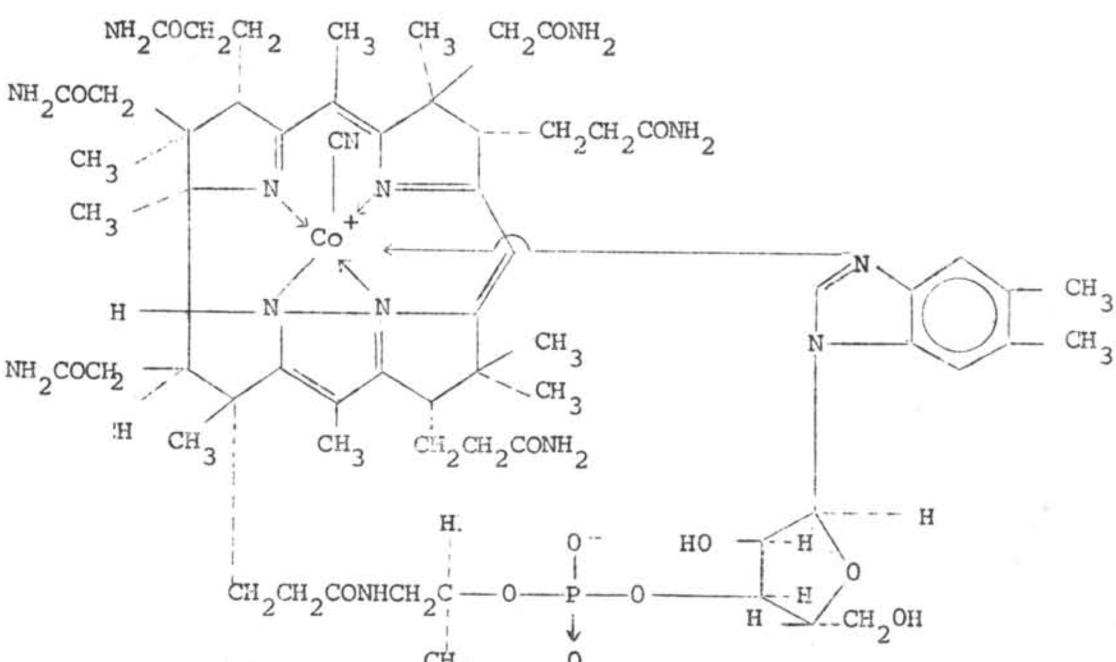
CHAPTER I

INTRODUCTION

Vitamin B_{12} the designation for cyanocobalamin, was the first form of the vitamin to be isolated in crystalline form (Rickes et al, 1948). It is a water-soluble compound which is crystallized as small red needles (Goodman and Gilman, 1965). The empirical formula is $C_{63}H_{88}N_{14}O_{14}PCo$ and it has a molecular weight of 1355.42 (Osol and Hoover, 1975). The compound is composed of two heterocyclic systems, a benzimidazole and a modified porphyrin nucleus, as shown below.



Cyanocobalamin (Vitamin B_{12})

The structure of chemical substance is unique and complex. X-ray crystallographic data were necessary in conjunction with degradative chemical evidence for the complete structural elucidation. Vitamin B_{12} is the only vitamin discovered to date that has an atom of a metal (cobalt) in its molecule. The cyanide group coordinated to the cobalt was the first form of the vitamin to be isolated. Vitamin B_{12} is therefore called cyanocobalamin while the name "Cobalamin" refers to all of the molecules except the cyano group. When the ligand is hydroxide instead of cyanide, the compound is Vitamin B_{12a} (Hydroxocobalamin); when it is water, the substance is Vitamin B_{12b} (Aquocobalamin); and when it is nitro, the compound is Vitamin B_{12c} (Osol and Hoover, 1970).

Vitamin B_{12} is slowly decomposed by ultraviolet as strong visible light. It is stable in air and, in dry form, is relatively stable at 100°C for a few hours. Aqueous solution of Vitamin B_{12} at pH 4.7 can be autoclaved at 120°C. Cyanocobalamin appears to be the most stable of the various Vitamin B_{12} analogs studied to date.

Vitamin B_{12} participates in nucleic acid synthesis. The recent finding of Luhby and Cooperman (1953) that patients with pernicious anemia excreted more aminoimidazolecabamide appeared as a further evidence of this role. This substance is a precursor of the purine, inosinic acid, which is a key intermediate substance in nucleic acid synthesis. The particular reaction blocked is one in which folic acid has also been thought to play an enzymatic role. The intermediate was not found in the urine, however, in patients with folate deficiency. Evidence also indicates the role of Vitamin B_{12} in propionic acid metabolism as a coenzyme A to isomerase in the conversion of methylmalonyl coenzyme A to a succinyl coenzyme A, a Kreb's cycle intermediate. The discovery of methylmalonate in the urine of patients with pernicious anemia indicates this block and it has been suggested as a possible index of Vitamin B_{12} deficiency. Vitamin B_{12} has other vital role in the biosynthesis of methyl group of glycine, serine or formate and in transmethylation reactions such as occur in the formation to ethanolamine. Rats, chicks, and other animals have been shown to require for optimum growth, a factor in liver extracts, packing plant meal, and fish meal that has been named the animal protein factor (APF). APF-deficient hens produce eggs with low hatchability and chicks with low viability and retarded growth. There is also high motality and retarded growth in the offspring of APF-deficient rats. Such signs can be prevented by addition to the diet of APF concentrate or, as more recently found, Vitamin B_{12}.

Intestinal absorption of dietary Vitamin B_{12} in many animals, included man, requires "intrinsic factor" (Castle and Towsend, 1929) a glycoprotein of molecular weight 50000 (Grasbeck et al, 1966) secreted by the gastric parietal cells (Hoedemaker et al, 1964). This combines with the Vitamin B_{12} produce a complex which is resistant to proteolysis by intestinal and pancreatic digestive enzymes (Williams et al, 1954) and therefore is able to traverse intact the length of the small bowel. In the distal small intestine, in the presence of divalent cations and a pH above 6 (Carmel et al, 1969) the Vitamin B_{12} -intrinsic factor complex (B_{12}~IF) attaches to a specific receptor present on the microvillous membrane of the mucosal cells (Donaldson et al, 1967). The subsequent transloca-tion of the large water soluble Vitamin B_{12} molecule (MW. 1355) across the lipid cell membrane is poorly understood, but clearly the process begins with the membrane bound receptor for B_{12}-IF complex. Toskes et al, (1971) have suggested that a pancreatic factor may be required for normal vitamin B_{12} absorption, though it is not clear at which level this factor might have a role.

There is evidence that with crystalline form of vitamin B_{12}, the amount which is absorbed from an oral dose of one form may be significantly different from that which is absorbed from the same dose of another form (Rosenblum et al, 1956; Heinrich et al, 1964; Herbert et al, 1964; Adams et al, 1971). It has been reported that the detected form of Vitamin B_{12} in food were adinosylcobalamin, hydroxocobalamin, methylcobalamin, cyanocobalamin and sulphitocoba-lamin. Adinosylcobalamin and hydroxocobalamin were predominant

In man, Vitamin B_{12} deficiency is manifested itself primarily in megaloblastic anemia and subacute combined degeneration of the spinal cord. The severest form of Vitamin B_{12} deficiency is pernicious anemia, in which absorption of the vitamin is impaired through lack of Castle's intrinsic factor. It can be restored to normal if the Vitamin B_{12} is given together with intrinsic factor. Other forms of Vitamin B_{12} deficiency, ordinarily complicated by some degree of folic acid deficiency, may occur even though intrinsic productive is normal. For example, the host may be deprived of Vitamin B_{12} by parasites such as the fish tapeworm. Abnormal bacterial growth in the small intestine, accompanying intestinal statis, also results in Vitamin B_{12} deficiency. Intestinal malabsorption, as in sprue, steatorrhea, regional enteritis, sometimes limits absorption of the vitamin below requirements. And dietary inadequacy of Vitamin B_{12}, although rare, has occured among persons (call "Vegan") who eliminate animal protein from their diet.

Man is wholly dependent on dietary B_{12}, since they appear unable to utilize any of the intestinally synthesized vitamin, it is either not formed or is not released from the cells of synthesizing organism, in regions of the gut from which absorption occurs (Ungley, 1955). The dietary source of Vitamin B_{12} is formed in all animal tissues and products. The natural form of Vitamin B_{12} has been found in coenzyme form linked to its specific protein (Toohey et al, 1961).

The importance of Vitamin B_{12} as accessory food in human is well known. Since man depends on an adequate intake of Vitamin B_{12} in order to maintain Vitamin B_{12} balance. The recommended daily dietary allowance of the Food and Nutrition Board for Vitamin B_{12} ranges from 1 to 8 µg, the lowest value is for infants up to 2 months and the highest value (3 µg higher than the average adult allowance) is for woman during pregnancy (Osol and Hoover, 1970).

The assay methods available for the determination of Vitamin B_{12} fall into three groups i.e., biological, microbiological and chemical. The biological methods are time consumming and difficult to perform from the stand point of personal and equipment. On the other hand, response of animals may give a specific measure of the amount of vitamin present as well as its availability in the test substance. The microbiological methods are more sensitive and less time consumming and require less highly trained personal once the method is established. Chemical methods are less sensitive than either of the growth assays but are much faster to perform and generally more reproducible (Freed, 1966).

It is well known that Vitamin B_{12}, widely disseminated in sea water, sewage sludge, and fermented products, is synthesized by a large number of microorganism. Higher animal, as far as is known, cannot synthesize Vitamin B_{12} and must necessarily obtain the vitamin directly or indirectly from bacterial source. Robbins

(1951) has speculated on the presence of Vitamin B_{12} in blue-green algae and phytosynthetic crysomonads and their ecological relationship to the production of the vitamin by microbial fermentations in pond muds. The substantial amount of the vitamin in these primitive life forms represents a cycle from microorganismal Vitamin B_{12} production through the food chain to higher animal. In terrestial form of life, substantial quantities of the vitamin are produced in ruminants by rumen bacteria in the presence of adequate cobalt nutrition (Dawbarn, 1952), ultimately returning to soil to support soil biota. Vitamin B_{12} is not present to a great extent in higher plants, and the small amounts present are assumed to be bacterial action or contamination.

The amount of Vitamin B_{12} in foods and food extracts have been measured by variety of methods which do not always give concordant results. The existence of factors related to Vitamin B_{12} that interfere with its estimation was not appropriated when early result were reported (Robinson, 1966). In 1949, Lewis et al reported that the liver, heart, small intestine and femoral muscles of Vitamin B_{12}-depleted rats contained no Vitamin B_{12} whereas the kidney had a substantial quantity. The average Vitamin B_{12} content of the milk of cows, sheep and goats studied was 6.6, 1.4 and 0.12 μg per litre, respectively. Commercial cow's milk was found to contain 1.6 to 6.5 ug of Vitamin B_{12} per litre (Collins et al, 1950). The mean Vitamin B_{12} content of the milk from 5 human subjects was found to be 0.41 μg per litre (Collins *et al*, 1950). Cow's milk contained 3.2 to 12.4 ug with an average of 6.6 μg per litre when assayed microbiologically (Halick *et al*, 1953) and 7.1 μg per litre determined by rat assay. Egg yolk contained 0.003 to 0.026 μg of Vitamin B_{12} per gram (Yacowitz, 1952) and the amount of Vitamin B_{12} was increased by injecting high levels of the vitamin into hen (Halick, 1953). Fish solubles contained 22 to 120 ug per 100 gm and fish meal 6 to 33 ug per 100 gm (Pritchard, 1951). The organ containing most Vitamin B_{12} in fish was the heart, the amount present brings up to 150 times as much as in the liver (Braekkan, 1958).

Studies on the Vitamin B_{12} content in Thai Foods are very scantly. Liver has been reported to contain larger amount than other protein foods, milk contains some, while fruits and vegetables contain very little or none (Hemindra *et al*, 1971). Fish sauce and fermented fish, a traditional Thai food, were found to be the rich source of Vitamin B_{12} (Areekul *et al*, 1975).

The objective of the present study is to determine Vitamin B_{12} content in some Thai Foods i.e., cereal and cereal products, meat, poultry, eggs, fishes, milk and milk products, fat and oil, vegetables and fruits. The data of Vitamin B_{12} content in some Thai Foods will provide an optimum daily food consumption to meet the need of daily requirement of Vitamin B_{12} for Thai people.