



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

Junjaree Siriwittayakorn, B.Sc. (Med. Tech.) \*

Boonlong Sivasomboon, M.D. \*\*

### 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.  $\pm 21$ ) and in 11 woman was 55 mcg/100 ml serum (S.D.  $\pm 16$ ). The average serum carotene was 85 mcg/100 ml serum (S.D.  $\pm 44$ ) in man and 147 mcg/100 ml serum (S.D.  $\pm 30$ ) 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 more susceptible to invasion of infection organisms. Keratinization of the vascular tis-

\* Section of Clinical Chemistry, school of Medical Technology, Faculty of Medicine, Chiang Mai University.

\*\* Department of Medicine, Faculty of Medicine, Chiang Mai University.

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 far 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 CALCULATIONS:

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 evaporated to dryness. Continue

the reaction with TFA reagent. The optical densities are read at 660 m $\mu$  and the standard curve is 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 m $\mu$  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 dividing 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  $\times$  200
2. mcg vitamin A/100 ml serum  
= (mcg/tube of vitamin A read from curve - (mcg/ml of carotene in the same sample  $\times$  F))  $\times$  200

## RESULTS

The samples were analyzed in duplicate. The average serum carotene and vitamin A levels in 109 healthy Thai males were  $85 \pm 44$  mcg % and  $63 \pm 21$

mcg % respectively. The average serum carotene and vitamin A levels in 11 healthy Thai females were  $147 \pm 30$  mcg % and  $55 \pm 16$  mcg % respectively. From these results, Thai males have a normal serum vitamin A levels higher than Thai females 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 very 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 different countries varies a great deal and is usually higher in men than in women (9). On the contrary, women usually have a serum vitamin A and carotenoids level higher than boys (11). In the undeveloped countries, normal serum vitamin A levels'

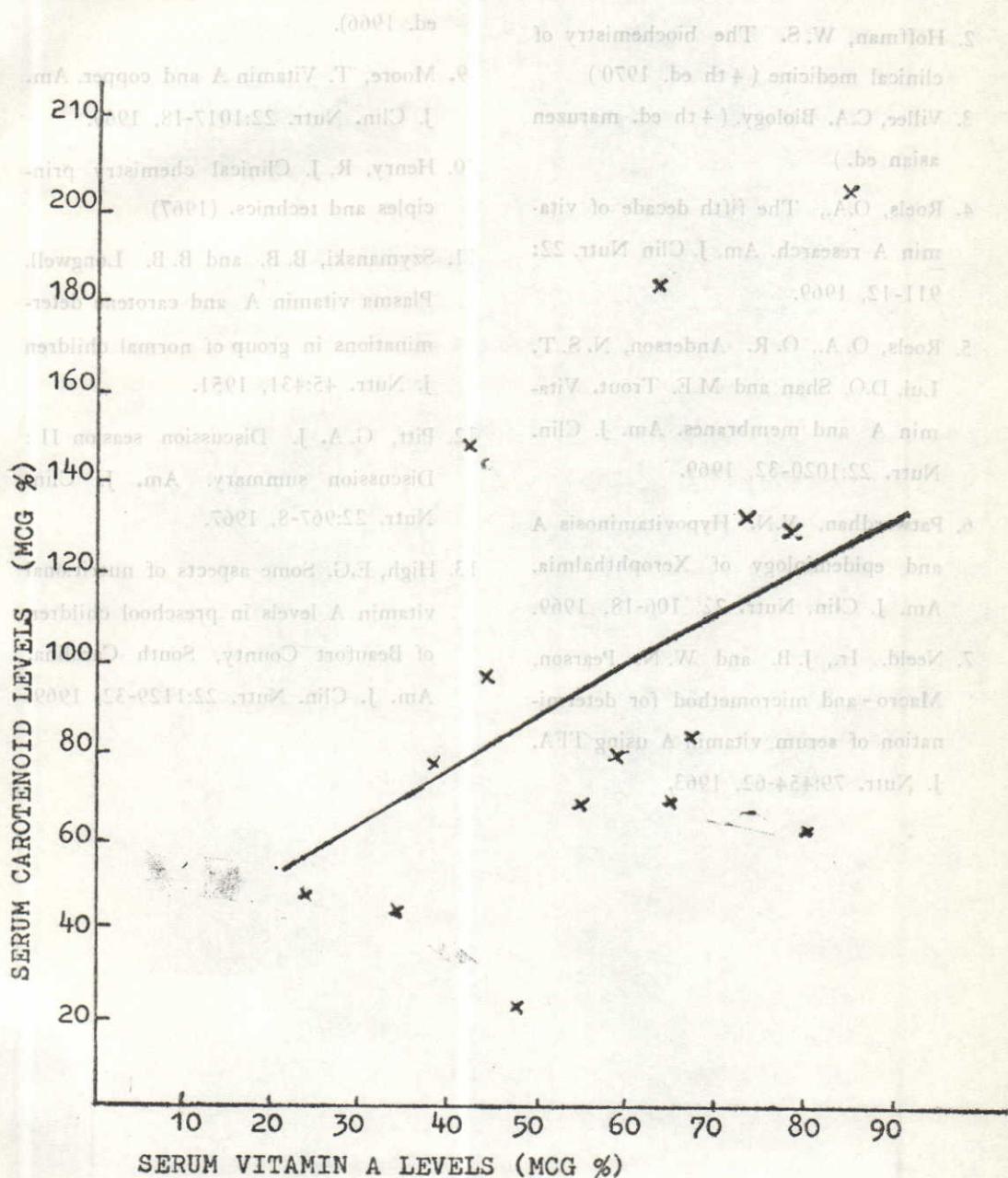
are lower than the others, and have a high percentages of people who have levels below 20 mcg% i.e. in the low and deficient range. From the INND surveys, 18% of Thai men and 25% of Thai women had vitamin A levels below 20 mcg%. May be the difference between these mainly depends on the nutritional conditions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

There are many reports showing the relationship between serum carotenoids and vitamin A levels. Some failed to find a relationship and some found a good relationship. Dr. Moodman (12) gave his advice that the relationship should depend

on nutrition. 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 relationship. We agree with the reason professor Edward G. High (13) gave before that a bad relationship was due to a great interference of non-pro-vitamin A carotenoids in the serum.

## References

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 techniques. (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 session 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.