# A Repository Vitamin $B_{12}$ Preparation: Cyanocobalamin Zinc Tannate

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INTRAMUSCULARLY administered crystalline cyanocobalamin is rapidly excreted into the urine, and the larger the dose, the smaller is the percentage retained in the body.[1] It would appear that regardless of the amount of vitamin $B_{12}$ injected, the body has a limited capacity to bind and retain the vitamin,[2] and the free or unbound fraction of an injected dose of cyanocobalamin is eliminated rapidly into the urine.

It is probable that the serum concentration of vitamin $B_{12}$ in man falls to deficiency levels (less than 100 $\mu\mu$g./ml.) only when the tissue reservoirs, chiefly the liver, have been almost depleted.[3] Indeed, in pernicious anemia the liver has been found to contain no vitamin $B_{12}$.[4] By calculation, the normal serum concentration (350 to 560 $\mu\mu$g./ml.) represents only 1 or 2 $\mu$g. of vitamin $B_{12}$ in the total circulation,[3] and hence, the injection of a very small amount of cyanocobalamin can restore circulating concentrations to normal. The mere restoration of the circulating quantities of the vitamin does not represent the true objective of therapy of a patient with deficiency and exhausted tissue reservoirs.

Regardless of the size of the intramuscularly administered dose of crystalline cyanocobalamin, only that fraction which is retained in the body can go toward repletion of body stores. It follows that prolongation of the time during which cyanocobalamin is circulated within the body favors retention. The relatively insoluble cyanocobalamin zinc tannate§ complex has the potentials of slow release from the site of injection and prolonged periods of time during which body proteins can bind and retain vitamin $B_{12}$.

The present study was directed toward discovering the extent to which cyanocobalamin zinc tannate merits the designation of a repository form of vitamin $B_{12}$.

## MATERIALS AND METHODS

The lyophilized, limitedly soluble cyanocobalamin zinc tannate complex is supplied in multidose vials, and when restored with the diluent, provides an aqueous suspension containing 500 $\mu$g. cyanocobalamin, 1.2 mg. zinc and 2.6 mg. tannic acid per ml.[5] There was minimal pain following its intramuscular injection in quantities of either 1 or 2 ml.

A series of twenty-five persons was studied, all of whom were healthy ambulatory adults, varying in age from nineteen to fifty-three years, and in weight from 108 to 230 pounds. The group consisted of twenty-one women and four men. Eleven persons received intramuscular injections of 500 $\mu$g. and fourteen were injected with 1,000 $\mu$g. Pretreatment blood samples were drawn, and after treatment, samples were obtained from some patients at one, three and six hours and then at four, eight, twelve, sixteen, nineteen, twenty-four and twenty-seven days thereafter, and from others at slightly different times, three, seven, eleven, fifteen, eighteen and twenty-one days. Blood samples were allowed to clot in the refrigerator overnight, the serum har-

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§ Cyanocobalamin zinc tannate is commercially available under the trademarks Bevitam® (Merrell) and Depinar® (Armour). The material used in this study was Depinar, Lot No. U202, supplied by Dr. Joseph Hubata of Armour Pharmaceutical Company.

TABLE I

Serum $B_{12}$ Concentrations ($\mu\mu$g.) Following a Single Intramuscular Injection of Vitamin $B_{12}$ Tannate* (500 $\mu$g.)

| Patient | Sex | Age | Weight (lb.) | Premedication | Time After Dose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Day 1 | | | Day 4 | Day 8 | Day 12 | Day 16 | Day 19 | Day 24 | Day 27 |
| | | | | | 1 hr. | 3 hr. | 6 hr. | | | | | | | |
| B | F | 49 | 245 | 520 | 2,300 | 2,400 | 2,200 | 900 | 1,000 | 490 | 490 | 460 | 460 | 340 |
| F | F | 33 | 165 | 570 | 1,200 | 1,400 | 2,000 | 3,800 | 2,100 | ... | 1,200 | 780 | 610 | 750 |
| K | F | 53 | 162 | 190 | 1,200 | 1,100 | 800 | 375 | 100 | 250 | 290 | 230 | 300 | 260 |
| P | F | 49 | 134 | 550 | 1,700 | 2,600 | 2,500 | 1,800 | 3,000 | 2,700 | 1,640 | 1,260 | 960 | 1,090 |
| SC | F | 38 | 168 | 580 | 2,800 | 2,300 | 1,900 | 2,600 | 2,100 | 420 | ... | ... | 410 | 490 |
| SH | F | 25 | 135 | 330 | 450 | 700 | 150 | 500 | 700 | 730 | 630 | 520 | 410 | 330 |
| Average | | | | 457 | 1,608 | 1,750 | 1,592 | 1,663 | 1,500 | 918 | 850 | 650 | 525 | 543 |

| Patient | Sex | Age | Weight (lb.) | Premedication | Time After Dose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Day 1 | | | Day 3 | Day 7 | Day 11 | Day 16 | Day 19 | Day 22 | Day 25 |
| | | | | | 1 hr. | 3 hr. | 6 hr. | | | | | | | |
| WMM | F | 27 | 110 | 640 | 2,700 | 1,750 | 1,000 | 1,050 | 800 | 920 | 980 | 640 | 560 | 720 |
| HW | M | 31 | 230 | 200 | 900 | 750 | 820 | 700 | 560 | 780 | 440 | 410 | 360 | 370 |
| BD | F | 30 | 138 | 140 | 1,200 | 1,000 | 1,500 | 1,600 | 1,800 | 2,075 | 1,700 | 1,130 | 1,200 | 1,200 |
| MMcL | F | 37 | 110 | <100 | 500 | 500 | 600 | 900 | 600 | 240 | 200 | ... | ... | ...† |
| RG | M | 27 | 150 | 240 | 1,000 | 4,100 | 1,500 | 2,700 | 1,000 | 920 | 1,260 | ... | 800 | 400 |
| Average | | | | 264 | 1,260 | 1,620 | 1,150 | 1,562 | 1,300 | 987 | 916 | 727 | 730 | 672 |

* Depinar Lot No. U202.
† On the thirty-first day after injection serum concentration of vitamin $B_{12}$ was 140 $\mu\mu$g.

vested into 10 ml. sterile serum vials, and stored at minus 20°c. until assayed.

Serum concentrations were estimated by microbiologic assay using the Lactobacillus leischmania method.[6] All specimens from any particular patient were assayed during the same test, so that assay conditions were constant for that patient.

## RESULTS

The serum vitamin $B_{12}$ concentrations following single intramuscular injections of 500 $\mu$g. of cyanocobalamin zinc tannate are presented in Table I, and those following single injections of 1,000 $\mu$g. of the same preparation are presented in Table II. The different time schedules for sampling are obvious.

It should be noted that patient M. McL., female, age thirty-seven, and L. W., female, age forty-two, both showed pretreatment serum $B_{12}$ concentrations less than 100 $\mu\mu$g./ml. No reason could be found for these abnormally low values; blood counts were normal, free gastric acidity was demonstrated, no neurologic symptoms were found, and there was no familial history of pernicious anemia. Further, observations on patient M. McL. are presented in Table III.

## COMMENTS

It has been well established that cyanocobalamin is preponderantly transported and bound by $a_1$ and $a_2$ globulins,[2,7,8] and free or unbound cyanocobalamin is rapidly excreted by the kidneys. Under normal conditions of health, it may be postulated that the serum concentrations of vitamin $B_{12}$ will be the resultant of (1) the small daily quantities of the vitamins derived from the diet that are absorbed through the gastrointestinal mucosa; (2) the modest daily metabolic requirement for the vitamin; and (3) the capacity of body tissue to bind and retain cyanocobalamin. A delicate balance is struck between these various factors under normal circumstances. The estimated quantities of vitamin $B_{12}$ in the average diet[9] are 0.2 to 3.5 $\mu$g.; the daily requirement[9] for the vitamin, 0.1 to 1

TABLE II

Serum $B_{12}$ Concentrations ($\mu\mu$g.) Following a Single Intramuscular Injection of Vitamin $B_{12}$ Tannate* (1,000 $\mu$g.)

| Patient | Sex | Age | Weight | Premedication | Time After Dose: Day 1 1 hr. | Day 1 3 hr. | Day 1 6 hr. | Day 3 | Day 7 | Day 11 | Day 16 | Day 19 | Day 22 | Day 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JW | M | 40 | 167 | 200 | 2,000 | 1,600 | 1,700 | 1,800 | 800 | 600 | 500 | 450 | 460 | 400 |
| EQ | M | 37 | 175 | 440 | 2,600 | 3,000 | 2,100 | 2,200 | 788 | 560 | 800 | 400 | 480 | 460 |
| RL | F | 30 | 130 | 200 | 2,600 | 2,300 | 1,650 | 1,350 | 1,000 | ... | 520 | 660 | 500 | 470 |
| LW | F | 42 | 108 | <100 | 400 | 3,000 | 1,500 | 1,100 | 1,300 | 880 | 580 | 400 | 360 | 460 |
| EB | F | 43 | 140 | 800 | 1,200 | 1,000 | 2,900 | 2,500 | 1,100 | 500 | 660 | 560 | 550 | 420 |
| DS | F | 39 | 135 | 630 | 3,200 | 2,800 | 2,250 | 2,000 | 2,500 | 2,200 | 3,150 | 2,000 | 1,300 | 580 |
| | Average | | | 395 | 2,000 | 2,283 | 2,017 | 1,825 | 1,248 | 948 | 1,035 | 745 | 608 | 465 |

| Patient | Sex | Age | Weight | Premedication | Time After Dose: Day 1 1 hr. | Day 1 3 hr. | Day 1 6 hr. | Day 4 | Day 8 | Day 12 | Day 16 | Day 19 | Day 24 | Day 27 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BL | F | 19 | 154 | 300 | 1,500 | 1,000 | 1,500 | 2,100 | 1,900 | ... | 1,460 | 600 | 690 | 200 |
| CO | F | 39 | 150 | 200 | 1,700 | 2,900 | 2,600 | 1,400 | 1,400 | 960 | 860 | 760 | 610 | ... |
| CO | F | 41 | 169 | 310 | 2,300 | 2,600 | 1,900 | 1,900 | 2,100 | 680 | 650 | 520 | 570 | 440 |
| DE | F | 36 | 138 | 250 | 3,300 | 3,100 | 1,100 | 600 | 950 | 250 | 230 | 280 | 390 | 100 |
| H | F | 42 | 136 | ... | 2,400 | 1,600 | ... | 1,200 | 350 | 340 | 360 | 300 | 280 | 180 |
| SM | F | 50 | 180 | 400 | 2,100 | 3,000 | 2,000 | 2,700 | 3,200 | 1,640 | 1,300 | 1,040 | 860 | 960 |
| ST | F | 33 | 150 | 310 | 1,400 | 4,200 | 1,700 | 800 | 1,200 | 1,130 | 980 | 1,620 | 1,010 | 1,030 |
| R | F | 20 | 112 | ... | ... | 1,800 | 1,600 | 850 | ... | 680 | 600 | 380 | 270 | 210 |
| | Average | | | 295 | 2,100 | 2,525 | 1,771 | 1,444 | 1,588 | 811 | 805 | 688 | 585 | 448 |

* Depinar Lot No. U202.

TABLE III

Serum $B_{12}$ Concentrations Observed during Treatment with Cyanocobalamin Zinc Tannate* over a Period of 191 Days

| Date | Amount ($\mu$g.) | Days after Injection: 0 (Control) | 3 | 4 | 5 | 7 | 8 | 10 | 11 | 14 | 16 | 30 | 31 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8/24/59 | 500 | <100 | 900 | ... | ... | 600 | ... | ... | 240 | ... | 200 | ... | 140 | <100 |
| 11/16/59 | 1,000 | ... | ... | 950 | ... | ... | 950 | ... | ... | (11/30/59) 530 | ... | ... | ... | ... |
| 11/30/59 | 1,000 | ... | ... | 2,080 | ... | 1,760 | ... | ... | 900 | (12/14/59) 660 | ... | ... | ... | ... |
| 12/14/59 | 1,000 | ... | ... | ... | 2,200 | ... | ... | (12/28/60) 2,800 | ... | ... | ... | ... | ... | ... |
| 12/28/59 | 1,000 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 380 | ... | ... |
| 2/1/60 | 1,000 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 840 | ... | ... |

* Depinar Lot No. U202.

$\mu$g. and the amount of circulating vitamin, 1 to 2 $\mu$g., which reflects the normal serum concentration of 350 or 560 $\mu\mu$g./ml.[3,10] In a healthy person the liver is always a storehouse containing 1,000 to 2,000 $\mu$g. of $B_{12}$[4,11] so that if all extraneous sources of the vitamin are cut off, it will be three to six years before a deficiency state is established.[11]

In a group of our patients followed up over a period of years, it has been established that each person has a more or less constant serum concentration of $B_{12}$, determined by his own physiology. Various schedules of therapy are capable of temporarily altering the levels at which vitamin $B_{12}$ circulates; however, shortly after the discontinuance of therapy, the

patient re-establishes his "normal." These observations clearly suggest a defined limitation of the patient to bind and retain cyanocobalamin. Interestingly enough, the two conditions in which serum concentrations of vitamin $B_{12}$ have most consistently been shown to be elevated, acute or chronic myelogenous leukemia[2,8] and hepatocellular jaundice,[12] are both conditions in which globulins are abnormal

There is a sharp limitation of time during which the body tissues and blood proteins have the opportunity of binding the vitamin when cyanocobalamin is administered by injection. One must assume either that (1) during the period of six to eight hours following the injection of crystalline vitamin $B_{12}$, all unsaturated body proteins bind the vitamin to their fullest capacity, or (2) the time during which excess quantities of vitamin circulate following an intramuscular injection is inadequate for the full saturation of proteins capable of binding $B_{12}$.

Extensive work[1] has shown that as intramuscular injections exceed 30 to 40 $\mu$g., the amount of the vitamin excreted into the urine progressively increases, certainly implying a limited capacity to bind and retain the vitamin. The question remains, however, as to whether the limited time during which the proteins have an opportunity to bind the circulating quantities of $B_{12}$ may be too short for optimal or complete saturation to occur. The interesting properties of cyanocobalamin zinc tannate seem to bear upon this inquiry.

In Tables I and II are the individual data showing serum concentrations of twenty-five persons over twenty-five and twenty-seven day periods following the intramuscular injection of 500 and 1,000 $\mu$g., respectively, of cyanocobalamin zinc tannate. Variations from person to person are obvious, but there is a general and uniform trend. From the premedication control level, there is a prompt rise to peak concentrations within the first six hours following injection, and then a slow decline to pretreatment level. This decline occurred in a few patients after as short a time as eight days, but in others it had not occurred within twenty-seven days, at which time the study was terminated. The *average* figures clearly show an elevation of serum concentration above pretreatment level for the entire duration of the study.

In previous publications, we have emphasized the inherent error of the microbiologic assay of vitamin $B_{12}$[13] and have joined others[14] in the statement that a 20 per cent plus or minus error should be conceded. If one applies the harshest criteria to the data in Tables I and II, the following adjustments can be made. If the *highest* average pretreatment concentration, 467, is adjusted upward by 20 per cent to 548 $\mu\mu$g./ml., and if the *lowest* average serum concentration, 805, following treatment is adjusted downward by 20 per cent to 644 $\mu\mu$g./ml., it still can be concluded that a single intramuscular injection of 500 or 1,000 $\mu$g. of cyanocobalamin zinc tannate elevates serum concentrations above pretreatment levels for at least sixteen days.

Peak serum concentrations, higher after 1,000 $\mu$g. than after 500 $\mu$g., are observed during the first three hours after injections. However, the average serum concentrations thereafter are not strikingly different. Our observation that serum concentration promptly reached its peak is at variance with previous observations that ". . .there was no large increase in serum level of vitamin $B_{12}$ activity until the end of the first day (1,600 $\mu\mu$g./ml.),"[15] but does conform with the statement of others that "a certain portion of the vitamin $B_{12}$ (about 10 to 15 per cent in the CZT suspension, is rapidly absorbed, as shown by the rat excretion during the first six hours after injection."[5] It should be emphasized that a "normal" serum concentration of vitamin $B_{12}$, 350 to 560 $\mu\mu$g. ml., represents only 1 to 2 $\mu$g. of the vitamin in the total circulation. It is clear then that the elevation of serum concentration to 1,000 or 2,000 $\mu\mu$g./ml. reflects an increase of only a few micrograms of cyanocobalamin.

*In vitro* studies indicate a limit to the amount of cyanocobalamin that blood proteins can bind[2] and this limit is approximately 350 to 400 $\mu\mu$g./ml. It seems reasonable to assume, therefore, that when values of serum concentration exceed this figure, those amounts in excess

of it represent free, or loosely bound, $B_{12}$. The method of assay employed in the present study measured only "total vitamin $B_{12}$ activity."

There are many analogies between the dose response curves following the intramuscular injection of vitamin $B_{12}$ and penicillin. The rapidity with which the serum concentrations decline following the intramuscular injection of crystalline vitamin $B_{12}$ is quite similar to that observed following the intramuscular injection of sodium or potassium penicillin G. The recovery of a major portion of the injected dose, 60 to 80 per cent, within six to eight hours following injection, is also similar. Despite the use of vehicles designed to retard the absorption of penicillin, little was accomplished until a relatively insoluble salt of penicillin was prepared. The analogy between the repository forms of penicillin and cyanocobalamin zinc tannate is inescapable. Indeed, prolonged penicillemia three to four weeks following the injection of dibenzylethylene diamine penicillin (DBED) is quite comparable to our findings of a two to four week elevation of serum concentrations of the vitamin.

Considering cyanocobalamin zinc tannate to be a repository vitamin $B_{12}$ preparation, it is interesting to make certain theoretic calculations. If it is granted that a normal serum vitamin $B_{12}$ concentration of 500 $\mu\mu$g. represents 1 to 2 $\mu$g. of the vitamin in the total circulation, and that this normal circulating value could be doubled by doubling the amount of available cyanocobalamin, it is clear that an additional 1 or 2 $\mu$g. of vitamin $B_{12}$ could give a considerably elevated serum concentration for a variable period of time, dependent upon the rate of utilization and/or elimination. If one arbitrarily assumes that the tabularized data on serum concentrations presented in this paper justify the statement that an injection of 500 $\mu$g. of cyanocobalamin zinc tannate produced an average elevation of serum concentrations for a period of twenty-five days, one can calculate that the release of approximately 1 $\mu$g. of the vitamin per hour from the depot at the site of injection would account for the observed results. Obviously, vitamin was not released from the site of injection at any such *regular* rate. All the observations suggest that there was a more rapid release in the first days following injection and thereafter a steadily decreasing effect upon serum concentrations.

The observations made on patient M. McL. are of particular interest (Table III). From a pretreatment level of less than 100 $\mu\mu$g./ml., regarded as the deficiency range, the patient showed a prompt peak concentration which persisted for some time following the intramuscular injection of 500 $\mu$g. of cyanocobalamin zinc tannate. At the end of thirty-one days, the pretreatment level was approximated and at some time prior to the fifty-fourth day, the patient had returned to her original status. If prolonged circulation of high concentrations of $B_{12}$ offers a better opportunity for body proteins capable of binding $B_{12}$ to saturate themselves, and the normal daily requirement for vitamin $B_{12}$ is approximately 1 $\mu$g., one might have anticipated that the patient would not have returned to pretreatment level so rapidly.

Following this first injection, "normal" (560 $\mu\mu$g./ml.) or above normal serum concentrations were attained for a period of at least seven days. Following the second injection of cyanocobalamin zinc tannate of 1,000 $\mu$g., the concentrations of "normal" or above were maintained for a period of approximately fourteen days.

Without waiting a return to pretreatment levels, this patient was given several additional intramuscular injections of 1,000 $\mu$g. of repository vitamin $B_{12}$. From the previous experience with this patient, it is suggested that approximately thirty days were required to deplete a particular depot. Accordingly injections given at intervals shorter than thirty days should give rise to a cumulative effect. This appears to have occurred in this patient.

It is postulated that each person has a finite quantity of protein in his body capable of binding and retaining cyanocobalamin. Although a repository form of vitamin $B_{12}$, such as cyanocobalamin zinc tannate, can produce high circulating serum concentrations for prolonged periods of time, thus furnishing an optimal period in which the body can saturate itself, it is the person's total pool of

protein capable of binding $B_{12}$ that constitutes the limiting factor of retention of the vitamin in the body. Amounts in excess of the ability of the body to bind, are excreted.

## SUMMARY AND CONCLUSIONS

Cyanocobalamin zinc tannate, when injected intramuscularly, is released slowly from the site of its injection and can appropriately be called a depot or repository type of vitamin $B_{12}$. When twenty-five persons were injected intramuscularly with doses of 500 or 1,000 $\mu$g., peak serum concentrations of vitamin $B_{12}$ were observed within three hours, and elevated concentrations persisted for periods up to twenty-seven days. A few patients showed a return of serum concentrations to pretreatment levels within eight and ten days.

Cyanocobalamin zinc tannate would appear to be a true repository vitamin $B_{12}$ preparation and one which should make unnecessary the repeated intramuscular injections of small quantities of vitamin $B_{12}$ for the repletion of the exhausted body stores that are observed in true vitamin $B_{12}$ deficiency. The slow release over a period of many days of quantities of vitamin $B_{12}$ that are greatly in excess of daily requirements, should make the treatment of "suboptimal nutritional states" that have been postulated (1) more economical, (2) more convenient and comfortable for the patient, and (3) less wasteful of personnel time in administering treatment. Perchance large quantities of circulating vitamin $B_{12}$ do exert pharmacologic effects over and above the essential enzymatic function, then this repository form of cyanocobalamin should lend itself to the pursuit of such inquiry. It is suggested that cyanocobalamin zinc tannate may have great value in the institutional practice of medicine and in the treatment of large undernourished populations, when available skilled hands are few and the need for economy in therapy is mandatory. There would appear to be an analogy between the mass use in public health of repository penicillins and the potentials for use of this new cyanocobalamin zinc tannate.

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