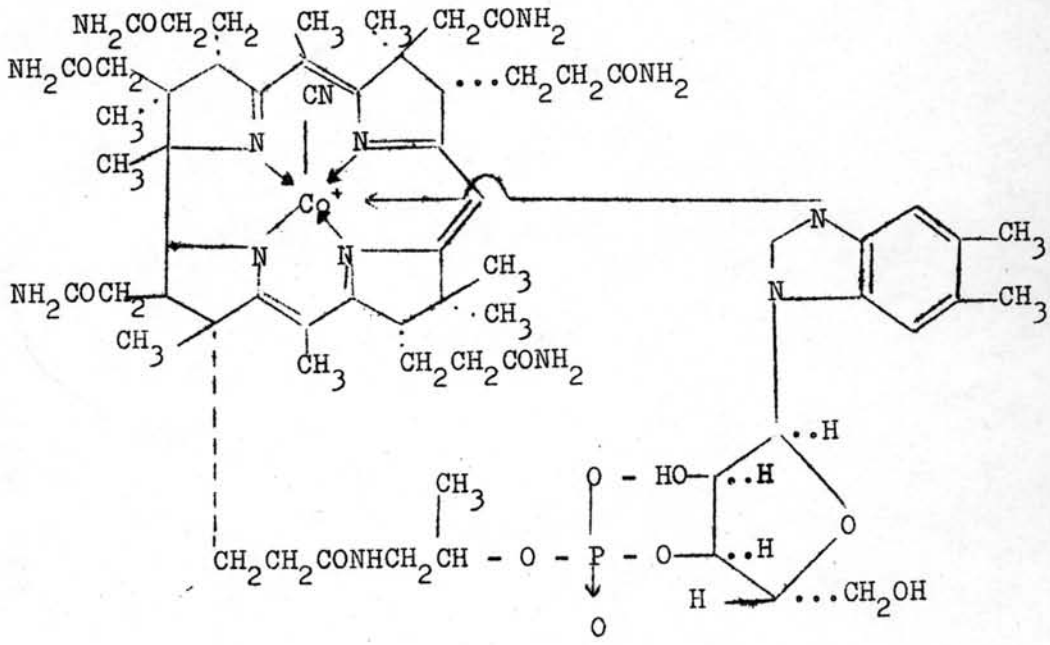
CHAPTER I

INTRODUCTION

Vitamin B_{12} is a complex water-soluble compound which is crystallized as small red needles (Goodman and Gilman, 1965). The formula of vitamin B_{12} is $C_{63}H_{88}CoN_{14}P$; and its molecular weight is 1355.42 (Osol and Hoover, 1970). The compound is composed of two heterocyclic systems, a benzimidazole and a modified porphyrin nucleus, as shown in the following structure :



Cyanocobalamin (Vitamin B_{12})

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 molecule 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 or strong visible light. By controlled irradiation with visible light, cyanide may be selectively liberated from cyanocobalamin without destruction of the cobalamin structure (Veer et al.,1950), but long exposure to light results in complete inactivation of the vitamin. Vitamin B_{12} is also inactivated by treatment with strong acids or alkalies.

Vitamin B_{12} has been found to be essential for the growth of many microorganisms. Among these microorganisms are Lactobacillus lactis Dorner, (Shorb et al., 1948), Lactobacillus leichmannii,(Hoffmann et al., 1948),a mutant strain of Escherichia coli, (Davis and Minigiole, 1950). Not only do some microorganisms require vitamin B_{12}, but many are capable of synthesizing relatively large quantities of the vitamin (Rickes et al., 1948). Streptomyces species such as griseus or aureofaciens and Propionibacterium species

are used in the commercial production of vitamin B_{12}. These may give yields of vitamin B_{12} as high as 24 mg per litre of fermentation liquor (Smith, 1965). The vitamin has been isolated as a cobamide peptides which may contain up to 23 % vitamin B_{12}. In general vitamin B_{12} is not found in the plant kingdom, it is synthesized by bacteria and vitamin B_{12} in higher animals arises indirectly from bacteria sources. An exclusively vegetarian diet by man or animals leads to vitamin B_{12} deficiency. Nevertheless under natural conditions, food is contaminated with soil and bacteria which probably provide a significant amount of vitamin B_{12} (Chanarin, 1969).

Man is wholly dependent on dietary sources of vitamin B_{12}. Detectable quantities of vitamin B_{12} activity are found in animal proteins and fermented products. Meats, especially liver, eggs, milks, cheese and fish are among the richer sources of the vitamin (Hollenbeck et al., 1955). In man, synthesis of the vitamin occurs by bacteria in the large bowel, from which site it is not absorbed. The feces of animals and man usually contain large amounts of cyanocobalamin. Both normal persons and pernicious anaemia patients in relapse excrete daily about 5 µg of vitamin B_{12} in the feces (Goodman and Gilman, 1965).

Vitamin B_{12} is essential for the normal functioning of all cells, but particularly for cells of the bone marrow, the nervous system, and the gastrointestinal tract. Evidence

exists that vitamin B_{12} is involved in protein, carbohydrate, and fat metabolism, but its chief importance in mammalian tissues seems to be, together with folic acid, in the anabolism of deoxyribonucleic acid in all cells. The biochemical fault in pernicious anaemia, a condition caused by a prolonged deficiency of vitamin B_{12}, is a failure of elaboration of the intrinsic factor, normally present in the secretions of the stomach mucosa. Vitamin B_{12} is a requisite for normal blood formation, and certain macrocytic anaemias respond to its administration. In pernicious anaemia, unless accompanied by intrinsic factor, the vitamin is not orally absorbed in effective amounts and must be administered parenterally in microgram quantities (Osol and Hoover, 1970). The maximum amount of vitamin B_{12} that is absorbed through the agency of intrinsic factor in man is between 2 and 3 µg (Chanarin, 1969).

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 women during pregnancy (Osol and Hoover, 1970). In an adult, the minimal daily hematopoietic requirement for crystalline vitamin B_{12} is in the range of 0.1 µg, the quantity of cyanocobalamin or coenzyme B_{12} that will produce a minimal hematological response in a patient with uncomplicated

vitamin B_{12} deficiency (Herbert and Sullivan, 1964). In an individual with normal vitamin B_{12} stores, the daily loss of the vitamin mainly via the billiary tract, is from 3 to 7 µg, of which all but approximately 1 µg is reabsorbed. A 1 µg quantity is approximately the one that will sustain the body vitamin B_{12} stores of the average adult, when administered daily, and is probably a resonable approximation of the human requirement (Bozian et al., 1963). Patients who lack of intrinsic factor secretion may require daily slightly more than normal individuals, since they are unable to reabsorb vitamin B_{12} secreted into the intestinal tract (Goodman and Gilman, 1965).

There are two separate and distinct mechanisms for the absorption of vitamin B_{12}. The more important of these two mechanisms is mediated by the gastric intrinsic factor of Castle. Interference with this mechanism produces the overwhelming majority of the megaloblastic anaemias due to vitamin B_{12} deficiency seen in the United States. The other mechanism of vitamin absorption is independent of intrinsic factor, and is primarity operative only in the presence of quantities of vitamin B_{12} much greater than those made available from the usual diet. The two mechanisms for vitamin B_{12} absorption overlap to a variable degree in normal man, depending on daily dietary vitamin B_{12} intake and the quantity of the vitamin released from its bound form in the food (Good-

man and Gilman, 1965).

Absorbed vitamin B_{12} is transported via the blood stream to the various tissues and especially to the liver, the main organ for vitamin B_{12} storage. The liver contains amounts varying from 50 to 90 % of the normal adult's total body stores of the vitamin, these stores range approximately from 1 to 10 mg in normal adults, with an average of about 4 to 5 mg. There is no evidence for significant catabolism of vitamin B_{12} in man, and it is probable that loss occurs only by excretion, mainly via the biliary tract. Injected cyanocobalamin or hydroxocobalamin is converted in the liver to coenzyme forms, and thus enters the common storage pool (Rosenblum et · al., 1963). Vitamin B_{12} circulates in the blood stream bound primarily to a specific α_1-globulin and, to a lesser degree, to other globulins (Hall and Finkler, 1963). The mean normal plasma concentration of vitamin B_{12} is 450 pg/ml ; the normal range is 200 to 900 pg/ml. Almost all of this vitamin B_{12} is bound to plasma protein; a small fraction (1 to 10 %) may be free or very loosely bound.

Approximately 3 to 7 µg of vitamin B_{12} is secreted into the alimentary tract daily, mainly in the bile, but also in gastric juice, pancreatic secretion, and other sources. The normal daily urinary excretion of vitamin B_{12} is in the range of 0.0 to 0.25 µg. Only the small amount of vitamin B_{12} not attached to plasma protein in normal subjects is available

for urinary excretion (Goodman and Gilman, 1965).

The assay methods available for the determination of vitamin B_{12} fall into three groups : biological, microbiological, and chemical. The biological methods are time-consuming and difficult to perform from the standpoint of personnel and equipment. On the other hand, the responses 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 consuming and require less highly trained personnel once the method is established. Chemical method are less sensitive than either of the growth assays but are much faster to perform and generally more reproducible (Freed, 1966).

The only established clinical use of cyanocobalamin is in the treatment of vitamin B_{12} deficiency. Claims have been made for the value of cyanocobalamin in the therapy of a variety of miscellaneous conditions, including infections hepatitis, multiple sclerosis, trigeminal neuralgia, poor appetite, miscellaneous neuropathies, poor growth, various psychiatric disorders, aging, thyrothoxicosis, sterility, and various forms of malnutrition (Goodman and Gilman, 1965).

The most common cause of vitamin B_{12} deficiency is a faulty absorption of the vitamin from the gastrointestinal tract. This may be due to lack of secretion of Castle's intrinsic factor, as in Addisonian pernicious anaemia, or in

patients with partial or total gastrectomy, or to some other disturbances of the intestinal milieu, such as diverticulosis or steatorrhoea . Deficiency may be caused also by insufficient dietary intake of the vitamin B_{12} as in some strict vegetarian diets, and finally by faulty storage in the body as may be the case with some disease of the liver (Lajtha, 1961).

Many tropical diseases especially the intestinal, liver and blood parasites may cause malabsorption or affect the storage of vitamin B_{12}. No such studies have been recorded in patients with malaria, fasciolopsiasis and gnathostomiasis infection.

In Thailand, iron-deficiency anaemia is common in rural areas due to the high incidence of hookworm infection (Areekul et al., 1972, Vajarasthira and Harinasuta, 1957). Nutritional megaloblastic anaemia is rarely seen in this country even in pregnant women or in people of low socio-economic status (Sundharagiati, 1957). Although patients with amoebic liver abscess and opisthorchiasis had low absorption of vitamin B_{12} and also patients with hookworm infection showed both impaired absorption and low vitamin B_{12} levels in serum, yet, they did not develop megaloblastic anaemia (Devakul et al., 1967) Areekul et al., 1971). This is probably due to the daily consumption of Thai diets which contains a considerable amount of vitamin B_{12} (Sundharagiati, 1957 ; Hemindra et al., 1971).

The objective of the present study is to determine vitamin B_{12} contents in fish sauce, soya-bean sauce and fermented fish that are available in the local markets and estimate the amount of vitamin B_{12} consumed daily by the average Thais from these kinds of food. Serum vitamin B_{12} levels will also be studied in patients with Plasmodium falciparum malaria, Gnathostomiasis and Fasciolopsis buski infection. The assay of vitamin B_{12} in human serum was based on the principle of radioisotope dilution and coated charcoal technique.