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Cobalt-57 Labeled Vitamin B-12 Plasma Levels for the Differential Diagnosis of Macrocytic Anemias[1,5]

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The value of isotopically labeled Vitamin B-12 as a diagnostic agent in the study of Pernicious Anemia and related disorders, is now well established. The work done during the early part of the past decade by Heinle (1) and Schilling (2) in this country, and Mollin (3) and Booth (4) in England, has advanced the basic understanding of the role of Vitamin B-12 in the pathogenesis of Pernicious Anemia as well as in other disorders of intestinal absorption.

Several methods have been developed for the determination of intestinal absorption of orally administered radiocyanocobalamin. Successful methods in use are: the fecal recovery procedure (1); measurement of hepatic uptake (5); urinary excretion technic (2); and the measurement of blood levels of labeled Vitamin B-12. The test most popular in the United States had been the urinary excretion procedure as described by Schilling (2). The necessity for patient cooperation and the difficulties inherant to any complete 24 hour urine collection especially in the elderly, have lead to a search for additional methods of evaluating Vitamin B-12 intestinal absorption.

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[5]Presented, in part, at the 7th annual meeting of the Society of Nuclear Medicine Estes Park, Colorado, June 23, 1960.

The measurement of blood levels of orally administered cobalt labeled Vitamin B-12 would appear to be an obvious answer to the problem of urinary collection. Unfortunately, the problems created by the large tracer amounts of cobalt-58 or cobalt-60 labeled Vitamin B-12 necessary to produce significant blood radioactivity have not allowed this procedure to be used routinely. The recent availability of another cobalt isotope, cyclotron produced, 270 day half-life cobalt-57, has made larger tracer doses possible. The fact that cobalt-57 emits no particulate energy and but one gamma-ray (0.123 MeV) makes it a suitable material for plasma level determinations.

METHODS AND MATERIALS

This report presents our experiences with cobalt-57 labeled Vitamin B-12 for the determination of plasma levels and urinary excretion in 98 hematologically normal individuals and in 38 patients with Pernicious Anemia. The diagnosis of Pernicious Anemia was established by clinical history and physical examination, peripheral blood and bone marrow studies, gastric analysis and, in a few patients, by measurement of serum Vitamin B-12. The urinary excretion procedure was performed on 150 hematologically normal individuals and in 38 patients with Pernicious Anemia. All of the latter patients were tested initially without and again with the addition of purified Intrinsic Factor concentrate. The original lot of cobalt-57 labeled Vitamin B-12[1] had a specific activity of 600 microcuries per milligram. Higher specific activity material (one microcurie per microgram) became available later in the study and it was this material which was used for the plasma level determinations.

URINARY EXCRETION PROCEDURE

The method is essentially that of Schilling (2) modified to our own laboratory equipment. Cobalt-57 labeled Vitamin B-12, (0.56 μg B-12 and 0.56 μC cobalt-57 was given to the fasting patient in a capsule. A 24 hour urine collection was begun simultaneously with administration of the tracer dose. Two hours later, one milligram of nonradioactive Vitamin B-12 was given parenterally to saturate the B-12 binding protein of the blood and allow the labeled material to be excreted in the urine. At the end of the collection period, total urine volume was recorded and a 100 ml aliquot placed in a volumetric flask and counted on top of a 2 x 2 inch crystal well-type scintillation detector. The corrected count was then applied to the total urine volume and divided by the counts obtained from a 100% dose standard counted under similar conditions. Counting error was less than five per cent using this method. Counting efficiencies for three cobalt isotopes counted in this manner were calculated; cobalt-60 = 1.2%; cobalt-58 = 4% and cobalt-57 = 7 per cent.

The per cent of administered dose appearing in the urine in a 24 hour period in 150 hematologically normal individuals ranged from 7%-to-37% (Mean = 30%).

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[1]Obtained from Mr. Geoffrey Gleason, Abbott Laboratories, Oak Ridge, Tennessee. (October, 1959)

None of the patients with Pernicious Anemia excreted more than one per cent of the administered dose prior to the administration of Intrinsic Factor concentrate. When the test was repeated with the addition of 60-to-100 mg purified Intrinsic Factor concentrate to the test dose, the per cent of administered dose appearing in the urine in 24 hours in the Pernicious Anemia patients rose to within the normal range.

PLASMA RADIOACTIVITY PROCEDURE

Ninety-eight hematologically normal individuals received oral tracer doses of 1.0 μC of cobalt-57 labeled Vitamin B-12 in capsule form. The test material had a specific activity of 1.0 μC per μC and was given with the patient in a fasting state. No nonradioactive Vitamin B-12 was administered to these patients. Initially, heparinized blood samples were obtained at 2, 4, 6, 8, 10, 12, and 24 hours after the tracer dose, and 4 ml aliquots of plasma counted for a significant number of counts in a standard well-type scintillation detector with a medical spectrometer[1] set to discriminate against gamma energies below 50 keV and above 150 keV. Later in the study, only the 8, 10 and 12 hour samples were found to be necessary. An aliquot of the 100% dose standard was counted in a similar manner and results expressed as per cent of administered dose per liter of plasma. The counting efficiencies for cobalt-57 = 90% and for cobalt-58 = 45% using this procedure. Cobalt-60 was not tested.

Eighty of the 98 normal individuals had maximum concentration of plasma activity in the eight-hour sample while the remaining 18 reached peak activity at 10 or 12 hours. The curve of plasma radioactivity is not significantly different from that reported for cobalt-58 Vitamin B-12 (8). The results obtained in normals are summarized in Table I.

TABLE I

PLASMA LEVELS OF COBALT-57 VITAMIN B-12 IN 98 NORMAL INDIVIDUALS (PER CENT OF DOSE PER LITER PLASMA)

Time in Hours	*8 Hours*	*10 Hours*	*12 Hours*
Mean (%)	0.76	1.05	0.95
Range (%)	0.55 0.95	0.88 1.27	0.78 1.15

The results obtained when the 38 patients with Pernicious Anemia were tested, are shown in Table II. The values obtained for the eight-hour samples from the group of normal individuals and from those with Pernicious Anemia were subjected to statistical analysis. The analysis of variance and regression of values between the two groups was found to be significant ($p = 0.0001$). In ad-

[1]Nuclear-Chicago Corporation Model 132B Analyzer-Computer.

dition, there was no evidence of crossover between the normal and Pernicious Anemia groups using the eight-hour plasma sample as a point of reference.

TABLE II

PLASMA LEVELS OF COBALT-57 VITAMIN B-12 IN 38 PATIENTS WITH PERNICIOUS ANEMIA

(PER CENT OF DOSE PER LITER PLASMA)

Time in Hours	*8 Hours*	*10 Hours*	*12 Hours*
Mean (%)	0.16	0.21	0.22
Range (%)	0.00 0.19	0.00 0.23	0.00 0.25

Each of the patients with Pernicious Anemia was given a second 1.0 microcurie tracer dose of cobalt- (57) Vitamin B-12 along with a capsule of 60-to-100 mg of purified Intrinsic Factor Concentrate[1] and heparinized blood samples obtained at 8, 10 and 12 hours. Four milliliter aliquots of plasma, as well as a 100% dose standard were counted in a manner similar to the initial test and the results expressed as per cent of dose per liter of plasma. As noted in Table III, all patients tested in this manner demonstrated an increase in plasma concentration of labeled Vitamin B-12 over the initial levels, although seldom equal to the level attained by the normals.

TABLE III

PLASMA LEVELS OF COBALT-57 VITAMIN B-12 IN 38 PATIENTS WITH PERNICIOUS ANEMIA FOLLOWING ADDITION OF 60-TO-100 MG INTRINSIC FACTOR CONCENTRATE

(PER CENT OF DOSE PER LITER PLASMA)

Time in Hours	*8 Hours*	*10 Hours*	*12 Hours*
Mean (%)	0.57	0.84	0.81
Range (%)	0.48 0.71	0.62 1.27	0.69 1.03

[1]Obtained from E. R. Squibb & Sons, New Brunswick, New Jersey.

DISCUSSION

The superiority of cobalt-57 labeled Vitamin B-12 over the other commonly employed cobalt isotopes, is not limited to determination of plasma levels. Prior to 1956, the biologic half-life of Vitamin B-12 in man was thought to be 8-to-10 days. Additional basic information concerning body distribution and biologic half-life of Vitamin B-12 have been supplied by the work of Reizenstein (8) and Schloesser (9). The more generally accepted figure for biologic half-life of Vitamin B-12 is now 200-to-400 days averaging about one year. In addition, it has been well established that about 40% of an absorbed dose of labeled Vitamin B-12 concentrates in the liver where it remains throughout decay. If this knowledge is applied to cobalt-60 labeled Vitamin B-12 with its five year physical half-life, a value for effective half-life for this material becomes 328 days. Considering that with each disintegration, cobalt-60 emits beta particles with an average energy of 0.306 MeV and two gamma rays of 1.172 and 1.332 MeV energies, the value of cobalt-60 Vitamin B-12 as a routine testing agent in man, appears to be limited.

TABLE IV

RADIATION DOSE DELIVERED TO THE LIVER BY ONE MICROCURIE OF COBALT LABELED VITAMIN B-12

Isotope	*Half-Life in Days*		*Energies in MeV*		*Liver Dose in Mrads*
	Physical	*Effective*	*Beta*	*Gamma*	
Cobalt-57	270	155		0.123	48
Cobalt-58	72	60	0.48	0.51 0.81	512
Cobalt-60	1900	329	0.31 0.48	1.17 1.33	646

SUMMARY

The results of studies of plasma levels and urinary excretion values following oral administration of tracer quantities of another cobalt isotope, the 270 day half-life cobalt-57, are presented. Tests were performed in 98 normal individuals and in 38 patients with Pernicious Anemia, without and with the addition of Intrinsic Factor Concentrate they remained lower than the normal values. Significant statistical separation between normals and Pernicious Anemia groups was demonstrated using the eight-hour plasma sample as a reference. Of the cobalt isotopes in general use, cobalt-57 delivers by far the smallest radiation dose to the liver and has the best counting efficiencies when standard hospital radioisotope laboratory equipment is used.

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