis, Amer. J. Trop. Med., 6:101-107, 1957.

20. Cox, C.D., Hemolysis of sheep erythrocytes sensitized with leptospiral extracts (22113), Proc. Soc. Exp. Biol. Med., 90:610-615, 1955.

21. Cox, C.D., Standardization and stabilization of an extract from Leptospira biflexa and its use in the hemolytic test for leptospirosis, J. Infect. Dis. 101:203-209, 1957.

22. Cox, C.D., The laboratory diagnosis of human leptospiresis, South Dakota, J. Med. Pharm., 13:1-15, 1960.

23. Galton, M. M., Powers, D. K., Hall, A. D., and Cornell, R. G., A rapid macroscopic - slide screening test for the serodiagnosis of leptospiresis. Amer. J. Vet. Res., 19:505-512, 1958.

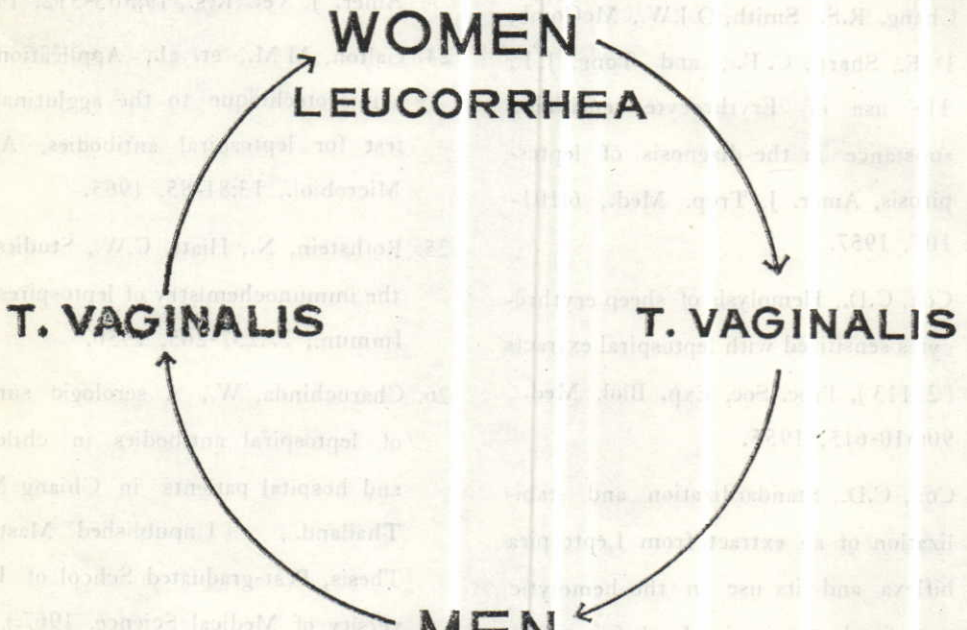
24. Galton, M M., et. al., Application of a microtechnique to the agglutination test for leptospiral antibodies, Appl. Microbiol., 13:81-85, 1965.

25. Rothstein, N., Hiatt, C.W., Studies of the immunochemistry of leptospires, J. Immun., 77:257-265, 1956.

26. Charuchinda, W., A serologic survey of leptospiral antibodies in children and hospital patients in Chiang Mai, Thailand., (Unpublished Master's Thesis, Post-graduated School of University of Medical Science, 1967.)

# โรคที่ต้องการบำบัดร่วมทั้งภรรยา และ สามี

# LEUCORRHEA




ตัดวงจรนี้ด้วย **FLAGISEPT** ORAL TABLETS

( METRONIDAZOLE )

และด้วย.. **VAGISEPT** VAGINAL TABLET

( THIMEROSAL )

## ราคาประหยัด เหมาะสำหรับเวชกรรมทั่วไป

**บริษัท แอด.พี.แสนการัด แลบบอราทอรี่ จำกัด**

218/1 ซอยศาลเจ้าโจ๊ด เจริญกรุง พระนคร โทร 31910

วารสารเทคนิคการแพทย์ เชียงใหม่
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May 1972
Vol. 5 No. 2

# REFRACTOMETRIC DETERMINATION OF TOTAL SERUM PROTEIN:

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Panja Kulapongs, M.D., Dip. Amer. Bd. Ped. ***

## Abstract

**A rapid and reliable estimation of total serum protein content can be made from the refractometric measurement using TS meter. Our experience indicated that the calibration standard provided by the manufacturer gives too low results compared to the standard biuret method. The new calibration scale is constructed for use in our lab. For practical purpose in nutritional field survey one can interprete the TS value ( in gm./Lit. ) as a total serum protein content ( in gm./100 ml. )**

## INTRODUCTION.

The utilization of the physical property of refractive index of a fluid to quantitate its content is common in industry. ( 1 ) When applying this theory in human serum one can obtain accurate and rapid estimates of the content of total serum solids (2) and total serum proteins. ( 1, 3, 4 ) Rubini and Wolf has demonstrated the accuracy of refractometric determination of total solids in serum with a protein content range of 1.77 to 13.9 gm/100 ml. and A/G ratio of 0.13 to 14.0. The estimation of the protein concentration of plasma or serum by refractometry has also been advocated for many year. (3,4,5) Although it is not as reliable as the measurement of total solids and specific gravity the accuracy of this technic in determination of total serum protein is satisfactory for clinical use. Extensive experimentation by many led to the improvement of technic and development of the widely used

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TS meter. The TS meter is a Goldberg refractometer which has been disigned specifically for medical use. The instrument is temperature compensated for temperature between 60° F. and 100° F.

From our recent field survey the wide discrepency between the total serum proteins values obtained from the TS meter readings and those from biuret method. (6) It is the purpose to report our findings with the proposal of the newer TS meter calibration scale for use in our country.

## Material and Method

The best group of patients who can provide a wider range of total protein content is children with protein calorie malnutrition were studied. Plasma samples were collected from microhematocrit (heparinized) tubes and the TSS value were read by the Goldberg TS meter. At the same time the venous blood total serum protein content was measure by the biuret method. (6)

## Results

From the results shown in Table I, II and Figures I, II it is evident that there is a good correlation between the TS readings and total serum protein values (biuret method). The TS reading can be directly interpreted as the total protein content. The calibration scale provided by the manufacturer gives too low total protein values.

Hydration state of the pateints exerts only minimal effect, if ever, to those correlation.

TABLE I. CORRELATION BETWEEN THE TSS READINGS AND TSP (Kjeldhal) VALUES IN DIFFERENT FORMS OF MALNUTRITION

| MARASMUS | | | MARASMIC-KWASHIORKOR | | | KWASHIORKOR | | |
|---|---|---|---|---|---|---|---|---|
| Case No. | TSS | TSP | Case No. | TSS | TSP | Case No. | TSS | TSP |
| 4 | 5.2 | 6.05 | 1 | 5.2 | 3.75 | 2 | 4.2 | 3.50 |
| 8 | 6.4 | 6.60 | 5 | 5.1 | 5.90 | 3 | 3.5 | 2.69 |
| 10 | 8.0 | 7 98 | 7 | 4.6 | 4.90 | 6 | 3.8 | 4.20 |
| 11 | 6.8 | 7.10 | 9 | 7.2 | 7.60 | 14 | 4.0 | 4.30 |
| 13 | 7.0 | 8.4 | 12 | 6.0 | 6.40 | 22 | 3.3 | 3.66 |
| 15 | 6.1 | 5.8 | 17 | 4.6 | 5.50 | 24 | 3.4 | 3.80 |
| 16 | 5.7 | 7.5 | 18 | 6.2 | 6.70 | 25 | 3.6 | 4.00 |
| 19 | 6.6 | 6.7 | 20 | 5.4 | 5.80 | 30 | 3.8 | 4.30 |
| 26 | 5.8 | 5.8 | 21 | 5.6 | 5.90 | 31 | 4.4 | 5.00 |
| 27 | 5.1 | 5.3 | 23 | 5.2 | 4.88 | 33 | 4.4 | 4.40 |
| 28 | 7.5 | 7.9 | 29 | 5.1 | 5.10 | 51 | 3.0 | 3.66 |
| 32 | 7.8 | 8.8 | 35 | 5.8 | 5.3 | 52 | 3.5 | 3.56 |
| 34 | 6.5 | 6.9 | 36 | 3.8 | 4.5 | 59 | 4.3 | 4.38 |
| 37 | 5.6 | 6.10 | 40 | 4.1 | 4.8 | 61 | 3.5 | 3.46 |
| 38 | 5.6 | 5.70 | 41 | 5.4 | 5.3 | 62 | 3.5 | 3.54 |
| 39 | 6.8 | 6.80 | 42 | 5.5 | 4.6 | 63 | 3.3 | 3.75 |
| 43 | 7.6 | 7.40 | 45 | 4.0 | 4.58 | 64 | 3.5 | 3.80 |
| 44 | 4.6 | 4.46 | 46 | 3.8 | 3.98 | 65 | 3.4 | 3.40 |
| 47 | 7.1 | 7.30 | 53 | 5.6 | 6.10 | 66 | 4.3 | 4.48 |
| 48 | 6.4 | 6.40 | 55 | 4.1 | 3.98 | 74 | 3.9 | 4.17 |
| 49 | 4.1 | 4.10 | 56 | 5.2 | 4.70 | | | |
| 50 | 6.5 | 7.50 | 57 | 4.1 | 4.24 | | | |
| 54 | 5.8 | 5.00 | 60 | 4.1 | 4.44 | | | |
| 58 | 5.5 | 6.00 | 67 | 5.3 | 5.50 | | | |
| 68 | 6.0 | 5.60 | 70 | 5.1 | 4.90 | | | |

| MARASMUS | | | MARASMIC-KWASHIORKOR | | | KWASHIORKOR | | |
|---|---|---|---|---|---|---|---|---|
| Case No. | TSS | TSP | Case No. | TSS | TSP | Case No. | TSS | TSP |
| 69 | 5.3 | 5.20 | 73 | 4.5 | 4.24 | | | |
| 71 | 6.5 | 7.14 | 76 | 6.1 | 4.8 | | | |
| 72 | 6.5 | 6.90 | 77 | 5.7 | 5.5 | | | |
| 75 | 7.0 | 6.46 | | | | | | |
| 78 | 6.6 | 6.35 | | | | | | |

NOTE: See Figure I.

**TABLE II. EFFECT OF HYDRATION ON REFRACTOMETRIC READINGS (TSS) AND TSP VALUES**

| Case No. | BEFORE HYDRATION | | AFTER HYDRATION | |
|---|---|---|---|---|
| | TSS | TSP | TSS | TSP |
| 32 | 7.8 | 8.8 | 6.6 | 7.0 |
| 33 | 4.4 | 4.4 | 4.5 | 4.7 |
| 34 | 6.5 | 6.9 | 6.5 | 6.0 |
| 35 | 5.8 | 5.3 | 5.7 | 6.1 |
| 36 | 3.8 | 4.5 | 4.7 | 5.1 |
| 37 | 5.6 | 6.1 | 6.0 | 6.8 |
| 38 | 5.6 | 5.7 | 6.7 | 7.1 |
| 39 | 6.8 | 6.8 | 5.5 | 6.2 |
| 40 | 4.1 | 4.8 | 4.6 | 5.4 |
| 41 | 5.4 | 5.3 | 5.2 | 5.3 |
| 42 | 5.5 | 4.6 | 4.5 | 4.67 |
| 43 | 7.6 | 7.4 | 6.3 | 6.9 |
| 44 | 4.6 | 4.46 | 4.2 | 4.62 |
| 45 | 4.0 | 4.58 | 4.4 | 4.4 |
| 46 | 3.8 | 3.98 | 4.4 | 3.99 |

| Case No. | BEFORE HYDRATION | | AFTER HYDRATION | |
|---|---|---|---|---|
| | TSS | TSP | TSS | TSP |
| 47 | 7.1 | 7.3 | 7.2 | 6.8 |
| 49 | 4.1 | 4.1 | 4.2 | 4.5 |
| 50 | 6.5 | 7.5 | 6.5 | 7.1 |
| 51 | 3.0 | 3.66 | 3.8 | 3.93 |
| 52 | 3.5 | 3.56 | 4.5 | 4.0 |
| 53 | 6.7 | 6.25 | 5.6 | 6.1 |
| 54 | 5.8 | 5.0 | 6.5 | 5.68 |
| 55 | 4.1 | 3.98 | 3.6 | 3.48 |
| 56 | 5.2 | 4 7 | 4.5 | 4.92 |
| 57 | 4.1 | 4.24 | 4.8 | 4.54 |
| 58 | 5.5 | 6.0 | 5.5 | 5.65 |
| 59 | 4.3 | 4.38 | 5.1 | 5.15 |
| 60 | 4.1 | 4.44 | 4.1 | 4.16 |
| 61 | 3.5 | 3.46 | 5.1 | 4.9 |
| 62 | 3.5 | 3.54 | 3.7 | 3.54 |
| 63 | 3.3 | 3.75 | 4.0 | 4.0 |
| 64 | 3.5 | 3.8 | 3.8 | 3.8 |
| 65 | 3.4 | 3.4 | 3.8 | 3.43 |
| 66 | 4.3 | 4.48 | 4.5 | 4.38 |
| 67 | 5.3 | 5.5 | 5.0 | 5.2 |
| 68 | 6.0 | 5.6 | 5.1 | 5.4 |
| 69 | 5.3 | 5.2 | 5.3 | 5.1 |
| 71 | 6.5 | 7.14 | 6 0 | 6.05 |
| 73 | 4.5 | 4.24 | 3.9 | 4.48 |
| 77 | 5.7 | 5.5 | 6.0 | 5.75 |

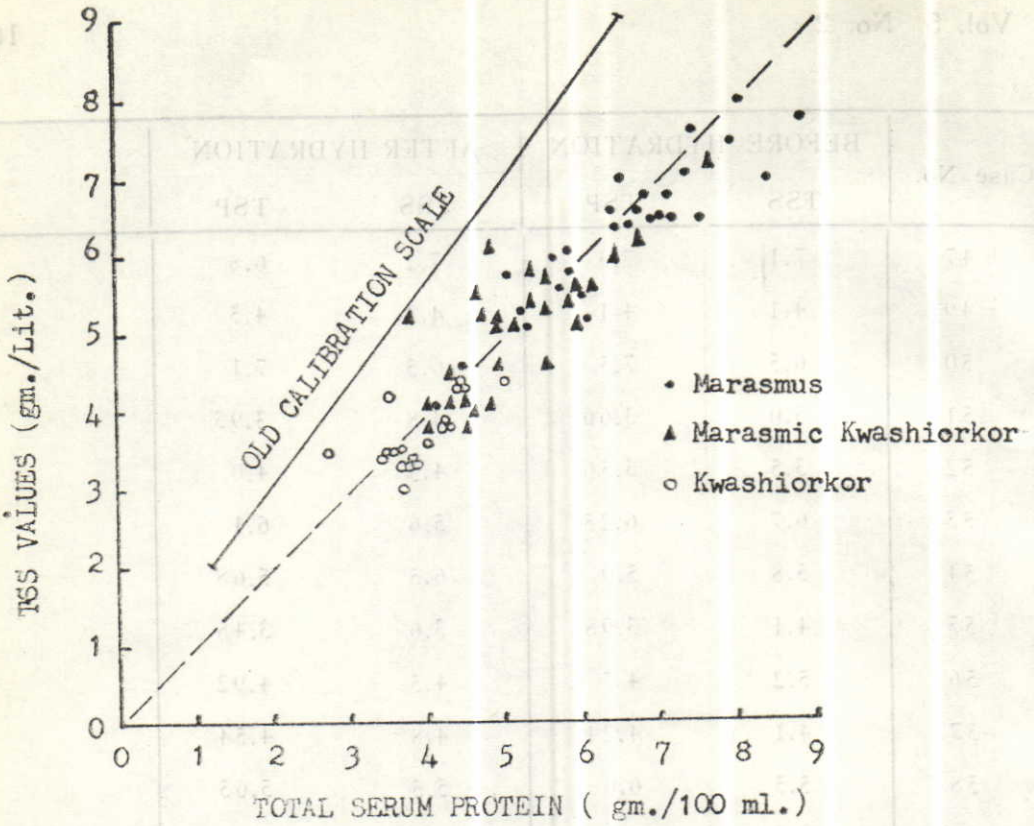


FIGURE I. CORRELATION BETWEEN THE TSS AND TSP VALUE

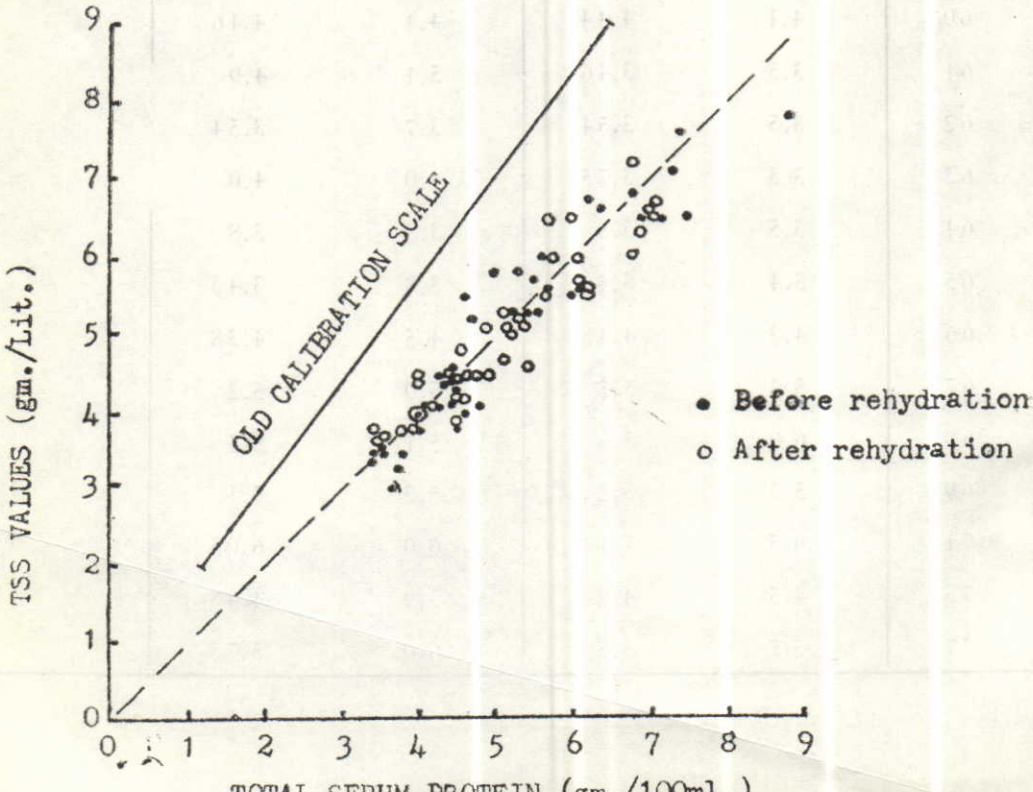


FIGURE II. EFFECT OF REHYDRATION ON THE CORRELATION BETWEEN

## COMMENTS.

The use of refractive index changes to measure serum protein concentration has been estiblished as a most rapid and useful technic for many years. ( 3, 7 ) and it is recently being adapted for auto-analytic produre. (7) It can be expected that refractometry will allow a greater variability of result in the determination of total serum protein than does the biuret method. The early reports on the use of refractive index for serum proteins determination stated that a correction factor must be applied for the non-protein constituents of serum. ( 1, 7 ) These components include electrolytes, glucose, urea, bilirubin, cholesterol and lipids. Marsh and Fingerhut recently demonstrated that glucose value up to 400 mg./ml. and urea N. values up to 200 mg./100 ml. caused changes of the refractive index which were within the tolerance. Bilirubin and turbidity either artificially produced or present as a result of pathological conditions, caused virtually no effect. (7, 8) The exact effect of very high cholesterol or lipid level was difficult to assess since artificially prepared solutions alter the protein nature of the test solution. (7) The validity of this method in the estimation of total serum proteins in specimens with varying albumin and globulin content and A/G ratios has been questioned but the recent study by Barry and associates indicated that its accuracy is entirely satisfactory for clinical use: Our experience also indicated degree of dehydration, albumin, and globulin content, jaundice and turbidity has no significant effect on the TS refractometry.

For practical pupose of nutritional survey it is suggested that we may adopt the TS reading directly as the total serum proteins in grams/100 ml. The confirmation of above findings is still required from other laboratories.

## REFERENCE


1. Neuhausen, B.J., and Rioch, D.M. : The refractometric determination of serum proteins, J. Biol. Chem. 55 : 353, 1923.
2. Rubini, M.E., and Wolf, A.V. : Refractometric determination of total solids and water of serum and urine, J. Biol. Chem. 225 : 869, 1957.
3. Sunderman, F.W. : A rapid method for estimating serum proteins : Formula for calculating serum protein concentration from the refractive index of serum. J. Biol. Chem. 153 : 139, 1944.
4. Sunderman, F.W., and Boerner, R. : Normal Values in Clinical Medicine. Phila., 1949.
5. Gettler, A.O., and Baker, W. : J. Biol. Chem. 25 : 211, 1916.
6. Kingsley, G.R.J. Lab. Clin. Med. 27 : 840, 1942.
7. Marsh, W.H., and Fingerhut, B. : Serum protein determination by automatic recording refractometry. Clin. Chem. 8 : 640, 1962.
8. Barry, K.G., McLaurin, A.W., and Parnell, B.L. : A practical temperature compensated hand refractometer (the TS meter) : Its Clinical used and application in estimation of total serum proteins. J. Lab. Clin. Med. 55 : 803, 1960.

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# Effect of Time and Temperature on Plasma Ammonia Determination

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## Abstract

**By means of this method it has been demonstrated that the distribution of ammonia in plasma of normal individuals increases after drawing. The influence of temperature on in-vitro generation of ammonia in plasma was evaluated. It was found that when blood was placed in a freezer with the temperature between -20° C and -25° C there was no significant rise of blood ammonia levels. There wes gradual rise when blood was placed in a refrigerator with the temperature about 4° C and more rapid rise at room temperature. The inhibition of ammonia production at temperature below -20° C facilitates a laboratory procedure when it is necessarily delayed.**

## Introduction

Blood ammonia is formed in the gastrointestinal tract and eliminated as urea by the liver. In cases of liver damage or disease, cirrhosis, and occasionally in severe heart failure, azotemia, corpulmonale, and erythroblastosis fetalis, the ammonia is not properly converted and the ammonia level of the plasma rises. Repeated ammonia determinations have been found especially useful in the monitering of patients suffering from hepatic coma. The method used to determine ammonia is fairly simple for any laboratory, but it must be performed as soon as possible following collection of the sample. Only freezing of the sample provides an adequate mean of storate as was demonstrated in this experiment.

* This experiment and report were presented by Chatchawadee Kunarak in fullfillment of the requirements for a B.Sc. (M.T.) from Chiang Mai University 71-72.

**Method**

The method used in this experiment represents a modification of the Seligson and Seligson microdiffusion method, the principle being that when saturated potassium carbonate is added to an aliquot of plasma, the alkali causes the release of any ammonia present in the plasma. The ammonia is trapped in hydrochloric acid in specially designed diffusion bottles. After an appropriate time interval, the acid drop and the absorbed ammonia are rinsed into cuvettes with Nessler's reagent and the optical density read. A standard of known concentration is prepared and treated in the same manner and the final results computed by the standard formula:

$$\frac{\text{O.D.}_{unk} \times \text{Value of Std}}{\text{O.D.}_{Std.}} = \text{Value unknown}$$

To show the effect of time and temperature on the level of ammonia in plasma, samples were taken from nine normal individuals with no history of liver damage or disease. EDTA has been found to be the ideal anticoagulant for ammonia studies and was used in this case. Plasma was separated immediately and an immediate determination done in order to determine a baseline value for each sample. Plasma must be separated before freezing because freezing and thawing of whole blood results in the hemolysis of red cells which causes a false elevation due to the presence of ammonia in the red cells. The samples were then divided into three sets. Set 1 was kept at room temperature (25° C) and retested 3 hours, 6 hours and 9.5 hours after collection. Set 2 was refrigerated and retested at 6, 12 and 23.5 hours. The third set was placed in the freezer and tested again at 24, 48 and 72 hours.

**Results**

It was found that the ammonia content of plasma prepared and maintained at room temperature is not constant. Plasma in contact with air in vitro continues to form ammonia. This reaction is markedly inhibited by keeping plasma in the freezer. It was observed that the ammonia levels of plasma frozen in the freezer immediately after shedding, if measured promptly after thawing, remain less changed than that of refrigerator stored or plasma kept at room temperature.

In Set 1, in which plasma was left at room temperature, there was a gradual rise of ammonia content starting at 10 minutes. At the end of 6 hours, the concentration of blood ammonia had risen to 2 or 3 times the normal level. When blood was kept in the freezer, there was no significant increase in ammonia level.

In Set 2, the temperature of ordinary refrigeration apparently could not prevent the enzymatic hydrolysis of blood, when

was a gradual increase of ammonia although not to the same degeee as in these specimens left at room temperature.

In Set 3, the period of freezing was extended to 4 days. There was no significant increase of ammonia content in the blood, when compared with the normal. A comparison of curves indicated that the ammonia levels were less altered during the period of freezing than storage at either room temperature or refrigeration.

The mean values of varied conditions were :

| % of plasma ammonia increase per hour | |
|---|---|
| Room temperature | 28.1 % |
| Refrigerator | 9.3 % |
| Freezer | 0.834 % |

**Conclusion :**

This investigation showed that the ammonia concentration of freshly drawn plasma was insignificantly altered by rapid freezing but that at room temperature, the plasma ammonia concentration remains constant for 15-40 minutes after blood is withdrawn from the body and then rapidly increases. Because ammonia levels increase rapidly on standing, the test should be run as soon as possible following collection of blood. If processing must be delayed more than 20 minutes, plasma should be quick frozen and kept in the freezer until ready for determination. Testing should not be delayed more than 3 days as even in the frozen state, the ammonia increases after that time. The sample should be thawed at 37°C for no more than 5 minutes and analysed immediately. Nowadays, freezing helps laboratories to be able to determine plasma ammonia when specimens have been drawn at night or on weekends because they may be stored for subsequent analysis. It also affords a means of preserving blood specimens during transportation from hospitals at which blood ammonia determinations are not available to central laboratories. In research laboratories it allows investigators to collect serial specimens for subsequent analysis without requiring repeated interruptions for immediate processing of individual samples. The determination of plasma ammonia has wide applicability in clinical medicine and research. Nevertheless, many hospital laboratories do not perform ammonia determinations because of difficulties encountered in the collection and analysis of specimens. A major reason for excluding this procedure has been the necessity of performing the analysis within 10-30 minutes of obtaining the sample.

Comparison of percentage increase in plasma ammonia at various times

Kept plasma at room temperature.

| Sample | Time | | |
|---|---|---|---|
| | 3 hours | 6 hours | 9.30 hours |
| 1 | 138.9% | 186.9% | 140.0% |
| 2 | 144.1% | 172.1% | 28.6% |
| 3 | 15.7% | 374.1% | 120.2% |
| Average/hour | 33.2% | 40.7% | 10.3% |

Comparison of percentages increase in plasma ammonia at various times.

Stored plasma in the refrigerator.

| Sample | Time | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| 1 | 7.02% | 70.2% | 71.4% |
| 2 | 5.4% | 0% | 108.6% |
| 3 | 148.9% | 257.1% | 274.4% |
| Average/hour | 12.5% | 9.1% | 6.4% |

Comparison of percentage increase in plasma ammonia at various times.

Stored plasma in the frozen state.

| Sample | Time | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| 1 | 0% | 0.3% | 90.9% |
| 2 | 58.4% | 0% | 178 9% |
| 3 | 14.83% | 0% | 74.6% |
| Average/hour | 1.01% | 0.002% | 1.5% |

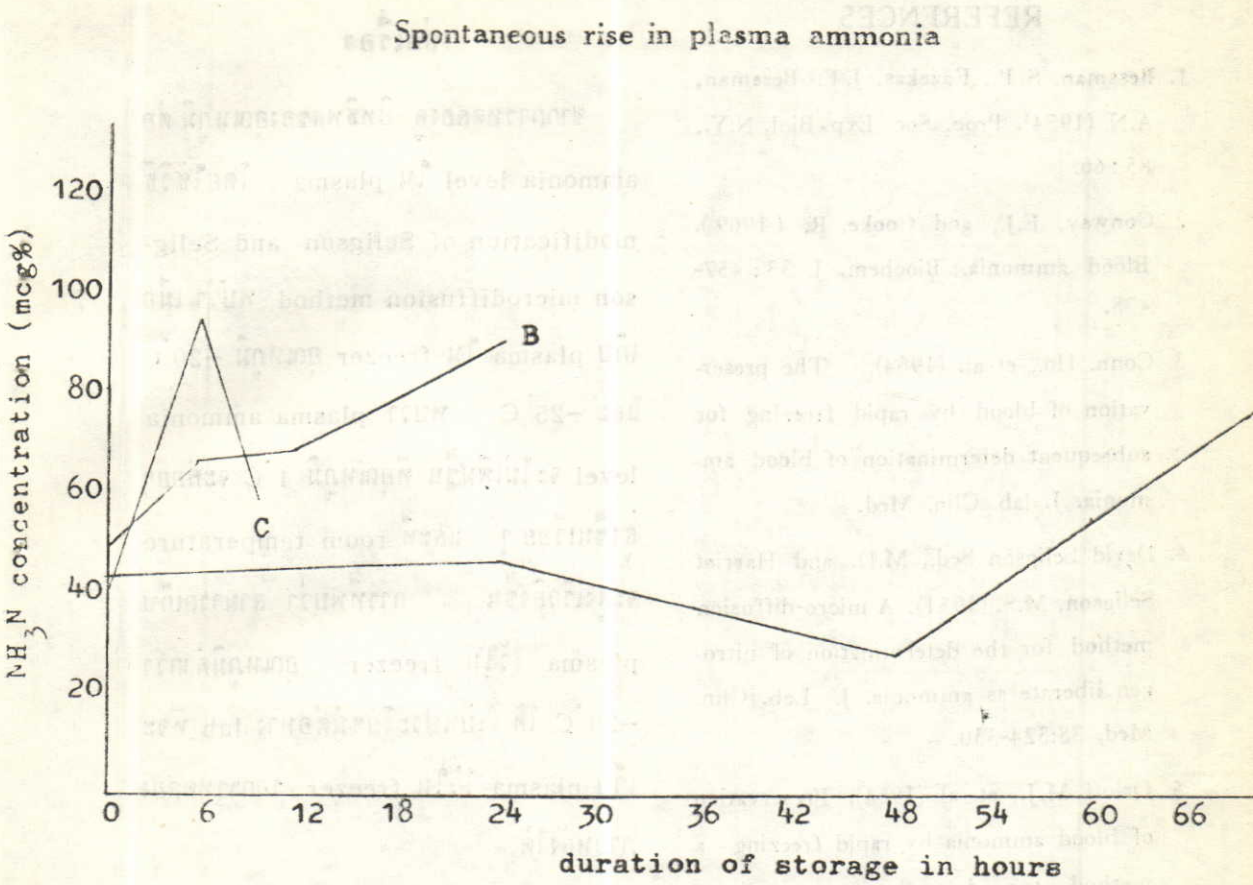


Fig II. Ammonia concentration in nine samples of blood as a function of time after shedding.

curve A. – plasma kept in the freezer

curve B. – plasma kept in the refrigerator.

curve C. – plasma kept at room temperature.

Each curve base on the mean plasma ammonia concentration of nine specimens determined serially after various period of storage.

## REFERENCES


1. Bessman, S. P., Fazekas, J. F. Bessman, A.N. (1954). Proc. Soc. Exp. Biol. N.Y., 85 : 66.
2. Conway, E.J., and Cooke, R. ( 1969 ). Blood ammonia. Biochem. J. 33 : 457-478.
3. Conn. Ho., et al. (1964). The preservation of blood by rapid freezing for subsequent determination of blood ammonia. J. lab. Clin. Med.
4. David Seligson Sed., M.D. and Harriet Seligson, M.S. (1951). A micro-diffusion method for the determination of nitrogen liberate as ammonia. J. Lab. Clin. Med. 38:324-330.
5. Orloff M.J., et. al. (1964). Preservation of blood ammonia by rapid freezing - a method for delayed determinations. Clin. Chem. 10 : 991-1001.
6. Seligson, D., and Hirahara, K. ( 1957 ). The measurement of ammonia in whole blood, erythrocytes and plasma. J. Lab. Clin. Med. 49 : 962.
7. Tobe, B.A., and Wood, D. (1962). The clinical significance of the formation of ammonia in shed whole blood. Blood. 20 : 802.


## ย่อเรื่อง


จากการทดลองดู อิทธิพลของอุณหภูมิ ต่อ ammonia level ใน plasma โดยใช้วิธี modification of Seligson and Seligson microdiffusion method พบว่า เมื่อเก็บ plasma ใน freezer อุณหภูมิ −20°C และ −25°C พบว่า plasma ammonia level จะไม่เพิ่มขึ้น ที่อุณหภูมิ 4°C จะค่อยๆ สูงขึ้นเรื่อยๆ และที่ room temperature จะสูงเร็วยิ่งขึ้น การที่พบว่า สามารถเก็บ plasma ไว้ใน freezer อุณหภูมิต่ำกว่า −20°C ได้ เป็นประโยชน์ต่อทาง lab ที่จะเก็บ plasma ไว้ใน freezer รอการทดลองภายหลังได้

วารสารเทคนิคการแพทย์ เชียงใหม่
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# The Relationship between Hookworm Infection and the Level of Serum Iron.

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Chucherd Sivasomboon, M.D., M.P.H. & T.M. ***

## Abstract

**A study on the relationship between hookworm infection and the serum iron level was carried out among the inmates of Chiang Mai provincial prison between November 1970 and January 1971. Quantitative egg counts were done in infected subjects and in normal persons as a control. The level of serum iron and the total iron binding capacity were determined among these subjects. The corelation between the degree of hookworm infection and the serum iron level was established.**

Hookworm infection is one of the most commonly found diseases in the world. Evidently, the disease has been present since prehistoric period. It has caused human to suffer and great economic loss in number of times.

During the year 1921 - 1923, the Rockefeller foundation in cooperation with the Thai government made a survey on hookworm infection in Thailand and found 57.3 % of the Thai population sufferred the disease. The data comprised 22.8 % from the Northeast, 26.9 % from the Center, 43 % from the South and 8 % from the North (5). Most of the hookworm infection found in Thailand are caused by the worm **Necator americanus** and only 0.86 % by **Ancyclostoma duodenale.**

Iron (Fe.) is one of the most important component of hemoglobin in blood.

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There is always a constant amount of iron in a normal healthy person. The rate of turnover is about 27 - 35 mg/day. Iron source does not come solely from intestinal absorption but also from hemoglobin catabolism in the reticuloendothelial system. Protein "transferrin" acts as the transportator of which one third is bound to "plasma iron" and the rest remains free. Plasma iron concentration is low in anemic conditions such as rhumatoid, certain kind of cancer. Plasma iron is high during the damage of red blood cells.

The main cause of anemia in tropical areas is due to malaria and hookworm infection. The symptom of hookworm infection is not drastic but long lasting and can be fatal if not treated.

Many records and evidences showed a strong linear relationship between number of hookworms infected persons and hemoglobin concentration ( 2, 4, 6, 7 ). The quantity of hookworms is related to the number of eggs in the feces of the infected person. People carrying hookworms in their intestines are divided into four catagories according to the number of eggs/unit of feces as follows.

1) Carrier - persons having less than 800 eggs/1 gm of feces or equivalent to 40 hookworms in the intestine.

2) mild case - persons having between 800-3000 eggs/1 gm of feces or equivalent to 150 worms.

3) moderate case - persons having between 3,000 - 7,500 eggs/1 gm of feces or equivalent to 400 worms.

4) severe case - persons having over 7,500 eggs/1 gm of feces or equivalent to 800 worms.

Investigations were done in patients sufferred severe infection and all of them were found to have low hemoglobin content in blood (3). The anemia is of hypochromic and microcytic type. Plasma iron was reduced greatly. The red cell / white cell ratio is twice as high as in normal. The loss of red blood cell is increased via intestine. A normal healthy persons losses 0.03% of iron per day while 4.79 % of iron per day is lost in severe cases. During the initial infection by hookworm, the hemoglobin content is not changed since iron is replaced by the reserved body iron. Later, when the iron reserve is depleted, the overall iron content begin to decline and hence anemic condition follows.

The purpose of this study is to investigate the relationship between iron content in serum and number of hookworms in the intestine ie. the number of eggs / gm of feces.

**Material and Methods** The volunteers were the inmates of Chiang Mai provincial prison. Stool examination was performed to detect infection and number of eggs was determined in infected sample by Stool's Technique (7). The multiplication

factor for calculating the number of eggs varies according to the condition of stool as follows.

| condition of stool | factor |
|---|---|
| hard formed | 1 |
| mushy, formed | 1.5 |
| mushy | 2 |
| mushy, diarrheic | 3 |
| frankly diarrheic | 4 |
| watery | 5 |

Determination of serum iron is achieved by modified technique of Caraway (1). The principle of the method is to reduce Fe and detach it from the heme component with ascorbic acid and hydrochloric acid containing sterox. The Fe is detected in the supernatant by adding 2-4-6-S- tripyridye-S-triazine (TPTZ) and ammoniumacetate. The quantity of Fe is then measured colorimetrically at the wavelength of 590 mu.

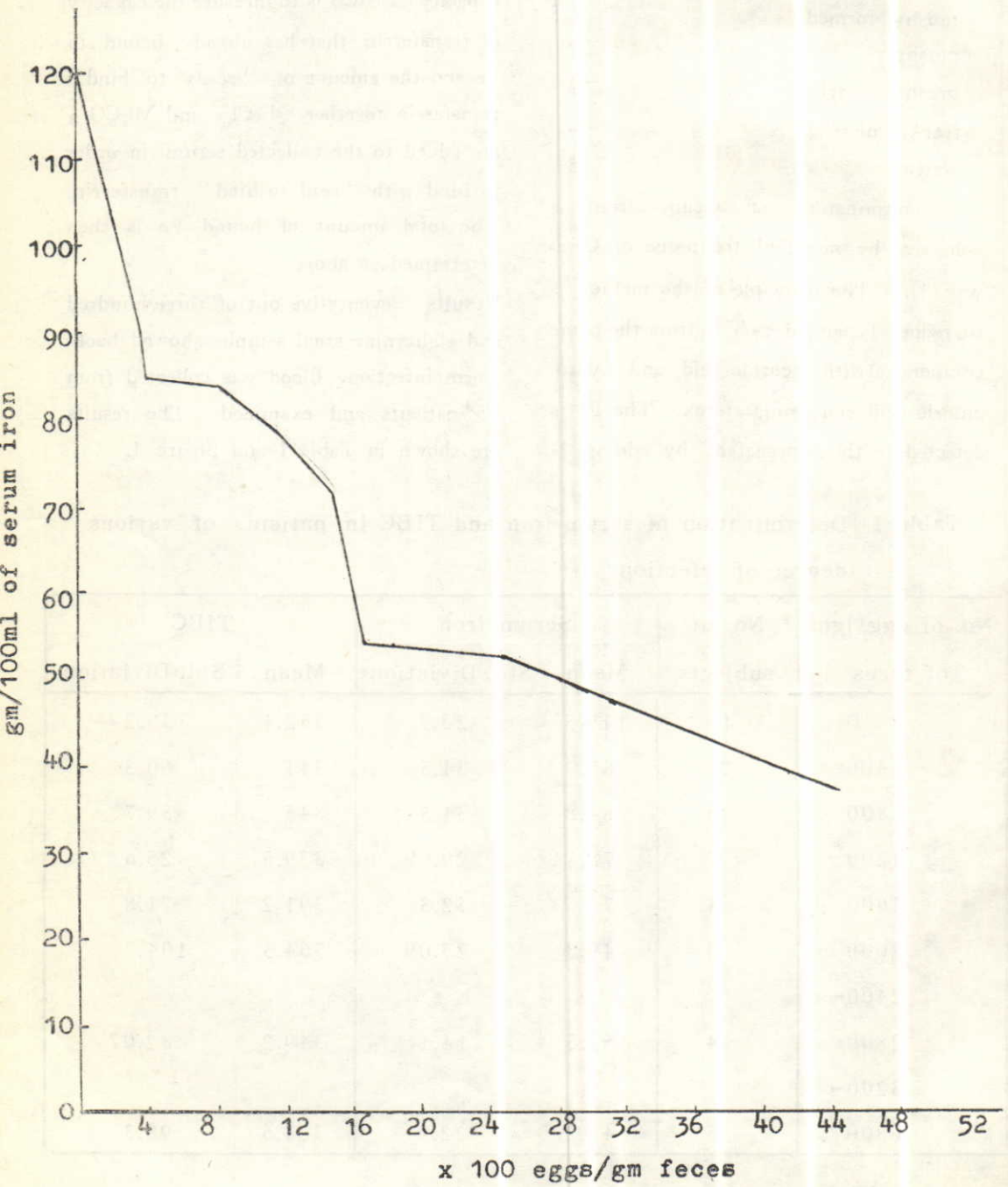
Determination of total iron binding capacity (TIBC) is to measure the capacity of transferrin that has already bound to Fe and the amount of "ready to bind" transferrin together. $FeCl_3$ and $MgCO_3$ are added to the collected serum in order to bind with "read to bind" transferrin. The total amount of bound Fe is then determined as above.

**Results** Seventyfive out of three hundred and eightynine stool samples showed hookworm infection. Blood was collected from 45 patients and examined. The results are shown in Table 1 and Figure 1.

Table 1 Determination of serum iron and TIBC in patients of various degree of infection.

| No. of egg/lgm of faces | No. of subjects | Serum iron | | TIBC | |
|---|---|---|---|---|---|
| | | Mean | Std. Diviation | Mean | Std. Diviation |
| 0 | 15 | 119.5 | 23.7 | 352.4 | 39.2 |
| 400 | 23 | 85 | 31.5 | 341 | 60.3 |
| 800 | 8 | 84.8 | 31.5 | 345 | 59.7 |
| 1200 | 7 | 78.14 | 29.09 | 339.5 | 25.6 |
| 1600 | 4 | 73 | 32.3 | 391.2 | 71.8 |
| 2000 | 3 | 49.3 | 23.09 | 364.3 | 104.7 |
| 2400–2800 | 4 | 52.5 | 14.1 | 349.2 | 82.07 |
| 3200–6800 | 5 | 45.2 | 22.9 | 354.6 | 99.3 |

Figure 1 Relationship between level of serum iron and the degrees of infection of hookworm in the subjects.



**Discussion** There is a gradual reduction of level of serum iron in the persons infected with hookworm. Although the initial drop of the iron level in very mild cases seems to be too great, it is still in the limit. The reduction of serum iron may not indicates the anemic condition since there may be enough iron from the body reservior to bind with hemoglobin. The data indicates the loss of overall Fe and the degree of Fe loss corelates with the degree of hookworm infection. In this experiment, the TIBC is rather constant in both normal and infected subjects. The power of binding of transferrin to Fe may be influenced by many factors such as diet, individual Fe reserve, daily turnover of Fe.

**Conclusion** The level of iron serum in hookworm infected persons is lower than normal. The degree of hookworm infection directly relates with the degree of reduction of iron serum.

**REFERENCE**


1. Caraway, W. T. Macro and Micro methods for determination of Serum Iron and Iron-Binding Capacity. Clinical Chemistry, **9,** 188-199 (1963).
2. Carr, H.P. Observation upon Hookworm Infection in Mexico. Amer. Jour. Hyg., **6,** ( supp. 2 ) ( 1963 ).
3. Layrisse, M.A. et al., Hookworm Anemia; Iron Metabolism and Erythrokinetic : Blood Jour. of Hemat. **18**, 16 - 72, ( 1961 ).
4. Layrisse, M. A. and Roche, M. The relationship between Anemia and Hookworm Infection. Amer. Jour. of Hyg. **73**, 279-301, ( 1964 ).
5. Sadun, E.H. Studies on the Distribution and Epidemiology of Hookworm, Ascaris and Tricuris in Thailand. Amer. Jour. of Hyg. **62**, 166-155, (1955).
6. Smillie, W.G. and Augusttine, D.L. In : Chandler, A.C. Hookworm Disease, New York : Macmillan Co., 1929, p. 268.
7. Stoll, N. R. Investigation on The Control of Hookworm disease XXI : On the use of egg counting method in soil culture studies of hookworm larvae. Amer. Jour. of Hyg. **3,** 339-442, (1923).

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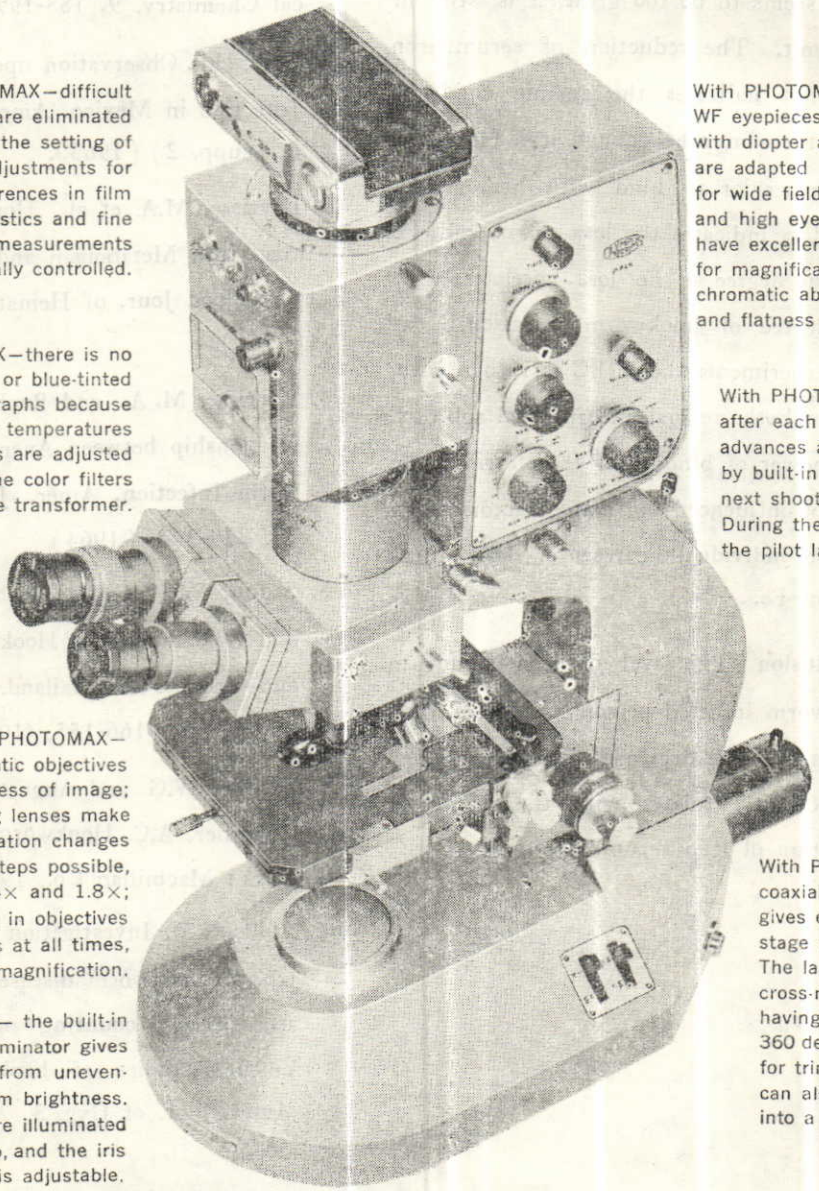

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# THE SUSCEPTIBILITY OF FIFTY STRAINS OF SHIGELLA TO EIGHT ANTIMICROBIAL AGENTS

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Kampol Panas ampol, M.D. **

## Abstract

Fifty strains of Shigella from patients in Nakorn Chiang Mai Hospital and McCormick Hospital were isolated during the period from December 15, 1970, to March 20, 1971. The identification of Shigella was carried out by biochemical reaction and serology. Of 50 isolated Shigella, altogether 32 strains of Sh. flexneri were identified. All of these strains were tested for antimicrobial sensitivity by the serial tube dilution methed with eight antimicrobial agents. The antimicrobial agents were Garamycin (Gentamycin), Terramycin (Oxytetracycline), Colimycin, Streptomycin, Chloramphenicol, Ampicillin, Kanamycin and Sulfadiazine. The results indicated that Shigella flexneri were sensitive to Gentamycin 12.4%, Colimycin 68.75%, Ampicillin 12.5%, Kanamycin 12.5%, Chloramphenicol 3.12% respectively. Fourteen strains of Sh. sonnei were sensitive to Colimycin 83.33% and to Ampicillin 7.14%. For 4 strains of Shigella dysenteriae, 75% of them have been shown to be sensitive to Colimycin and 25% of them were sensitive to Kanamycin. None of Shigella were sensitive to Sulfadiazine, Streptomycin and Terramycin.

## INTRODUCTION

Intestinal tract infections are among the most common disease usually found in tropical countries, particularly in poor hygenic populations. The infections agents may be parasitic, such as Hookworm, Entamoeba histolytica, or may be bacterial.

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Among bacterial intestinal tract infections, Shigellosis or bacillary dysentery, caused by Shigella, is one of the most common disease in Thailand. Shigella is divided by biochemical reactions into two major groups, mannitol fermenters and non-mannitol fermenters, and into four groups by serological reaction, group A (**Shigella dysenteriae**), group B (**Shigella flexneri**), group C (**Shigella boydii**), and group D (**Shigella sonnei**).

The transmission of this disease may occur by direct contact through fecal-oral transmission; or indirectly, by eating contaminated foods, vegetables, or drinking contaminated water or milk (1,2,3). The disease begins with acute diarrhoeae, accompanied by fever and often vomitting, cramps and tenesmus. In severe cases the stool may contain blood, mucus and pus.

Many antibiotics are introduced in the treatment of Shigellosis. Formerly sulfaguanidine was very effective, however later many strains developed resistance. Some enteric bacilli could transfer resistance factors to Shigella and make it resistant to the antibiotics (4,5,6,), Therefore, the antimicrobial sensitivity should done before treatment. But sometime the doctor can not wait for the sensitivity results. In this case the drug of choice shoul be given. This experiment tried to study the antimicrobial susceptibility of Shihella and it will be useful for the doctor to choose the drug for treatment of Shigellosis.

## MATERIALS AND METHODS

I. **Organism.** Shigella used in this experiment were isolated from the patients in Chiang Mai Hospital and McCormick Hospital. Most of the patients came to the hospital with the symptoms of diarrhoeae or dysentery.

II. **Antimicrobial agents.** Eight antimicrobial agents, sulfadiazine, colimycin, terramycin, garamycin, ampicillin, chloramphenicol, streptomycin, and kanamycin were tested for susceptibility of Shigella.

These agents were dissolved in sterile distilled water to give concentration of 2000 mcg or unit/ml and kept frozen in 4 ml aliquots. Before uging the stock solution was thawed and the remainder discarded after use.

III. **Susceptibility testing method.** Stock solutions of antimicrobial agents were thawed and diluted 1 : 10 with sterile distilled water, the final concentration being 200 mcg or unit/ml. The serial dilution of antimicrobial agents were done using tryptic case soy broth to make concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781 and 0.39 mcg or unit/ml, the final volume was 0.5 ml. Then 0.5 ml. of 1 : 1000 dilution of overnight culture of Shigella

in trypticase soy broth was added to each antimicrobial dilution. Incubate 37° C overnight and read MIC (Minimal Inhibitory Concentration).

RESULTS

A total of 50 strains of Shigella were isolated, 32 strains were **Sigella flexneri**, 14 strains were **Shigella sonnei**, and 4 strains were **Shigella dysenteriae**.

The results of the antimicrobial susceptibility are shown in Table I, II and III. The percentage of sensitive and resistance are shown in Table V. These results are compared with blood level of the antimicrobial agents (Table IV). None of the Shigella were sensitive to streptomycin, sulfadiazine and terramycin. Colimycin seems to be effective.

Table I Minimal Inhibitory Concentration (MIC.) of Shigella flexneri 32 strains to 8 Antimicrobial agents

| Concentration of Antimicrobial agents mcg/ml or unit/ml | MIC. to Antimicrobial agents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Caramycin mcg/ml | Colimycin mcg/ml | Terramycin mcg/ml | Chloram-phenicol mcg/ml | Streptomycin mcg/ml | Ampicillin mcg/ml | Kanamycin mcg/ml | Sulfadiazine mcg/ml |
| > 100 | – | 10 | – | 1 | 5 | – | – | 32 |
| 100 | – | 2 | 2 | 6 | 1 | – | – | – |
| 50 | – | 7 | 5 | 15 | 8 | 1 | 1 | – |
| 25 | – | 7 | 15 | 6 | 3 | 1 | 3 | – |
| 12.5 | – | 5 | 8 | – | 14 | 4 | 8 | – |
| 6.25 | 5 | 1 | 2 | 3 | 1 | 14 | 16 | – |
| 3.125 | 23 | – | – | – | – | 8 | 4 | – |
| 1.562 | 4 | – | – | – | – | 4 | – | – |
| 0.781 | – | – | – | – | – | – | – | – |
| 0.39 | – | – | – | – | – | – | – | – |
| < 0.39 | – | – | – | – | – | – | – | – |

Table II Minimal Inhibitory Concentration ( MIC. ) of Shigella sonnei 14 Strains to 8 Antimicrobial agents

| Concentration of Antimicrobial agents mcg/ml or unit/ml | MIC. to Antimicrobial agents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Garamycin mcg/ml | Colimycin mcg/ml | Terramycin mcg/ml | Chloram-phenicol mcg/ml | Strepto-mycin mcg/ml | Ampicillin mcg/ml | Kanamycin mcg/ml | Sulfadia-zine mcg/ml |
| > 100 | -- | 2 | -- | -- | -- | -- | -- | 14 |
| 100 | -- | 2 | -- | 3 | -- | -- | -- | -- |
| 50 | -- | 4 | 1 | 6 | 3 | -- | -- | -- |
| 25 | -- | 5 | 5 | 1 | 8 | 1 | -- | -- |
| 12.5 | -- | 1 | 2 | 4 | 3 | 1 | -- | -- |
| 6.25 | 4 | -- | 6 | -- | -- | 9 | 10 | -- |
| 3.125 | 10 | -- | -- | -- | -- | 2 | 4 | -- |
| 1.562 | -- | -- | -- | -- | -- | 1 | -- | -- |
| 0.781 | -- | -- | -- | -- | -- | -- | -- | -- |
| 0.39 | -- | -- | -- | -- | -- | -- | -- | -- |
| < 0.39 | -- | -- | -- | -- | -- | -- | -- | -- |

Table III Minimal Inhibitory Concentration ( MIC. ) of Shigella dysenteriae 4 strains to 8 Antimicrobial agents

| Concentration of Antimicrobial agents mcg/ml or unit/ml | MIC. to Antimicrobial agents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Garamycin mcg/ml | Colimycin mcg/ml | Terramycin mcg/ml | Chloram-phenicol mcg/ml | Strepto-mycin meg/ml | Ampicillin mcg/ml | Kanamycin mcg/ml | Sulfadia-zine mcg/ml |
| > 100 | -- | 1 | -- | -- | 1 | -- | -- | 4 |
| 100 | -- | -- | -- | -- | -- | -- | -- | -- |
| 50 | -- | 1 | 4 | -- | -- | -- | -- | -- |
| 25 | -- | 2 | -- | 3 | -- | -- | -- | -- |
| 12.5 | -- | -- | -- | 1 | 3 | 1 | -- | -- |
| 6.25 | 1 | -- | -- | -- | -- | 3 | 3 | -- |
| 3.125 | 3 | -- | -- | -- | -- | -- | 1 | -- |
| 1.562 | -- | -- | -- | -- | -- | -- | -- | -- |
| 0.781 | -- | -- | -- | -- | -- | -- | -- | -- |
| 0.39 | -- | -- | -- | -- | -- | -- | -- | -- |
| < 0.39 | -- | -- | -- | -- | -- | -- | -- | -- |

Table IV. The limit of Minimal Inhibitory Concentrations for "Sensitive", "Intermediate" and "Resistant" strains.

| Antibiotics | Sensitive (mcg/ml) | Intermediate (mcg/ml) | Resistant (mcg/ml) |
|---|---|---|---|
| Streptomycin | 5.0 | 5.0–25 | 25 |
| Kanamycin | 5.0 | 5.0–25 | 25 |
| Gentamycin | 2.5 | 2.5–10 | 10 |
| Chloramphenicol | 5.0 | 5.0–25 | 25 |
| Tetracycline | 2.5 | 2.5–50 | 5.0 |
| Colimycin | 100U/ml | 100–500U/ml | 500 U/ml |
| Ampicillin | 2.5 | 2.5–10 | 10 |

Table V Percentage of sensitive (S), intermediate (I), and resistance (R) to 8 antimicrobial agents of Shigella.

| Antimicrobial agents | | Sh. flexneri | Sh. sennei | Sh. dysenteriae |
|---|---|---|---|---|
| Streptomycin | %S | 0 | 0 | 0 |
| | %I | 12.5 | 71.4 | 75 |
| | %R | 87.5 | 28.6 | 25 |
| Ampicillin | %S | 12.5 | 7.14 | 0 |
| | %I | 43.75 | 75.4 | 75 |
| | %R | 47.75 | 17.46 | 25 |
| Kanamycin | %S | 12.5 | 0 | 25 |
| | %I | 84.38 | 100 | 75 |
| | %R | 3.12 | 0 | 0 |
| Sulfadiazine | %S | 0 | 0 | 0 |
| | %I | 0 | 0 | 0 |
| | %R | 100 | 100 | 100 |
| Garamycin | %S | 12.5 | 0 | 0 |
| | %I | 87.5 | 100 | 100 |
| | %R | 0 | 0 | 0 |
| Colimycin | %S | 68.75 | 83.33 | 75 |
| | %I | 31.25 | 16.66 | 25 |
| | %R | 0 | 0 | 0 |
| Terramycin | %S | 0 | 0 | 0 |
| | %I | 0 | 0 | 0 |
| | %R | 100 | 100 | 100 |
| Chloramphenicol | %S | 3.12 | 0 | 0 |
| | %I | 18.25 | 0 | 75 |
| | %R | 78.63 | 100 | 25 |

## DISCUSSION

Antimicrobial susceptibility is very important in antibiotic therapy (7). Each organism is different in its susceptibility to drugs. There are many methods for determining antibiotic susceptibility such as the disc agar diffusion method, the agar plate dilution method, and the test tube serial dilution method. The test tube serial dilution method seem to be the best with the result explessed in quantitative terms as MIC (Minimal Inhibitory Concentration), which is easy to compared with other antibiotics. However, this method is time consuming, laborious and needs skilled technicians. There are many factors affection the accuracy of the test : for example, medium pH, stability of antibiotics, inoculum, and and incubation time.

From our result, all of the 50 Shigella strains were resistant to sulfadiazine, streptomycin, and terramycin. This may be because these drugs have been used commonly for a long time, and patients usually buy and take them by themselves. So the posibility that the organisms have been exposed to and have developed resistance to these drugs is higher. Haltalin et. al. (8) have shown that only 25 of 52 strains were sensitive to sulfadiazine while 47 of 52 strains were sensitive to ampicillin. The other may have resulted from the small amount of our sample.

Colimycin and kanamycin seem to be the most effective drugs for Shigellosis, because only a few strain of Shigella were resistant to these drugs.

## REFERENCES


1. Davies, J.B.M., Symptomless carriers in home contacts in Sonne dysentery, Brit Med. J., 2 : 191, 1952.
2. Siwasilp, C., Survival of the Enteropathogens in the Various sources of water in Chiang Mai, Personel Communication. Thesis for Master Degree (Unpublished).
3. Suksuvan, M., Role of Fruit and Vegetables in the Transmission of Enteric Infection in Chiang Mai, Personel Communication, Thesis for Master Degree ( Unpublished )
4. Aden, D.P., Reed, N.D., Underdahl N. R., and Mebus, C.A., Transferable drug resistance among Enterobacteriaceae isolated from case of neonatal diarrhoea in calves and piglets, Appl. Microbiol. 18 : 961-963, 1969.
5. Koonkhamlert, C., and Sawyer, W. D., Infectious drug resistance in Enterobacteria, J. Med. Ass. Thailand, 53 : 712-731, 1970.
6. Misuhashi, S., Hashimoto, H., Egawa, R., Tanaka, T. and Nagai, Y., Drug resistance of Enteric bacteria, IX. Distribution of R factors in Gram negative bacteria from clinical sources. J. Bact., 93 : 1242, 1967.
7. Petersdorf, R.G. and Plorde, J.J., The usefulness of in vitro sensitivity tests in antibiotic therapy, Ann. Rev. of Medicine, 14 : 41-56, 1963.
8. Haltalin, K.C., Nelson, J.D., Ring III, R., Sladoje, M., and Hinton, L. V., Double-blind treatment study of Shigellosis comparing ampicillin, sulfadiazine and placebo. J. Pediat., 70 : 970, 1967.

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# ย่อ และ รีวิวเอกสาร

**Improved Colorimetric Method for Assay of Amphetamines in Urine**

Christopher S. Frings, Cecelia Queen, and Lowell B. Foster

Clinical Chemistry vol. 17 No. 10 p: 1016-1919 Oct. 1971.

การตรวจหา amphetamines ในปัสสาวะมีหลายวิธีด้วยกัน เช่น Gas chromatography ซึ่งต้องการอุปกรณ์พิเศษและมีหลาย steps ในการทำจึงเสียเวลามาก ส่วนวิธี fluorometric และ spectrophotometric ส่วนมากขาด sensitivity และ specificity วิธี spectrophotometric เดิมใช้ methyl orange method ซึ่งมี interferences จากยาต่างๆ มาก ผู้เขียนได้เสนอวิธี modified methyl orange สำหรับหา amphetamines ใน urine โดย pre-extraction ของ urine ซึ่ง adjusted ให้มี pH 5.5 ด้วย chloroform เสียก่อนเพื่อ remove drugs ส่วนมากซึ่งจะ interfere ออกแล้วจึงให้ amphetamines รวมกับ methyl arange ได้ chromogen ซึ่ง เมื่อวัดที่ 515 mu จะ follow Beer's law จนความเข้มข้นของ amphetamines ถึง 10 mg/100 ml วิธีนี้เป็นวิธีที่ง่าย, pricise และ sensitive.

พัตราภรณ์ ชมเชิงแพทย์
B.Sc. (Med. Tech.), C (ASCP)

**Glucose Assay Systems : Evaluation of a Colorimetric Hexokinase Procedure**

Walter R. Weight, John C. Rainwater, and Lawrence D. Tolle

CLINICAL CHEMISTRY vol. 17 No. 10 p. 1010-1015, Oct. 1971

การหา glucose ในเลือดโดยวิธี enzymatic มี advantage คือ มี specificity ดี จึงเป็นที่นิยมใช้กันมาก enzyme ตัวแรกที่ใช้สำหรับหา glucose คือ Glucose oxidase product ที่เกิดขึ้น คือ hydrogen peroxide มี biological materials หลายอย่างที่ interfere การหาปริมาณ hydrogen peroxide ฉะนั้นจึงทำให้ specificity ของวิธีนี้ลดลง

ต่อมาได้มี developed วิธี enzymatic ขึ้นใหม่ใช้ enzymes Hexokinase และ Glucose-6-phosphate dehydrogenase วิธีนี้ไม่มี interferences ใดๆ ที่จะทำให้ specificity ลดน้อยลง

เพื่อที่จะ evaluate วิธี Hexokinase procedure ผู้ทดลองจึงได้ทดลองหา glucose ใน sesum ด้วยวิธีนี้ เปรียบเทียบกันอีก 3 วิธีคือ

1. Ferricyanide (ซึ่งใช้ Auto Analyser)
2. O–Toluidine (manual procedure)
3. Neocuproine (ใช้ SMA–12/30)

หลักการของวิธี Hexokinase มีดังนี้ ใช้ Glucostrate Kit ของบริษัท Warner-Lambert ซึ่งประกอบด้วย Buffered enzyme reagent, Color developer และ Diluent–stabilizer raagent. เมื่อ incubate serum กับ buffered serum reagent (ซึ่งประกอบด้วย hexokinase, gulcose–phosphate dehydrogenase ATP, NADP. และ Tris buffer) แล้วให้ทำปฏิกริยากับ Color developer (Phenazine methosulfate) ผลสุดท้ายเติม stabilizer ซึ่งคือ 0.1 N HCI วัดสีที่ได้ที่ 520 mu เทียบกับสีที่เกิดจาก known standard glucose solution

จากการทดลองเปรียบเทียบกันทั้ง 4 วิธี พบว่าวิธี Glucostrate มี precision มากกว่า o–Toluidine, เท่ากับ Ferricyanide (Auto Analyser) และน้อยกว่า Neocuproine (SMA–12/30) และวิธี Glucostrate, o–Toluidine และ Ferricyanide มี correlation กันดี แต่ค่า neocuproine มีค่าต่างจาก 3 วิธีข้างต้น อาจเนื่องจาก 3 วิธีข้างต้นนั้น standardized ด้วย aqueous standard solution แต่ neocuproine standardized ด้วย Hyland reference serum.

พัตราภรณ์ ชมเชิงแพทย์
B.Sc. (Med. Tech.), C (ASCP)

**A Case Study of the Detection and Identification of Anti-Vel within a Family.**

Margaret L. Ailes. The American Jounal Medical Technology. Vol. 37 No. 5

Vel–antigen มีอยู่ในเลือดซึ่งพบได้บ่อยนั้น ได้พบโดย Sussman และ Miller ในปี ๑๙๕๒ สำหรับ Antibody ต่อ Antigen ชนิดนี้ ได้พบในหญิงคนหนึ่ง ซึ่งเกิด severe hemolytic reaction โดยได้ทำ cross–maching ๑๐,๐๐๐ ราย พบว่า serum ของคนไข้นี้เกิด agglutination ทุกรายยกเว้น ๔ ราย และจากการใช้วิธี absorption test จึงทราบว่ามี antibody ชนิดเดียวในคนไข้ซึ่งคือ Anti–Vel นั่นเอง

การศึกษาครั้งนี้ได้ศึกษา ใน คน ไข้ หญิง อายุ ๕๒ ปี ที่มี Antibody ซึ่งได้โดยตรงกับ "public" Antigen Vel โดยที่เลือดของเธอไม่ compatible กับของ donor เลย เนื่องจากมี Antibody ใน plasma ของเธอ เธอเคยตั้งครรภ์อย่างปลอดภัยมาแล้วถึง ๔ ครั้ง แต่ในครรภ์ที่ ๕ นี้มีการผิดปกติขึ้น จึงได้ทำการศึกษาแบบของกรรมพันธุ์ ในครอบครัวปัจจุบันของเธอ ซึ่งมี คน ๖ คน ในครอบครัว โดยใช้ Cells จากเลือดมาทำปฏิกิริยากับ serum ของเธอเอง ซึ่งผลปรากฏว่า น้องสาวของเธอเพียงคนเดียวเท่านั้นที่เป็น Vel–negative (No Vel–antigen) ส่วนลูกชายอีก ๓ คนเป็น Vel–positive แต่ลูกคนหนึ่งพบว่ามี Antigen ที่อ่อน, การขาด Vel–antigen ใน inheritated trait เนื่องจาก Vel–antigen ถูก control โดย recessive gene ซึ่งต้องเป็น gene ที่ homologous ซึ่งมี genotype เป็น Ve Ve และ Vel–positive เป็น $Ve^a$ $Ve^a$ ส่วน Vel–negative จะมี gene เป็น Ve Ve แม้ว่า Vel–negative phenotype จะหายากแต่ก็เคยพบ anti-vel หลายราย ซึ่งจะสะท้อนให้เห็นถึงการให้ Transfusion ในรายรีบด่วน โดยที่อาจมี antibody นี้เกิดขึ้นได้ดังนั้น การทำ antibody นี้จึงจำเป็นการจะหา denor ต้องทำ cross–matching แบบสุ่มโดยจะพบได้ 1 รายในทุกๆ 10,000-25,000 units ของเลือด

สุรภา เดชะ
B.Sc. (Med. Tech.)

**Comparison of Two Commercially Available Media for Detection of Bacteremia**

John A. Washington II
Applied Microbiology, Volume 22, October 1971, No. 4

จากการใช้ media ที่มี Sodium polyanetholesulfornate คือ thiol Broth และ media ที่ไม่มี sodium polyanetholesulfonate คือ tryptic soy broth เปรียบเทียบการเจริญของเชื้อ ในการทำ Blood culture ระหว่างเดือนกรกฎาคม 1968 จนถึงเดือน ธันวาคม 1970 โดยเจาะเลือดด้วยวิธี Venipuncture จากคนไข้ที่มารับการรักษาที่ Mayo Clinic แล้ว inoculated ลงใน Media ที่ 35°C แล้ว sub culture ทุกวันลงใน chocolate blood agar plates แล้ว incubated ที่ 35°C ใน 10% $CO_2$ 48 ชั่วโมง ถ้าไม่พบเชื้อขึ้นก็ sub culture จนครบ 14 วัน

ผลของการ Culture และ isolate พบว่า คนไข้ 171 คน Hemo-culture 3795

ราย พบเชื้อ Actinobacillus และ Pseudomonas แยกได้จาก tryptic soy broth เป็นจำนวนมากกว่า แยกได้จาก thiol broth และในขณะเดียวกัน แยกเชื้อ Streptococcus กับ Corynebacterium spp. ทั้ง aerobic และ anaerobic ได้จาก thiol broth เป็นจำนวนมากกว่าที่แยกได้จาก tryptic soy broth.

ใน positive culture พบจำนวน anaerobic bacteria 11 % และในจำนวนนี้ 20% ผู้ป่วยเป็น Bacteremia และ 78% ของ positive culture พบ พวก Bacteroidaceae ซึ่งเป็นคนไข้ Bacteremia 69% นอกจากนี้พบว่าคนไข้เป็น Polymicrobial bacteremia 11% ซึ่งปรากฏว่ามีมากกว่าที่เคยมีรายงานไว้

ดังนั้นจึงสรุปจากการทดลองไว้ว่า trybtic soy broth เป็น media ที่ดีสำหรับเชื้อ Actinobacillus, Enterobacter และ Pseudomonas ส่วน thiol broth เป็น media ที่ดีสำหรับเชื้อ Proteus และ Streptococcus และใน thiol broth จะมีเชื้อ corynebacterium group ทั้งที่เป็น aerobic และ anaerobic contaminate ได้มากกว่าใน tryptic soy broth สำหรับเชื้ออื่น ๆ พบว่าขึ้นได้คล้ายกันใน media ทั้ง 2 ชนิด.

จิราภรณ์ ดวงบาน

B.Sc. (Med. Tech.)

**Intracranial Abscesses**

by D. Balakrinan and M. Natarajan from I. India Med. Asso. 58:87-90, 1971

จากการศึกษาระยะเวลา 4 ปี คือปี 1963-1967 ที่แผนกศัลยกรรมประสาท โรงพยาบาลเออร์สไกน์ มาดูไร ประเทศอินเดีย มีคนไข้ที่ป่วยด้วย Intracranial Abscesses 45 ราย ได้ศึกษาถึงสาเหตุของโรคนี้พบว่าเกิดจาก

1. หูอักเสบเรื้อรังและลุกลามไปถึงเยื่อหุ้มสมอง

2. เป็นการอักเสบที่เกิดขึ้นจากส่วนต่าง ๆ ของร่างกาย และลุกลามไปยังเยื่อหุ้มสมอง

จากผู้ป่วย 45 ราย ทำการเพาะเลี้ยงเชื้อ 22 ราย มีเชื้อขึ้น 19 ราย ไม่ขึ้น 2 ราย smear พบเชื้อ แต่เพาะไม่ขึ้น 1 ราย ที่เพาะขึ้น พบเชื้อ Staphylococci 4 ราย Staphylococci coagulase negative 1 ราย hemolytic Streptococci 3 ราย Streptococci faecalis 2 ราย non hemolytic Streptococci 2 ราย Proteus vulgaris 3 ราย Aerobic spore bearers 1 ราย coliform bacilli 3 ราย

Pneumococci 1 ราย เชื้อทั้งหมดนี้มีความไวต่อยา streptomycin และ chloramphenicol.

เนตร สุวรรณคฤหาสน์

B.Sc. (Med.Tech.)

**Cary-Blair, a Transport Media for Vibrio Parahemolyticus**

David A, Neumann, M.D., CPTMC, Michael W. Benenson, M.D., CPT MC, Edward Hubster, SSG, USA, and Ngugen Thi Nhu Tuan.

Amer. J. Clin Path. 57 : 33-34, 1972.

ในปี 1952 ชาวญี่ปุ่นพบว่า 30–40 % ของคนไข้ที่เป็น "Summer Diarrher" จะพบ Vibrio parahemolyticus อยู่ด้วย

ในปี 1964 United States Army Medical Research Team (WRAIR) ได้มีการ survey หา organism ตัวนี้ โดยใช้ cary–Blair เป็น Transport Media ซึ่งประกอบด้วย Sodium thioglycollate, 1–5 gm, $Na_2HPO_4$ 1.1 gm, NaCl 5 gm, Bacto agar 5 gm ละลายน้ำ demineriliz ed 991 ml ต้มให้ agar ละลายแล้วแบ่งใส่ขวดที่มีฝาปิดแล้ว autoclave mediaพบว่า transport media ชนิดนี้ไม่มี nutrient ที่จะทำให้ enteric bacteria โดยเฉพาะอย่างยิ่ง Escherichia coli ขึ้นได้ จากการทดลองพบว่า Shigella และ Salmonella จาก rectal swab สามารถขึ้นมาได้ใน media นี้ซึ่ง incubate ที่อุณหภูมิห้อง 30 วัน

อย่างไรก็ตาม Cary–Blair เป็น media ที่คิดว่าดีที่สุด สำหรับเป็น Transport media ของ fecal specimen ซึ่งพบว่า survival time ของ Vibrio parahemolyticus ใน cary–Blair transport media สามารถอยู่ได้ถึง 35 วัน ซึ่งเพียงพอสำหรับการ survey หาเชื้อตัวนี้ในกรณีที่มีการระบาดเกิดขึ้น.

ยุพา สุภาเลิศ

B.Sc. (Med. Tech.)

**Detection of Hemoglobin S Utilizing Sickledex Solubilily, Reduecd Oxygen Tension, and Electrophoresis.**

Judson Moses Ravi, BBA, BS.

American journal of Medical Technology Vol. 38, No. 1 Jan. 1972 P : 7—8

จากการทดลองหา Hemoglobin S เปรียบเทียบทั้ง 3 วิธีคือ Sickledex Solubility, Reduced Oxygen tension และ Electrophoresis พบว่าวิธี oxygen tension ได้ผล sensitive น้อยที่สุด (67%) และอ่านผลยากที่สุด ซึ่งถ้าหากมี poikilo-

cytes อยู่ด้วยโดยวิธีนี้จะให้ผล false positive

สำหรับวิธี Sickledex จะ sensitive มากขึ้น (83%) ข้อดีของวิธีนี้คือการทดลองทำได้ง่ายและไม่ต้องมีเครื่องมือพิเศษอะไร วิธีนี้จะแยก H(S). ออกจาก Hb อื่น ๆ แต่ถ้ามี H(C) และ Hb อื่น ๆ ที่หายาก Multiple myeloma และ hyperproteinemia จะให้ผล false positive นอกจากนี้ false negative อาจเกิดได้ถ้าหากความเข้มข้นของ Hb ต่ำ ดังนั้นเมื่อใช้ anemic blood ควรเอา plasma ออกเพื่อให้ Haematocrit สูงหรือเพิ่มเลือดเข้น 2 เท่า แต่วิธีนี้ไม่สามารถแยกระหว่าง Homozygous HbSS และ Sickle trait (AS) ได้

วิธีที่ดีที่สุดคือ Hemoglobin Electrophoresis ซึ่งสามารถหา Sickle trait ที่ไม่สามารถหาได้โดยวิธี screening method นอกจากนี้ยังแยกระหว่าง HbSS และ AS, เราสามารถหาปริมาณของ HbS ได้โดยเอา acetate strip มาอ่านใน densitometer.

ยุพา สุภาเลิศ
B.Sc. (Med. Tech.)

**Enzymatic Diagnosis of Megaloblastic Anemia**

R. M. Winston, M. D, F.C. Warburtion, M.D., and A Stott, M.D.

Brit. J. Haematology, 19:587-592, 1970.

ได้มีการทดลองเปรียบเทียบ isoenzyme Lactate Dehydrogenase (LDH) ในเม็ดเลือดของคนไข้ ซึ่งเป็น Megaloblastic Anemia ซึ่งอาจมีสาเหตุมาจากการขาดวิตามิน $B_{12}$, folate deficiency หรือมีอาการรวมทั้งการขาดเหล็กและ hemolytic anemia กับคนปกติพบว่าคนไข้ที่เป็น Megaloblastic anemia จะมี $LDH_1$–$LDH_2$ isoenzyme ตรงกันข้ามกับคนปกติ

พบว่าในเม็ดเลือดแดงของคนปกติและของคนโลหิตจางทั่วไป ยกเว้น Megaloblastic anemia $LDH_2$ จะมี activity สูงกว่า activity ของ $LDH_1$ แต่ใน Megaloblastic anemia ทุกราย พบว่า activity ของ $LDH_1$ จะสูงกว่า activity ของ $LDH_1$ จะสูงกว่า activity ของ $LDH_2$ ซึ่งสามารถพบได้ใน serum ของคน Megaloblastic anemia เหมือนกัน

จากการใช้ Simple chloroform inhibition test พบว่า สามารถหาได้ว่า LDH จะสูงมากกว่า 900 unit ได้พอ ๆ กับที่ใช้หา

ในเม็ดเลือดแดง ซึ่งจะให้ผลตรงข้ามกับคนปกติ

ยุพา สุภาเลิศ

B.Sc. (Med. Tech.)

**Normal values for mean corpuscular volume as determined by the model S coultor counter**

Silver H - and Frankel S. - Dept. path., Jew - Hosp. St. Louis Mo. Amer. J. Clin. Path. 1971 55/4 (438-441)

เป็นที่ยอมรับกันมานานแล้วว่า ค่าปกติของ mean corpuscular volume (MCV) อยู่ระหว่าง 82–92 cuu. แต่ในสหรัฐอเมริกาพบว่า ค่านี้ต่ำเกินไป โดยทำการทดลองจาก blood specimens 200 ราย หาค่า RBC. count ด้วยเครื่องนับเม็ดเลือด model S coulter counter

หลังจากคำนวณหาค่า MCV แล้ว ปรากฏว่าได้ MCV ถัวเฉลี่ย 91.4 cuu คิดเป็น mormal rang= 84–99 cuu.

ชลอ บัวน้ำจืด

B.Sc. (Med. Tech.)

**Erroneous values on the model S coulter counter due to high titer cold autoagglutinins Hattersley**

P.G., Gerard P.W., Caggiano V. and Nash D.R. - Dept. Med., Sch. Med., Univs. California, Davis, Calif. - Amer. J. Clin. Path. 1971 55/4 (442-446)

Blood specimen ที่มี cold aggluti-nins สูงๆ เมื่อนำไปทำ RBC. count ด้วยเครื่อง Model S coulter counter ใน lab ที่อุณหภูมิต่ำๆ จะได้ค่า RBC. count ต่ำ เนื่องจากการเกิด microagglutinates ในรายที่เป็นเช่นนั้น การคำนวณหา MCV, MCH จะได้ค่าสูง ด้วยเหตุนี้จึงมีผู้แนะนำว่าการใช้เครื่อง Model S coulter counter จะต้องควบคุมอุณหภูมิให้พอเหมาะด้วย.

ชลอ บัวน้ำจืด

B.Sc. (Med. Tech.)

วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY
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Vol. 5 No. 2

# ข่าว

## พระราชทานเครื่องราชอิสริยาภรณ์

เทคนิคการแพทย์ ผู้ได้รับพระราชทานเครื่องราชอิสริยาภรณ์ ในโอกาสเฉลิมพระชนม์พรรษา วันที่ ๕ ธันวาคม ๒๕๑๔ มีดังนี้

จัตุรถาภรณ์ช้างเผือก นายสวัสดิ์ ลังการ์สิทธิ์

จัตุรถาภรณ์มงกุฎไทย นายไพโรจน์ สภาวจิตร

นางเพ็ญศรี วรรณฤมล

## กลับจากต่างประเทศ

– ดร. สนิท มกรแก้วเกยูร เทคนิคการแพทย์ รุ่นที่ ๘ ได้เดินทางกลับจาก ประเทศสหรัฐอเมริกา มาถึงประเทศไทยเมื่อวันที่ ๒๕ กุมภาพันธ์ ๒๕๑๕ และขณะนี้ได้กลับเข้ารับราชการที่ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ตามเดิมเรียบร้อยแล้ว

ดร. สนิท มกรแก้วเกยูร ได้รับปริญญาวิทยาศาสตร์บัณฑิต(เทคนิคการแพทย์) วิทยาศาสตร์มหาบัณฑิต (จุลชีววิทยา) แล้ว ได้เดินทางศึกษาต่อขั้นปริญญาเอก ณ ประเทศสหรัฐอเมริกา จนสำเร็จการศึกษา และได้รับปริญญาเอกสาขา Bacterial Immunity เมื่อวันที่ ๑๑ มกราคม ๒๕๑๕

– ดร. เวคิน นพนิตย์ เทคนิคการแพทย์ นครหลวงกรุงเทพฯ–ธนบุรี รุ่นที่ ๗ ได้เดินทางกลับจากสหรัฐฯ เพื่อกลับมาเยี่ยมบ้านชั่วคราวเมื่อเดือนมกราคม ๒๕๑๕ และได้กลับเข้าทำงานต่อ ที่ประเทศสหรัฐอเมริกาในตำแหน่ง Director, Training Program in Electron Microscopy, School of medicin, University of north carolina, chapel Hill, N.C., U.S.A. ในราว ๆ เดือนมิถุนายน พ.ศ. ๒๕๑๕

ดร. เวคิน นพนิตย์ จบการศึกษา ที่คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล แล้วไปทำปริญญาตรี, โท, เอก ที่ University of North carolina, U.S.A. โดยได้ปริญญาเอกสาขา Electron Microscopy

## ทุนองค์การอนามัยโลก

องค์การอนามัยโลก ได้จัดสรรทุนดูงานและศึกษาต่อให้แก่ภาควิชาเทคนิคการแพทย์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ตามที่อาจารย์ชั้นพิเศษ นายแพทย์ชัยโรจน์ แสงอุดม ได้ติดต่อขอไปและได้รับทุนดังกล่าวมาดังนี้

ปี ๑๙๗๔

Medical Microbiology (24 months)

๑ ทุน

Clinical Chemistry (24 months) ๑ ทุน

Clinical Microscopy (24 months) ๑ ทุน

และให้เงินสำหรับซื้อหนังสือเข้าห้องสมุด ๓๐๐๐ เหรียญสหรัฐฯ

ปี ๑๙๗๕

Blood Bank (12 months) ๑ ทุน

และให้เงินสำหรับซื้อหนังสือเข้าห้องสมุด ๒๐๐๐ เหรียญสหรัฐฯ

ปี ๑๙๗๖

Serology (12 months) ๑ ทุน

และให้เงินสำหรับซื้อหนังสือเข้าห้องสมุด ๒๐๐๐ เหรียญสหรัฐฯ

**แต่งตั้งอาจารย์ฝ่ายนักศึกษา**

คำสั่งคณะแพทยศาสตร์ ที่ ๒๖/๒๕๑๕ ลงวันที่ ๑๙ เม.ย. ๑๕ ได้แต่งตั้งอาจารย์ฝ่ายนักศึกษา เพื่อคอยให้การดูแล และให้คำแนะนำแก่นักศึกษา ในเรื่องกิจกรรมนอกหลักสูตร ตลอดจนความเป็นอยู่ต่าง ๆ ซึ่งสาขาวิชาเทคนิคการแพทย์ อาจารย์ฝ่ายนักศึกษาได้แก่ อาจารย์เนตร สุวรรณฤหาสน์

**แต่งตั้งอาจารย์ที่ปรึกษาของนักศึกษาเทคนิคการแพทย์**

คำสั่งคณะแพทยศาสตร์ ที่ ๒๘/๒๕๑๕ ลงวันที่ ๒๔ เมษายน ๒๕๑๕ แต่งตั้งอาจารย์ที่ปรึกษาเทคนิคการแพทย์ดังนี้

ชั้นปีที่ ๔

อาจารย์สวัสดิ์ ลังกาโสิทธิ์

อาจารย์สุชาติ ศิริทูล

ชั้นปีที่ ๓

อาจารย์สุวีระ รณวิชญ

อาจารย์พัตราภรณ์ ชมเชิงแพทย์

อาจารย์เพ็ญศรี วรรณฤมล

ชั้นปีที่ ๒

อาจารย์ชลอ บัวน้ำจืด

อาจารย์ผาสุก ชมเชิงแพทย์

อาจารย์จันจรี ศิริวิทยากร

ชั้นปีที่ ๑

อาจารย์วารุณี คุณาชีวะ

อาจารย์อัญชลี กิตติชนม์ธวัช

อาจารย์มาลินี เชาวพันธ์

นักศึกษาที่เข้าศึกษาในมหาวิทยาลัยก่อนปี ๒๕๑๕

อาจารย์ไพโรจน์ สภาวจิตร

โดยอาจารย์ที่ปรึกษาจะมีหน้าที่

– แนะนำและให้คำปรึกษาในการเลือกวิชาที่จะลงทะเบียนเรียนในแต่ละภาคการศึกษา

– รับผิดชอบในการเก็บประวัติการศึกษาของนักศึกษา ในความดูแลตั้งแต่ต้นจนจบการศึกษา

– จัดทำใบเสนอขออนุมัติ ปริญญา (มช. ๒๒) เมื่อนักศึกษาได้ศึกษาครบหลักสูตรแล้ว เพื่อเสนอให้คณบดีพิจารณา

– รับปรึกษาปัญหาทั่วไปแก่นักศึกษา

**ข้าราชการลาออก**

อาจารย์จันทรา ณ เชียงใหม่ อาจารย์ตรี ภาควิชาเทคนิคการแพทย์ คณะแพทยศาสตร์ ซึ่งได้ลาไปศึกษาต่อที่สหรัฐอเมริกา เมื่อต้นปี พ.ศ. ๒๕๑๓ และขณะนี้ยังคงอยู่ ณ ประเทศนั้น ได้ขอลาออกจากราชการ ซึ่งทางมหาวิทยาลัยเชียงใหม่ ได้อนุมัติให้ลาออกแล้ว ตั้งแต่วันที่ ๑ มิถุนายน พ.ศ. ๒๕๑๕ เป็นต้นไป.

**สมรส**

เทคนิคการแพทย์ ที่เข้าสู่พิธีมงคลสมรสในระยะนี้คือ

– รต. สุวรรณ ละออง (เทคนิคการแพทย์ กรุงเทพฯ รุ่นที่ ๑๒) แห่ง รพ.อนันทมหิดล สมรสกับคุณเพ็ญจันทร์ ณ อ.โคกสำโรง ลพบุรี เมื่อวันที่ ๒๕ กุมภาพันธ์ พ.ศ. ๒๕๑๕

– คุณสุรภา กันธากร (เทคนิคการแพทย์ เชียงใหม่ รุ่นที่ ๓) แห่ง ร.พ. นครเชียงใหม่ สมรสกับ รท. ฒะนัฐ เดชะ แห่งค่ายกาวิละ ณ อ. เมือง เชียงใหม่ เมื่อวันที่ ๑๑ มีนาคม ๒๕๑๕

– คุณสุพรรณ บริสุทธิ์ (เทคนิคการแพทย์ กรุงเทพฯ รุ่นที่ ๑๐) แห่งบางกอกเนอร์สชิง โฮม สมรสกับ คุณอรุณี โชตรนพัสดุ์ (รังสีเทคนิครุ่น ๑) แห่งสถาบันมะเร็งแห่งชาติ ณ นครหลวงกรุงเทพ–ธนบุรี เมื่อวันที่ ๑๕ เมษายน ๒๕๑๕

– คุณณรงค์ อรุณาทิตย์ (เทคนิคการแพทย์ กรุงเทพฯ รุ่น ๙) แห่ง รพ.ประสาทพญาไท สมรสกับ คุณพรทิพย์ ณ นครหลวงกรุงเทพฯ–ธนบุรี เมื่อวันที่ ๗ พฤษภาคม ๒๕๑๕

**อุปสมบท**

ได้ทราบข่าวที่น่ายินดีว่า อาจารย์ประยูร อินบริบูรณ์ เทคนิคการแพทย์กรุงเทพฯ รุ่นที่ ๑๐ อาจารย์โท ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ และประจำกอง บรรณาธิการ วารสารเทคนิคการแพทย์เชียงใหม่ จะอุปสมบทในวันที่ ๓ มิถุนายน พ.ศ. ๒๕๑๕ ณ วัดนากระรอก อ. พนัสนิคม ชลบุรี.

อีกท่านหนึ่ง คือ อาจารย์สุวีระ รณวิชญ์ เทคนิคการแพทย์กรุงเทพฯ รุ่นที่ ๙ อาจารย์โท

ภาควิชาเทคนิคการแพทย์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ จะอุปสมบทในราว ๆ กลางเดือนมิถุนายน ๒๕๑๕ ณ จังหวัดสุราษฎร์ธานี

**ข้าราชการลาศึกษาต่อ**

อาจารย์สุพัตรา พีราคม เทคนิคการแพทย์ เชียงใหม่ รุ่นแรก อาจารย์โท ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ได้ลาไปศึกษาต่อชั้นปริญญาโท ทางจุลชีววิทยา ณ มหาวิทยาลัยมหิดล นครหลวงกรุงเทพฯ – ธนบุรี มีกำหนด ๒ ปี ตั้งแต่เดือนมิถุนายน ๒๕๑๕ เป็นต้นไป และทางบัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล ได้ส่งตัวให้มาทำปริญญาโทต่อ ที่ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

อาจารย์ดำรง พิณตานนท์ เทคนิคการแพทย์เชียงใหม่ รุ่นแรก อาจารย์โท ภาควิชาเทคนิคการแพทย์ ได้ลาไปศึกษาต่อชั้นปริญญาโท ทางพยาธิวิทยา ณ มหาวิทยาลัยมหิดล นครหลวงกรุงเทพฯ–ธนบุรี มีกำหนด ๒ ปี นับตั้งแต่เดือนมิถุนายน ๒๕๑๕ เป็นต้นไป.