Chapter I.

Introduction

Cyanocobalamin is a compound of cobinamide cyanide phosphate 3'-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-benzimidazole inner salt or 5,6-dimethyl benzimidazolyl cyanocobamide. In addition to being a polyacidic base with six weakly basic amide groups, cyanocobalamin is a co-ordination compound of cobalt, with cyano group is replaceable by other groups, e.g. hydroxo or nitroso groups and by acetate, chloride, nitrate and other anions to form other B_{12} analogs. The structure of vitamin B_{12} was first announced at the British Chemical Society Symposium in July, 1955 and at the 3rd International Biochemical Congress in Brussels in August, 1955 by Alxander Todd and team (Cambridge), Dorothy Hodgkin and team (Oxford) and by E. Lester Smith (Glaxo).[1,2]

Cobalamin is refered to all parts of the molecule except the cyano group. A new system for the analogs and degradation products has been proposed to reduce confusion. Systematic nomenclature was introduced by International Agreement in 1959.[3] The term cobalamin (introduced before the chemical structure was known) is frequently used to refer to the vitamin B_{12} molecule without the cyanide group. Vitamin B_{12} itself then become cyanocobalamin. Semisystematic and trivial names are convenient and entirely acceptable. Some of the many compounds related to vitamin B_{12} appear in Table 9. in the

appendix. The schematic structure shown in the table conveniently depicts the two ligands of cobalt lying above and below the plane of the corin ring, which is understood to be a plane paralled to the top and perpendicular to the plane of the page.

In the Table 9, cyano group and 5,6-dimethylbenzimidazole are two ligands of the cobalt atom. Under appropriate conditions, cyano group can be replaced by a ligand with greater affinity for cobalt, yielding hydroxycobalamin with hydroxyl (or the basic product of its combination with H^+, aquocobalamin), nitrocobalamin with nitrite, and similar compounds. The lower ligand can also be replaced. In strong acid, a second H_2O displaces 5,6-dimethylbenzimidazole to form diaquocobalamin in which the nucleotide is bound to the ring system by the phosphate ester and the amide linkages only.

The substitution described above is associated with a significant drop in potency of vitamin B_{12} and is considered to be the decomposition of vitamin B_{12}. The exact mechanism of this decomposition, which is correlated with a significant drop in potency, is not clearly understood.

In 1949, Gakenheimer and Feller observed that a solution containing ascorbic acid and vitamin B_{12} exhibited more than 50 % decomposition of vitamin B_{12} after storage at 28^{o} to 30^{o} for 24 hours[4] . In addition, it has been observed that the presence of 0.5 % phenol does not significantly alter the rate of vitamin B_{12} decomposition resulting from its incompatibility

with ascorsic acid.

In 1950, Trenner, et al.[5] observed some connection between the incompatibility of the vitamin B_{12} and ascorbic acid. Aqueous solution containing about 20 mcg. of pure vitamin B_{12} per ml. and about 20 mg. of pure ascorbic acid per ml. were found to loss approximately 1.5 % of their vitamin B_{12} content per day when stored at room temperature. Such solutions were found to have a pH between 2.5 and 3.0

Bartilucci, et al. [6,7] have found that decomposition products of ascorbic acid (dehydroascorbic acid) facilitated the run down of vitamin B_{12}. Gerbe, et al. [8] have successfully used 70 % sorbitol as a vehicle for products containing ascorbic acid and cyanocobalamin. Stability of these products was attributed specifically to their sorbitol vehicle [8,9]

Bartilucci and Foss [6] reported about the effect of pH up on the stability of solutions of cyanocobalamin and ascorbic acid, alone and in combination. The optinum pH for stabilizing ascorbic acid in solution was found to be above 6.0 but below 7.0. The optimum pH for cyanocobalamin was between 4.5 and 5.0. The most favorable pH for a mixture of cyanocobalamin and ascorbic acid appeared to be between 6.0 and 7.0. In the same report also showed the stability study of these vitamins in combination with some stabilizers such as sorbitol and propylene glycol.

In 1952, Macek and Feller [10] reported some pharmaceutical properties of crystalline vitamin B_{12}. In the anhydrous solid is hygroscopic and may absorb about 12 % moisture.

In 1954, Blitz, Eigen and Gunsberg [11] studied the stability of vitamin B_{12} in a-B-complex solution and reported that vitamin B_{12} was not stable in a-B-complex solution. The destruction of vitamin B_{12} took place in the presence of thiamine and niacinamide at pH 4.25. Oxidation appeared not to be the only factor in the decomposition of vitamin B_{12} in a B-complex solution. The large losses of vitamin B_{12} were found to be a function of the concentration of both thiamine and niacinamide in the solution.

In 1955, Macek and Feller [12] studied the effect of thiamine hydrochloride on the stability of solution of crystalline vitamin B_{12} at pH 3.5 to 4.5. Decomposition of vitamin B_{12} occured at elevated temperatures in the presence of thiamine decomposition products or the thiazole moiety. The amount of decomposed vitamin B_{12} was shown to be related to the stability of the thiamine hydrochloride. Vitamin B_{12} was stable in solution of thiamine HCl and niacinamide at pH 3.5 to 4.5 and in B-vitamin solution at pH 3.0 and 4.5 at normal temperatures. Destruction of vitamin B_{12} in these solution occured at elevated temperature [12]. An apparant cause of the incompatibility was found by Mukherjee and Sen [13,14] that the effect and the presence of thiamine decomposition products i.e.

thiazole compounds may function as reducing agents for cyanocobalamin. Zuck, et al. [15] have found that solutions containing thiamine hydrochloride cyanocobalamin and other factors of the vitamin B_{12} complex, breakdown products of thiamine hydrochloride cause rapid destruction of cyanocobalamin Petel, et al. [16] have reported that riboflavin may absorb light which causes rapid and complete photooxidation of cyanocobalamin. This photolysis is accelerated by niacinamide and inhibited by antioxidants such as thiourea and ethylhydrocaffeate.

In 1956, Blitz, Eigen and Gunsberg [17] studied the stability of vitamin B_{12} in B-complex injectable solutions. The mechanism of decomposition of vitamin B_{12} in the presence of thiamine and niacinamide was discussed and also found that both partially decomposed thiamine and the thiazole moiety of thiamine destroyed the vitamin B_{12} in aqueous solution at pH 4.25 in the absence of niacinamide.

In 1958, Gambier and Rahn [18] studied the vitamin B_{12} in the presence of vitamin B_1 and niacinamide in aqueous combinations and obtained the best results for vitamin B_{12} at pH 3.3. From the observation of Macek and Feller indicated that the ideal range of pH was from 3.0 and 4.0 [12,19]

Numerous attempts have been made to find a suitable stabilizer to maintain the constant amount of vitamin B_{12}. However, none has been found. This failure may be accounted

to the fact that not only the presence of other B-vitamins but also the reducing effect of vitamin C containing in the preparation and the relatively high ambient temperature of the storage condition in our country. All of these can accelerate the degradation of vitamin B_{12}. Therefore, it is nearly impossible to obtain a vitamin B complex containing vitamin C which meets standard set by the FDA. However, it is generally known among the manufacturers that if one wants to produce a vitamin B-complex preparation containing vitamin B_{12} that meets the standard of the FDA, one has to put the extra amount of vitamin B_{12} to compensate for the degraded product. This practice is not beneficial both to the manufacturers and to the public while there is no technical know how available yet, this malpractice is still persisted.

In order to solve this problem straight forwardly, this study is designed to search for a new and effective stabilizer. The hypothesis is that the effective stabilizer should have some chemical structure similar to Vitamin B_{12}. Therefore, to prove this hypothesis, xanthine which has structure similar to vitamin B_{12} should be selected. Other substances will also be used for comparison for example, the effects of various agents such as amino acids, sugar alcohols on vitamin B_{12} stability in the vitamin B-complex containing vitamin C injection at a designed pH will be studied. The concentration of vitamin B_{12} will be checked spectrophotometrically from time to time for the period of 2 months at room temperature after

separation by column chromatography using Amberlite CG-50 Type II as an adsorbent. The suitable concentration of the stabilizer should be the effective lowest concentration.

It is well recognized that vitamin B complex containing vitamin C is very unstable. To make preparation without available effective stabilizer is a big loss. The manufacturers have to pay more for the extra amount of vitamin B_{12} and at the same time risk the legal problems while the patients who need the therapeutic value of the vitamin are in great disadventage.