CHAPTER IV

DISCUSSION AND CONCLUSION

Discussion

There are two problems which will be considered in the discussion of the result of this work. They are the problems involved in the method of determination of vitamin B_{12} concentrations in various samples and the interpretation of these values.

The assay of vitamin B_{12} in serum, tissues and foodstuffs can be carried out by microbiological assay or by isotope dilution techniques. The development and simplification of isotope dilution methods for the assay of vitamin B_{12} recently have been such that these methods are likely, to replace microbiological assay methods.

Herbert et al (1960) demonstrated that vitamin B_{12} and IF were stiochiometrically related, and suggested applying this relationship to a radioisotope dilution assay for vitamin B_{12} in biologic fluid. Rothenberg (1961, 1963) and Barakat and Ekins (1961) used IF concentrate to bind B_{12}, followed by protein precipitation to separate free from bound B_{12}. Barakat and Ekins (1961) used serum to bind B_{12}, followed by dialysis to separate free from bound B_{12}. Subsequently, Grosswicz et al (1962) reported an assay using serum to bind B_{12} and

unaware of the coating effect of serum proteins, used charcoal to separate free from bound B_{12}. Frenkel et al (1966) used DEAE cellulose for uptake of unbound vitamin B_{12}. Various procedure were used to provent B_{12} loss due to protein precipitation.

These methods showed that radioisotope dilution could be applied to assay of serum B_{12} level. The present procedure (Lau et al., 1965) avoids the difficulties of previous method by the use of : (1) strong hydrochloric acid (0.25 N) and heat to free the bound B_{12} in serum without protein coagulation (2) intrinsic factor concentrate to bind the released B_{12}, since it bind B_{12} at low pH whereas serum cannot (Herbert et al.,1960, Rothenberg, 1961). (3) coated charcoal to provide effective and rapid separation of free from bound B_{12}, (4) a control obviated the need for a calibration curve.

Bioassay procedures cannot be used easily with bacterially contaminated or turbid sera and such procedures also give false low results if the serum contains a high level of many antibiotic and other noxious drugs. Charcoal assay does not have these advantages. The presence of autoantibodies to intrinsic factor in the test serum does not interfere with charcoal assay, because these antibodies are destroyed when the serum is heated in the boiling water bath.

The haemoglobin coated charcoal appears to be economical, less complicated and the material is indeed more readily

available. There are, however, a number of factors that should be considered. These include the temperature and the length of incubation. It was found that although more than half of the total binding of B_{12} by intrinsic factor concentrates is reached within 15 seconds of incubation at room temperature, 30 minutes should be allowed for nearly complete saturation of the binding capacity. The quantity of intrinsic factor concentrate to use is that amount which will bind nearly 80 per cent of 500 picograms of B_{12}. If the amount bound is greater than 90 per cent degree of saturation is uncertain. The only requirements to make such assay feasible are: (1) the agent must be adsorbable by charcoal when in the free form, (2) a binder for the agent must exist whose molecular weight and configuration is sufficiently different from that of the agent so that only the free agent attached to its binder will be adsorbed by appropriately coated charcoal, (3) the agent to be assayed must be available in a radioactive or otherwise labeled form to serve as a marker. Recently, ^{57}cobalt-vitamin B_{12} can be obtained with very high specific activity (microcurie per microgram) and its low gamma photon energy 0.122 Mev. and the half-life of 270 days makes it very convenient for <u>in vitro</u> studies.

Vitamin B_{12} concentrations in samples of fish sauce, fermented fish, soya-bean sauce and serum were therefore determined by the radioisotope dilution and coated charcoal tech-

nique. The method, in the present studies, shows the excellent reproducibility and recoveries (see Table 2 and 3).

Fish sauce and fermented fish were found to contain a considerable amount of vitamin B_{12} (i.e, 1.91 µg % and 2.27 µg % respectively). These results resembled the data reported earlier (Sundharagiati, 1957; Areekul et al., 1972).

The vitamin B_{12} content varied greatly in fish sauce samples and a relationship between the vitamin B_{12} content and the price which has been reported earlier was also demonstrated in the present studies.

Fish sauce and fermented fish are the important traditional food products used extensively in this country. Fish sauce is used as a condiment, as flavouring material and sometimes as a substitute for solid salt. Fermented fish is widely used in the Northeast of Thailand. It was served as a condiment and a kind of the preserved food.

The daily consumption of fish sauce in Thailand was found to have a range from 10 to 25 ml/person (ICNND, 1960; Areekul et al., 1972). Assuming that the average daily consumption of fish sauce in Thailand is 15 ml/person, one should obtain 0.1 - 0.9 µg vitamin B_{12} per day as a supplement from fish sauce.

Fermented fish is also a traditional food product commonly consumed daily by the people resides in the Northeast of Thailand. An average daily consumption was estimated to

be 15 g. per person and vitamin B_{12} obtained should therefore be 0.1 - 1.0 µg/person/day. These figures of vitamin B_{12} consumed from fish sauce and fermented fish were considerably high in terms of the daily requirement of 1 - 2 µg.

Soya-bean sauce is obtained from an elaborated process of fermentation of soya-bean with various fungi, bacteria and yeast. Finding of low vitamin B_{12} concentrations in soya-bean sauce and 13 out of 48 samples contained no vitamin B_{12} were not unexpected. Since soya-bean contained no vitamin B_{12}, therefore the vitamin B_{12} contents in these samples must come from the contamination from the bacteria or from the processing method. The daily consumption of soya-bean sauce in this country is not known but it was expected that the figure may be very low.

Serum vitamin B_{12} levels in patients with P. falciparum malaria were found to be significantly lower than that of the normal subjects. This finding confirmed the result reported earlier (Areekul et al., 1972). Low serum vitamin B_{12} in these patients indicated a low intake or malabsorption of this vitamin. The most likely explanation was found to be the latter one (Areekul et al., 1972). They have shown that patients with P. falciparum malaria had impaired absorption of vitamin B_{12} during the acute episode of malaria and the absorption returned to the normal level during a convalescent stage.

The finding that serum vitamin B_{12} levels in patients with Gnathostomiasis was significantly lower than that of the normal subjects was very interesting. Unfortunately, vitamin B_{12} absorption was not studied in these patients. Serum vitamin B_{12} levels in patients with F. buski showed no significant difference from the normal value.

Iron deficiency anaemia is common in rural areas of Thailand while nutritional megaloblastic anaemia is rarely seen even in pregnant women or in people of low socio-economic status (Vajarasthira and Harinasuta, 1957; Areekul et al., 1972; Sundharagiati, 1957). Recent studies in Thailand showed that (a) patients with amoebic liver abscess and opisthorchiasis had low absorption coupled with normal serum vitamin B_{12} level (Devakul et al., 1967; Areekul et al., 1971), (b) patients with hookworm infection showed low absorption and low serum vitamin B_{12} level (Devakul et al., 1970). However all these patients showed no signs or symtoms of vitamin B_{12} deficiency.

It has been well established that if only 1 µg of vitamin B_{12} transferred across the intestinal mucosa into the blood vessel, it could prevent the pernicious anaemia. Assuming that the maintenance dose for patients with pernicious anaemia of 1 µg vitamin B_{12} per day represents an adequate daily requirement and if the average weight of liver is 1,500 grams, then on the basis of the average value of vitamin B_{12}

of approximate 0.7 µg/g of liver, sufficient vitamin B_{12} would be present in the liver to last for a 3-year period. This indicated that there is a considerable storage of vitamin B_{12} in terms of nutritional requirement. It would appear, therefore, that a decrease in the absorption of vitamin B_{12} from the gastrointestinal tract in patients mentioned above would not result in the immediate development of a deficiency state especially in Thai people who consumed fish sauce and/or fermented fish for almost the whole of their lives. This could be illustrated by a Thai patient with nutritional macrocytic anaemia who has been successfully treated with fish sauce containing vitamin B_{12} 1 µg for only 25 days (Sundharagiati, 1958). It could, therefore, concluded from this therapeutic response that fish sauce and fermented fish, the traditional dietary supplement in Thailand, could be the major sources of vitamin B_{12} that prevents nutritional megaloblastic anaemia in Thai people.

Conclusion

This thesis presents the vitamin B_{12} contents in fish sauce, soya-bean sauce, fermented fish and serum vitamin B_{12} levels with P. falciparum malaria, Gnathostomiasis and F. buski infection using radioisotope dilution and Hb-coated charcoal technique. The advantages of this method over previously described classical bioassay procedure are greater

simplicity, rapidity and reproducibility. The sensitivity, reproducibility and recoveries appeared to be very satisfactory.

Vitamin B_{12} concentrations in fish sauce, fermented fish, soya-bean sauce and serum of patients with malaria, Gnathostomiasis and Fasciolopsis buski were determined by this method. Vitamin B_{12} concentrations were found to be 1.91 µg per cent (range 0.30 - 5.92 µg per cent) and 2.27 µg per cent (range 0.33 - 6.43 µg per cent) in fish sauce and fermented fish respectively. The soya-bean sauce had a low content of vitamin B_{12} i.e, ranging from 0.01 to 0.53 µg per cent with a mean of 0.13 µg per cent. Thirteen samples out of 48 samples contained no vitamin B_{12}. If the average daily consumption of fish sauce and fermented fish in a Thai is 15 ml per day or 15 grams per day, one would obtain vitamin B_{12} 0.1 - 1.0 µg per day which was considerably high in comparison with a daily requirement of 1 - 2 µg per day.

Serum vitamin B_{12} levels in patients with P. falciparum malaria, Gnathostomiasis were found to be lower than that of the normal subjects. This might be due to the impaired absorption of vitamin B_{12} in these patients. Serum vitamin B_{12} level in patients with F. buski was found to be within the normal limit.

Fish sauce and fermented fish, a traditional food supplement in the diet in Thailand, should be considered as con-

tributing the major source of vitamin B_{12}. It seems to prevent megaloblastic anaemia in Thai people and even in pregnant women or in patients with liver diseases. This could explain the findings that have been known for a long time that megaloblastic anaemia was rarely encountered among the Thais.