# CHAPTER IV

## DISCUSSION

In the present study, Vitamin $B_{12}$ content was determined in some Thai foods by a method of Newmark et al (1973) which was modified by Grossowicz (1976). It is now an acceptant method which is generally replaced the more previously microbiological assay. The reproducibility of Vitamin $B_{12}$ concentration in a sample in our hand was found to have a mean value $\pm$ one standard deviation of 1184 $\pm$ 40 pg/ml with a mean standard error of 9.05 pg/ml. This indicated that the reproducibility of this method was quite satisfactory especially in view of the minute quantities involved.

Skeggs (1967) commented that the validity of the published assay procedures of Vitamin $B_{12}$ was based upon the recovery of cyanocobalamin added to the sample during extraction or preparation could no longer be accepted as an adequate criterion in retrospect. Many of the discrepancies in assay values of Vitamin $B_{12}$ resulted from the failure to extract the total cobalamin present in the sample and convert it to cyanocobalamin (the standard form) against which it was to be compared. An attempt had been made to stress the importance of considering sample treatment in terms of the less stable coenzyme $B_{12}$ and hydroxocobalamin components that might be present in the sample,

rather than in terms of the relatively stable cyanocobalamin, when concerning with the analysis of total Vitamin $B_{12}$ activity.

Cyanocobalamin labelled with Cobalt-57 was prefered in the present study because it had a conveniently long half life, no particulate radiation and less $\gamma$ - radiation, which provided greater safety in handling than $^{60}Co$. It has also a higher detection sensitivity in sodium iodide - scintillation counter and lower self - irradiation exposures to give greater stability to the radioactive Vitamin.

Results of Vitamin $B_{12}$ content in various foods in the present study as well as results from other reports are shown in Table 20 and Table 21. Our results were relatively lower than those values determined by the microbiological method. This was possibly due to the fact that the early assay method of Vitamin $B_{12}$ content in foodstuff were done with Lactobacillus leichmannii which is known to respond also to thymidine and other deoxyribosides. Furthermore corrections were some times applied by a second assay after treatment with hot alkali to destroy the Vitamin $B_{12}$ and not the nucleoside and the correction was often much larger than the difference figure ascribed to Vitamin $B_{12}$.

Vitamin $B_{12}$ content in the beef spleen did not show a great variation. Result in the present study showed a mean value of 2.41 µg/100 gm while it was found to be 2.7 - 5.2 µg/100 gm in another study (Scheid et al, 1954). In beef heart, the Vitamin $B_{12}$ content

was much higher, i.e. ranged from 3.0 to 17.0 µg/100 gm (Thompson et al, 1950; Tostaldi, 1950; Scheid et al, 1954). The highest Vitamin $B_{12}$ content was found in the beef liver which was reported to be as high as 152 µg/100 gm (Guttamann & Vandenhenvel, 1952). The greater variation of Vitamin $B_{12}$ content was found in the pork liver with the range of 2.3 to 51.2 µg per 100 gm with a mean value of 36.19 µg per 100 gm in the present study. Tappan et al (1950) reported the Vitamin $B_{12}$ content in pork spleen 7.0 µg per 100 gm and 4.0 µg per 100 gm was reported by Scheid et al (1954). Since the liver is the major site for storage of Vitamin $B_{12}$ the content of Vitamin $B_{12}$ in liver is therefore higher than that of the other organ.

In egg yolk, the Vitamin $B_{12}$ contents were nearly identical. Hemindra et al (1971) reported the higher content of Vitamin $B_{12}$ in the hen egg yolk (2.4 µg/egg yolk) than that of duck egg yolk (1.4 µg/egg yolk) while results in the present study showed almost the same value of Vitamin $B_{12}$ in both hen egg yolk (1.01 µg/100 gm egg yolk) and duck egg yolk (1.42 µg/egg yolk). The difference of Vitamin $B_{12}$ content in egg yolk may be due to the variation of the egg.

The Vitamin $B_{12}$ content in fresh cow's milk showed the values of 0.32 to 0.66 µg per 100 ml which was identical to a value of 0.52 µg per 100 ml reported in the present study. Vitamin $B_{12}$ content in human milk had lower value than that of cow's milk. Hemindra et al

(1971) showed the Vitamin $B_{12}$ content in human milk 0.05 µg per 100 ml compared to 0.03 µg per 100 ml in the present study. Gruyer cheese in the present study contained lower Vitamin $B_{12}$ (0.45 µg per 100 gm) than that (1.60 µg per 100 gm) reported by Lichtenstein et al (1961). Vitamin $B_{12}$ content in local cheese Chiengmai was found to be 0.13 µg per 100 gm. Lichtenstein et al (1961) reported the Vitamin $B_{12}$ content in scallop, 1.14 µg per 100 gm which was comparable to our study (1.05 µg per 100 gm). Droop (1955) reported that sea water from the open sea contained 0.1 to 1.0 µg of Vitamin $B_{12}$ per litre and in shore water 5 to 10 µg per litre which was sufficient for the requirement of marine algal flagellates and diatoms. Thus the considerable amount of Vitamin $B_{12}$ was found in the sea foods. In the present study, sea fishes contained higher Vitamin $B_{12}$ (1.40 $\pm$ 0.56 µg per 100 gm) than that of fresh water fishes (1.10 $\pm$ 0.92 µg per 100 gm).

Fermented foods showed a considerable amount of Vitamin $B_{12}$. Fish sauce, one of traditional food used extensively in Thailand, contained a relatively high amount of Vitamin $B_{12}$, i.e. ranged from 0.04 µg to 1.56 µg per 100 ml (Mean $\pm$ one S.D. 0.675 $\pm$ 0.525). The highest concentration of Vitamin $B_{12}$ was found in the high price sample of fish sauce, which was in accordance with result reported by Areekul et al (1975).

Cereals and cereals products contained no Vitamin $B_{12}$. Fruits and vegetables were also found to contain no Vitamin $B_{12}$ except, shallot and garlic which are tubers. Finding of the Vitamin $B_{12}$ content in some fruits and vegetables were possibly due to Vitamin $B_{12}$ contamination from the soil since it has been found that determinations by refined methods on clean samples revealed only negligible Vitamin $B_{12}$ levels in most plant materials. These findings confirmed the fact that Vitamin $B_{12}$ deficiency had been found in several species of animals and in some human subjects on exclusively vegetable diets (Vegans). Groundnuts presented a special case. Ford and Holdsworth found no Vitamin $B_{12}$ in freshly harvested groundnuts but after they had been allowed to stand in heaps (as often occurs) then the Vitamin was present, arising presumably through bacterial fermentation. The present study also found no Vitamin $B_{12}$ in groundnuts.

Table 19 Vitamin $B_{12}$ Content in Various Foods.

| Foods | Vitamin $B_{12}$ µg / 100 gm | References | |
|---|---|---|---|
| Beef spleen | 2.7 - 5.2 | Scheid et al | (1954) |
| Beef spleen | 2.41 | Present study | |
| Beef heart | 17.0 | Tostaldi | (1950) |
| Beef heart | 8.8-10.6 | Scheid et al | (1954) |
| Beef heart | 3.0-14.0 | Thompson et al | (1950) |
| Beef heart | 2.06 | Present study | |
| Beef liver | 105 | Tostaldi | (1950) |
| Beef liver | 120 | Tappan et al | (1950) |
| Beef liver | 20-26 | Shenoy and Ramasarma | (1954) |
| Beef liver | 152 | Guttamann and Vandenhenvel | (1952) |
| Beef liver | 31-99 | Scheid and Schweigert | (1952) |
| Beef liver | 40.95 | Present study | |
| Pork liver | 2.3 | Guttamann and Vandenhenvel | (1952) |
| Pork liver | 19-27 | Scheid et al | (1954) |
| Pork liver | 36.19 | Present study | |
| Pork spleen | 7.0 | Tappan, et al | (1950) |
| Pork spleen | 4.0 | Scheid, et al | (1954) |
| Pork spleen | 2.75 | Present study | (1954) |

Table 19 Vitamin $B_{12}$ Content in Various Foods

| Foods | Vitamin $B_{12}$ µg / 100 gm | References |
|---|---|---|
| Egg Yolk | 0.20-1.56 | Yacowitz, et al (1952) |
| Hen Yolk | 1.01 | Present study |
| Duck Yolk | 1.42 | Present study |
| Fresh Cow's milk | 0.66 | Halick et al (1953) |
| Fresh Cow's milk | 0.32 | ST-Pierre et al (1963) |
| Fresh Cow's milk | 0.48 | Banerjee and Challerjea (1963) |
| Fresh Cow's milk | 0.52 | Present study |
| Gruyer cheese | 1.60 | Lichtenstein et al (1961) |
| Gruyer cheese | 0.45 | Present study |
| Scallop | 1.14 | Lichtenstein et al (1961) |
| Scallop | 1.05 | Present study |

Table 20 Vitamin $B_{12}$ Content in Some Thai Foods.

| Thai Foods | | Radioisotope Method Vitamin $B_{12}$ ug/100 gm | |
|---|---|---|---|
| | | Hemindra et al (1971) | Present study (1977) |
| Meat, | Beef | 6.4 | 5.17 |
| | Beef liver | 113.0 | 40.95 |
| | Beef kidney | - | 12.87 |
| | Beef heart | - | 2.06 |
| | Pork | 4.5 | 5.24 |
| | Pork liver | 51.2 | 36.19 |
| | Chicken | - | 2.06 |
| | Chicken liver | 6.0 | 14.28 |
| | Duck (cooked) | 0.68 | 0.94 |
| Egg, | Chicken (whole egg) | 2.4 | 1.013 |
| | Duck (whole egg) | 1.4 | 1.420 |
| | Turtle (Jaramed) | 4.2 | 0.190 |
| | Turtle | 0.04 | 0.720 |
| | Quail's egg | 0.15 | 0.266 |
| Salted Egg (Whole egg) | | - | 1.00 |

Table 20 (Cont.) Vitamin $B_{12}$ Content in Some Thai Foods.

| Thai Foods | Radioisotope Method Vitamin $B_{12}$ ug/100 gm | |
|---|---|---|
| | Hemindra et al (1971) | Present study (1977) |
| Shellfish, Sea fish & Fresh water fish | | |
| Shrimp | 2.0 | 2.36 |
| Crab | 4.5 | 2.29 |
| Scallop | - | 1.05 |
| Sea mussel | - | 5.70 |
| Sea mussel (dried) | - | 11.49 |
| Horse mussel | 26.2 | 5.06 |
| Ark shell | - | 4.89 |
| Striped mackere | - | 1.23 |
| Salted Striped mackere | - | 2.15 |
| Cooked Striped mackere | - | 1.83 |
| Rakegilled mackerel | - | 2.34 |
| Red snapper | - | 1.02 |
| Squid | - | 0.97 |
| Sipat Siam | - | 1.04 |
| Sipat Siam (dried) | - | 1.86 |
| Climbing perch | - | 1.02 |

Table 20 (Cont.) Vitamin $B_{12}$ Content in Some Thai Foods.

| Thai Foods | Radioisotope Method Vitamin $B_{12}$ ug/100 gm | |
|---|---|---|
| | Hemindra et al (1971) | Present study (1977) |
| Fresh water fish | | |
| Pla nil | - | 1.02 |
| Pla mae sadang | - | 3.40 |
| Fermented Food | | |
| Paste (Ka Pi) | 2.0 | 1.466 (0.48 - 3.16) |
| Fish sauce | 0.88, 1.0, 3.5 | 0.678 (0.04 - 1.56) |
| Fermented fish | 7.0 | 1.208 |
| Fermented small fish | - | 0.766 |
| Fermented internal organ fish | - | 0.664 |
| Fermented crab | 3.1 | 0.802 |
| Fermented ark shell | - | 3.393 |
| Fermented Oyster | - | 27.245 |
| Fermented Soy bean | 0.27 | 0.822 |
| Nam Dudu | - | 16.543 |

Table 20 (Cont.) Vitamin $B_{12}$ Content in Some Thai Foods.

| Thai Foods | Radioisotope Method Vitamin $B_{12}$ ug/100 gm | |
|---|---|---|
| | Hemindra et al (1971) | Present study (1977) |
| Milk & Milk products | | |
| Human milk | 0.521 | 0.032(0.010-0.062) |
| Fresh Cow's milk | - | 0.519(0.298-0.776) |
| Powder milk | - | 0.442(0.02-1.25) |
| Cheese | 1.33 | - |
| Gruyer cheese | - | 0.35 |
| Chiengmai cheese | - | 0.13 |
| Butter (Australia) | 0.55 | 0.08 |
| Fruits,Vegetables,Rice,Oils | | |
| Morning Glory | 0.006 | 0 |
| Garlic | 0 | 0.02 |
| Chinese radish | - | 0 |
| Chilli | 0 | 0 |
| Glutinous rice | 0.12 | 0 |
| Rice | 0.2 | 0 |
| Soya bean | 0.8 | 0 |

Table 20 (Cont.) Vitamin $B_{12}$ Content in Some Thai Foods.

| Thai Foods | Radioisotope Method Vitamin $B_{12}$ ug/100 gm | |
|---|---|---|
| | Hemindra et al (1971) | Present Study (1977) |
| Fruits, Vegetable, Rice, Oils | | |
| Groundnut | 0 | 0 |
| Mung bean | - | 0 |
| Mungbean sprout | - | 0 |
| Soybean sprout | - | 0 |
| Soy bean curd | 0.86 | 0.09 |
| Soya bean sauce (Tao Cheo) | 0.27 | 0.16 |
| Lard | 0.02 | 0 |
| Vegetable oil | 0.02 | 0 |
| Shell oil | - | 0.04 |