CHAPTER I

INTRODUCTION

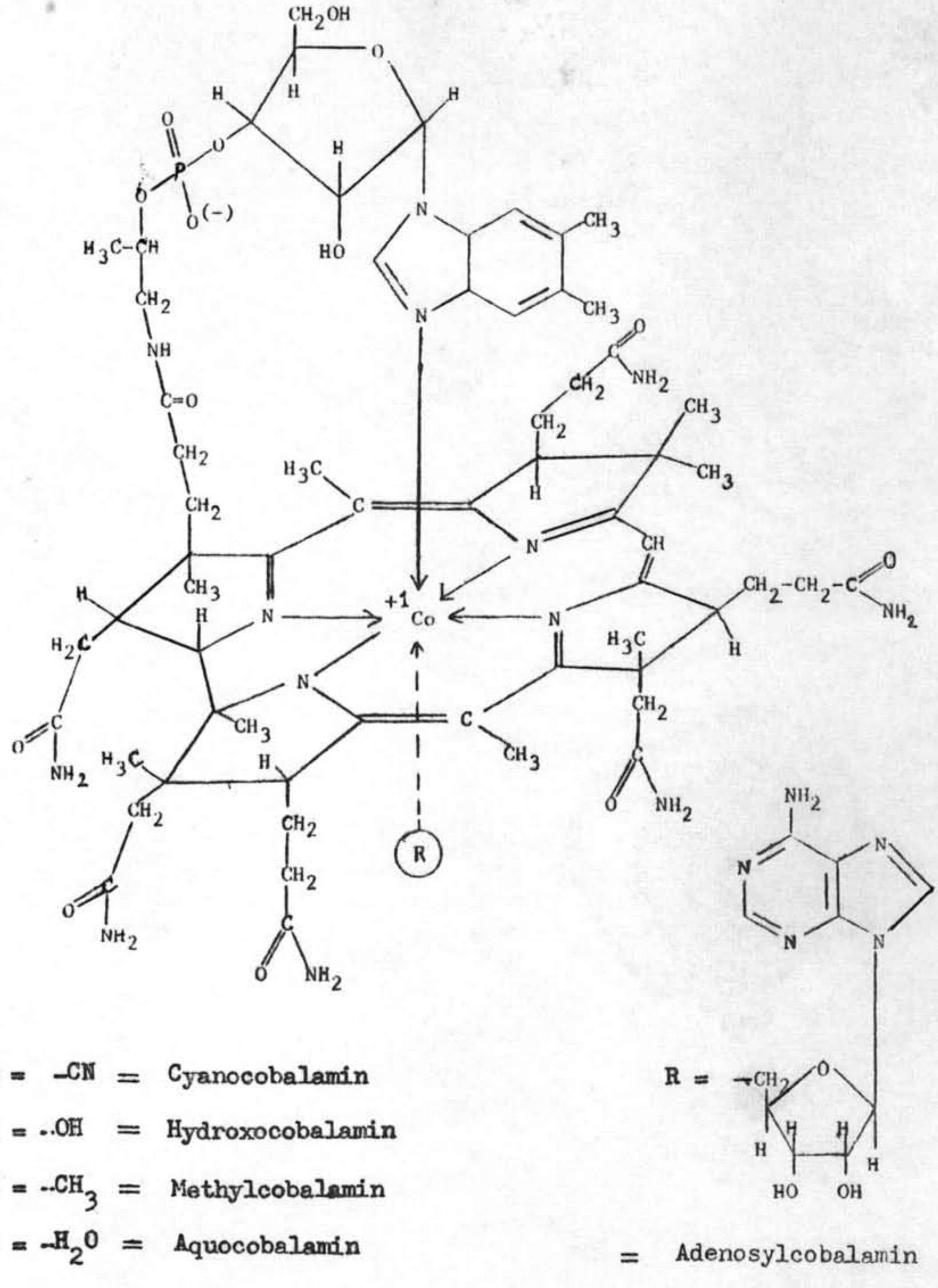
In 1948, a red crystalline compound was isolated from ox liver (Smith and Parker, 1948; Rickes et. al., 1948 a.) and proved to be active in the treatment of pernicious anemia (Berk et. al., 1948 b; West, 1948) and in subacute combined degeneration of the cord (Berk et. al., 1948 a.). It was named vitamin B_{12} and was found to contain about 4% cobalt as well as phosphorus and nitrogen. The cobalamin is a generic name which has been used for this group of compounds. Its formula is $C_{63}H_{88}O_{14}N_{14}PCo$. with a molecular weight of 1355.42 (Freed, 1966). Laboratory synthesis of this complex substance was accomplished in 1973 by R.W. Woodward and a team of 99 coworkers (Science, 1973).

Vitamin B_{12} molecule is devided into two main portions.

a) a planar group, a tetrapyrrole ring structure, resemble porphyrin, which surrounds the cobalt atom.

b) a nucleotide group, containing a base 5,6-dimethylbenzimidazole attached to phosphorylated ribose by an alpha-glycoside linkage.

Vitamin B_{12}, cyanocobalamin, was the first active isolated cobalamin because it is the most stable form of a series of compounds. Other cobalamins differ from cyanocobalamin in the nature of the ligand attached to the cobalt atom in place of CN^- as :



R = $-CN$ = Cyanocobalamin

R = $-OH$ = Hydroxocobalamin

R = $-CH_3$ = Methylcobalamin

R = $-H_2O$ = Aquocobalamin

R = = Adenosylcobalamin

Figure 1 Structural for various forms of cobalamins and cobamide coenzymes

- CH (Hydroxocobalamin, vitamin B_{12} a)
- H_2O (Aquocobalamin, vitamin B_{12} b)
- NO (Nitroso- or nitrocobalamin, vitamin B_{12} c)

All compounds have the same biological activity when tested in man (Smith, 1965)

Cobamide coenzymes (5-deoxyadenosyl cobalamin and methylcobalamin are two metabolically active forms in man and constitute the dominant forms of B_{12} in mammalian (Herbert, 1973). Methylcobalamin is the major form of B_{12} in plasma and 5-deoxyadenosyl cobalamin in the liver, and probably also in other tissues (Toohey and Barker, 1961; Stahlberg et. al., 1967; Linnell et. al., 1969: 1971). However, cobamide coenzymes appear equipotent therapeutically with cyanocobalamin (Wasserman et. al., 1960; Sullivan and Herbert, 1965).

Vitamin B_{12}, a complex water soluble compound, crystallized as small red needle. Its activity is destroyed by heavy metal and strong oxidising or reducing agents. It is soluble in water in the ratio 1:80. Aqueous solutions are neutral with maximal stability at pH 4.5 to 5.0 (Hashmi, 1973; Herbert, 1973). On exposure to light, cyanocobalamin is converted to hydroxocobalamin, but this change is reversed by keeping the solution in the dark. Prolonged exposure to sunlight degrades the vitamin B_{12} irreversibly (Smith, 1965). Cobamide coenzymes are unstable, cyanocobalamin or aquocobalamin is readily formed by exposure to cyanide or light (Herbert, 1973; 1975)

The original and sole source of vitamin B_{12} in nature is synthesized by microorganisms. It is absent in plants. The dietary sources of vitamin B_{12} are meat and meat products (including fish, shellfish and poultry) and, to a lesser extent, milk and milk products. The natural occurring cobalamins in foodstuff are predominant in adenosylcobalamin and hydroxocobalamin (Toohey and Barker, 1961; Farquharson and Adams, 1976), in which they are generally attached to polypeptides (Hodbom, 1960).

Vitamin B_{12} is required for normal blood formation, maintenance of neural function, normal growth and other fundamental metabolic processes. Wherever nucleic acid synthesis occurs in cells reproducing themselves, cobalamin is required (Herbert, 1975). It has been found to be essential for the growth of many microorganisms, as Lactobacillus lactis Dorner;(Shorb, 1948); Lactobacillus leichmannii (Hoffmann et.al., 1948); and many are capable of synthesizing relatively large quantities of the vitamin B_{12} (Rickes et. al., 1948 b). Streptomyces species such as griseus or aureofaciens and Propionibacterium species are used in the commercial product of vitamin B_{12} (Robinson, 1966; Channarin, 1969; Osal and Hoover, 1975). Like as other B-vitamin, coenzyme B_{12} is indeed the functional form in most, though not all, of the reaction mediated by vitamin B_{12} (Smith, 1965)

Absorption of vitamin B_{12} in the gastrointestinal tract takes place in two ways as follow:

a) Physiologic mechanism, mediated by gastric intrinsic factor of Castle, is capable of handling of maximal of 1.5 to 3.0 µg. of free vitamin B_{12} at anytime, per meal or per dose (Heyssel et. al., 1966; Berlin et. al., 1968; Herbert, 1973; 1975). Vitamin B_{12} is the only nutrient of man known to require a specific secretion of gastric mucosa to facilitate its absorption and also requires calcium ion and pH above 5.7 (Herbert and Castle, 1961; Carmel et.al., 1969; Goldberg and Fudenberg, 1969; McKenzie et. al., 1972). Interference with this mechanism produces human much vitamin B_{12} deficiency.

b) The pharmacologic mechanism which is a passive diffusion. It accounts for absorption of approximately 1.0 to 1.2% of oral dose administration (Berlin et. al., 1968; Carcino et. al., 1970; Shinton, 1972). Thus it is possible to increase the uptake to any desired level and this direct uptake is found to be of the same magnitude irrespective of whether the patients have a normal absorption or not (Berlin et. al., 1968; Carcino et.al., 1970)

Vitamin B_{12} is widely distributed throughout body tissues. The liver contains amount varying from 50 to 90 per cent of normal adult's total body stores of vitamin (Herbert, 1973), and average total stores range from 2 to 5 mg. (Heyssel et. al., 1966; Bozian et.al., 1964). Kidney and adrenal also have high vitamin B_{12} level (Rappazo et.al., 1970)

Excretion is in the bile (there is active enterohepatic circulation) via the kidney, and normal daily urinary excretion is neglible, i.e., 0 to 0.25 ug. (Herbert, 1975). Faecal excretion is always high, but much of this arises from bacterial synthesis in the lower gut. Both normal persons and pernicious anemia patients in relapse excrete daily about 3 to 6 µg. of vitamin B_{12} in the faeces (Shinton, 1972; Herbert, 1975)

It has been estimated that the minimal daily adult requirement for the vitamin B_{12} is 0.6 to 1.2 µg. (Heyssel et.al., 1966; Bozian et.al., 1963). This amount prevents all signs and symptoms of deficiency and will maintain serum vitamin B_{12} level. However, daily loss of vitamin B_{12} is between 0.1 and 0.2% of body stores, regardless of pool size. Thus normal subject 2.0 to 10.0 µg. are lost per day and must be replaced to maintain the stores (Bozian et.al., 1964; Heyssel et. al., 1966; Adams and Boddy, 1968; Shinton, 1972).

Recommended dietary daily intake of vitamin B_{12} in µg. is as follow: infant, 0.3; age 1-3 years, 0.9; age 4-9 years, 1.5; age 10 years and ever, 2.0; adult, 2.0; pregnancy, 3.0; and lactation, 2.5 (FAO/WHO Expert group, 1970)

Deficiency of vitamin B_{12} in the body leads to: megaloblastic anemia (Addisonian or pernicious anemia); subacute combined degeneration of the cord (degeneration of the brain, spinal cord and peripheral nerves, symptoms may be psychiatric or physical) and abnormalities of epithelial tissues particularly of the alimentary tract (eg. sore tongue, malabsorption)

The causes of vitamin B_{12} deficiency are: (Castle, 1970)

a) dietary deficiency (animal protein lack, alcoholism)

b) gastric defect: lack of intrinsic factor (Pernicious anemia and total or partial gastrectomy)

c) intestinal defect: malabsorption (ileal resection, sprue and celiac disease, and fish tapeworm infestation)

Vitamin B_{12} deficiency is ususlly due to intestinal malabsorption of various etiology. The most common is pernicious anemia in which gastric mucosa defect decreases intrinsic factor synthesis.

The assay methods available for the determination of vitamin B_{12} fall into three groups: (a). biological methods which employ two different animals, weaning rats and chicks; (b). microbiological methods which is carried out by determining ability to promote growth of various vitamin B_{12}-dependent microorganisms as Euglena gracilis, Lactobacillus leichmannii; and (c). chemical methods which are much faster to be performed and generally more reproducible, including colourimetrically, spectremetrically and isotope dilution methed (Freed, 1966)

Man depends on an adequate intake of vitamin B_{12} in order to maintain vitamin B_{12} balances. The amounts of vitamin B_{12} in tissues and tissue extracts have been measured by variety of methods which do not always give concordant results. Estimation of the dietary intake of vitamin B_{12} content of diet and referring to information on the vitamin content of foodstuff were presented by Robinson (1966); Chanarin (1969); and Adams et. al., (1973). The following values obtained by several groups of workers for vitamin B_{12} content of various meats: canned tuna 46 µg./kg.; ox, braised liver, 759 µg./kg.; ox, braised kidney, 58 µg./kg.; boiled egg, 10.3 µg./kg.; scrambled egg, 3.0 µg./kg.; fried egg, 6.1 µg./kg.; whole fresh milk, 6.5 µg./l.; pasteurized whole milk, 3.9 µg./l. (Adames et. al., 1973); cow's milk, 6.6 µg./l.; pasteurized cow's milk, 4.0 µg./l.; goat's milk, 0.12 µg./l. (Collins et. al., 1951); canned beef, 10-25 µg./kg.;

canned pork, 10-20 µg./kg.; canned beef liver, 200-600 µg./kg.; whole egg, 1.5 µg./egg.; whole milk, 3-5 µg./l.; evaporated milk, 1-3 µg./l.; condensed milk, 3-5 µg./l.; whole milk powder, 10-26 µg./kg.; skimmed milk powder, 25-40 µg./kg. (Merck Service Bulletin, 1958); boiled egg, duck, 60 µg./ kg,; cow's milk, boiled, 4.8 µg./l.; goat's, raw liver, 400 µg./kg. (Banerjee and Chatterjea, 1963): Cheddar cheese, 9.9 ug./kg.; Gruyere cheese, 16.0 µg./kg.; Swiss cheese, 20.8 ug./kg.; condensed milk, 3.1 µg./l.; evaporated milk, 1.3 µg./l.; nonfat dry milk, 37.6 µg./kg.; whole pasteurized milk, 3.6 µg./l. (Rosenthal, 1968)

The vitamin B_{12} content in some items of Thai diet obtained from previous workers: fish sauce, 39, 15, 0.6, 0.3 µg./kg.; fermented fish, 42 and 16 µg./kg. (Sundharagiati, 1957); cheese, 13.2 µg./kg.; pasteurized cow's milk, 1.5 µg./l. (Hemindra *et.al*., 1971); fish sauce, 2.0 to 33.2 µg./kg. (Areekul *et.al*., 1972); fish sauce, 19.1 µg./kg.; fermented fish, 22.7 µg./kg.; soya-bean, 1.4 µg./kg. (Areekul *et. al*., 1975); pasteurized milk, 1.49 ng./ml,; fresh cow's milk, 1.38 ng./ml.; condensed milk, 3.85 ng./ml.; evaperated milk, 0.32 ng./ml. (Areekul *et. al*., 1977)

Milk is the most complete food in nature. It is the ideal food of the infants, an excellent food for growing child and a very good food for the adult. The same nutrients are presented in the milk of all species, although in different proportions. Such quantitative differences appear to be an adaptation to the nutritive requirements of the young of each species. The usual assumption is that human milk offer the optimum physiological nourishment for the infants.

Human milk is not constant in composition from one to another at all periods of lactation or even hourly through the day. The composition of milk is related not only to amount secreted and the stage of lactation, but also to the timing of its withdrawal and to the individual variation among lactating mothers (such as maternal age, parity, health and social class). It has been proposed that the quantity and quality of breast milk are adversely affected by maternal undernutrition. The vitamin contents of human milk appear to be largely dependent of the state of nutrition of the mother (Harfouche, 1970; Jelliffe, 1976), but hitherto conducted studies are inconclusive.

The vitamin B_{12} in milk are strongly and specifically bound to miner whey protein (Ford, 1974). The binding protein is generally present in excess. The unsaturated binding capacity of vitamin B_{12} varies between milks of different mammalian species. The vitamin B_{12} binders in milk may have a complex physiological role, they may act in the mammary glands as trapping mechanism to accumulate the vitamin B_{12} from blood plasma into milk and facilitate vitamin B_{12} absorption in the gut and protect it against the intestinal microflora in the infants (Ford, 1974)

Previous studies of vitamin B_{12} content of human milk were as follow: 100 to 1500 pg./ml., average 410 pg./ml. (Collins <u>et. al</u>., 1951); 410 ± 390 pg./ml. (Areekul <u>et. al</u>., 1977); 200 to 950 pg./ml. (Hemindra <u>et. al</u>., 1971); 40 to 390 pg./ml., average 100 pg./ml. (Jathar <u>et.al</u>., 1970); 1100 ± 200 pg./ml. (Lampkin <u>et.al</u>., 1966); 605 ± 185 pg./ml. (Craft <u>et.al</u>., 1971); 55 to 160 pg./ml., average

80 pg./ml. (Srinivasamurthy et. al., 1953); 300 pg./ml. (Karlin, 1956) 300 pg./ml. (Gregory and Holdsworth, 1955); 82 to 113 pg./ml., average 97 pg./ml. (Deodhar and Ramakrishnan, 1959); and 110 to 480 pg./ml. (Lancet, 1965).

Intake of vitamin B_{12} in infants could be probably culculated on the assumption that the daily milk output of breast milk is known. In women of low socio-economic group would be about 500 to 600 ml. (Baker et. al., 1962; Srikantia and Reddy, 1967), and in well nourished mother, production of milk gradully increases from an approximately of 500 to 600 ml. in the early part and 700 to 800 ml. in the latter part of the lactation (Lonnerdal et. al., 1976). Diurnal variation exist, the maximum yield is in the morning and the lowest in the evening. (Jelliffe, 1976).

The estimated requirement of the infants for nutrients are, therefore, based on the composition of breast milk. The artificial substitutes, although, nutritionally adequate for maintenance of growth, lack some of the unique features of human milk, for examples, substantial amounts of secretory immunoglobulin-A (Mata and Wyatt, 1971); iron-binding protein, lactoferrin (Bullen et. al., 1972); and bifidus factor (Mata and Wyatt, 1971), all of which are known to enhance resistance to infection. Breast milk also contains comparatively high levels of the lysozyme which is capable of degradating bacterial cell wall (Mata and Wyatt, 1971) and relatively large amounts of a B_{12}-binding protein that has been shown to compete with B_{12}-removing microorganisms thereby acting bacteriostatically (Gullberg, 1973). The most significant of the many differences from cow's milk appears to be the abundant supply of nutrients most needed for the rapid growth and development of the central nervous system, including the brain. Particularly high levels of lactose, cystine and cholesterol, and specific patterns of polyenoic fatty acid are found in human milk (Jelliffe and Jelliffe, 1975)

Bottle-fed or breast-fed infants receive their vitamin B_{12} mainly by milk, the only source of nutrients for the majority of infants. The purpose of this study is to determine the vitamin B_{12} in human milk before and after receiving orally cyanocobalamin tablet; powdered milk; condensed milk; evaporated milk; and cow's milk and its proparations by radioisotope dilution technique.