$Co^{58}B_{12}$ ABSORPTION, PLASMA TRANSPORT AND EXCRETION IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS, SOLID TUMORS AND NON-NEOPLASTIC DISEASES *

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Several investigators have documented the fact that chronic myelocytic leukemia (CML) is associated with increases in serum vitamin B_{12} concentrations which may be of the order of 20 to 40 times the normal value (1–4). Less striking and less consistent elevations have been found in other myeloproliferative disorders. These include acute myelocytic leukemia (1, 3, 4), myeloid metaplasia (2–4), polycythemia vera associated with myelocytic leukemia (4) and Di Guglielmo's disease (5). Elevations have also been reported in patients with solid tumors when there are metastases to the liver (3, 6). The present study was designed to investigate *in vivo* the role that gastrointestinal absorption, plasma transport and renal excretion of vitamin B_{12} play in producing this abnormality.

Previous *in vivo* studies have dealt with the fate of a large intravenously administered dose of radioactive B_{12} (7, 8). In the present studies a dose of approximately 0.5 μg of $Co^{58}B_{12}$ was chosen, since this is within the limits of the estimated daily requirement of B_{12} (9). Oral administration was used so that gastrointestinal absorption could be evaluated and a physiologic pattern of plasma concentrations and excretion could be obtained. Twenty-four subjects have been studied, six of whom had CML.

MATERIALS AND METHODS

Materials. $Co^{58}B_{12}$ [1] had an original specific activity of 3.0 μc per μg. The biologic activity of the material used was confirmed by microbiologic assay (*Lactobacillus leichmannii*) and found to agree within 93 per cent of that given by the manufacturer.

Dosage and administration. The dose administered ranged between 0.33 and 0.56 μg of $Co^{58}B_{12}$. One subject, J.G., received 2.7 μg. Because of the relatively short 72 day half-life of Co^{58}, the calculated activity of the administered dose ranged between 0.3 and 1.4 μc, depending on the time of its administration. After an overnight fast, the subject drank the labeled vitamin along with 50 ml of distilled water. Breakfast was deferred for 1 hour.

Plasma concentrations. Ten ml of heparinized blood was drawn from an antecubital vein at 0, 3, 4.5, 6, 9, 12, 24, 36, 48 and 72 hours and then daily for at least 10 days. In a few studies, bloods were drawn at intervals up to 8 weeks. Plasma was separated by centrifugation and transferred to a 4 ml counting vial for radioassay.

Fecal excretion. Individual stools were collected in metal containers and were assayed for gross activity over the open well of a scintillation counter to evaluate the day-to-day pattern of excretion. Stools were then combined into 4-day pools and homogenized. A 100 g aliquot of each homogenized pool was placed in a 500 ml bottle and counted in the open well of a scintillation counter. A standard was diluted to 100 ml and counted in the same manner. The total fecal excretion over a 12 to 16 day period following administration of $Co^{58}B_{12}$ was determined and expressed as percentage of dose administered. This value subtracted from 100 per cent gave the percentage of dose absorbed.

Urine excretion. Twenty-four-hour urines were collected for 14 days, 300 ml aliquots of each 24-hour specimen were counted in a manner similar to that used for fecal homogenates.

Radioassay. Plasma, feces and urine were counted in a thallium-activated sodium iodide well-type scintillation counter.[2] Plasma samples were counted for a sufficient period of time to give a counting error of less than 5 per cent. Plasma activity was at a low level but at the time of peak activity was always 8 to 30 SD above background. The average background during the course of these studies was 183 cpm. Twelve cpm was equal to twice the SD of the average background. A suitably diluted 4 ml standard of $Co^{58}B_{12}$ was counted each day, and

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[1] Obtained from Merck and Co., Rahway, N. J.

[2] Nuclear Chicago scaler, model number D181, and radiation analyzer, model number 1810.

the radioactivity of each plasma sample was converted to $\mu\mu$g of $Co^{58}B_{12}$ per ml plasma. Fecal specimens were counted with an error of less than 3 per cent. Urine specimens were counted for 20 minutes to demonstrate negligible radioactivity.

Microbiologic assay. Blood specimens from fasting patients were drawn on the morning of the study prior to administration of the tracer dose and assayed in quadruplicate for their B_{12} concentration by a modification, described in detail elsewhere (3), of the USP method using *L. leichmannii,* A.T.C.C. 7830. With this method the mean serum B_{12} concentration for 31 normal subjects was 533 $\mu\mu$g per ml of serum with a range of 260 to 850.

Subjects. The 24 patients chosen for this study, along with pertinent clinical data, are listed in Table I. All diagnoses were established both clinically and histologically. None of the patients studied had received oral or parenteral vitamins for at least 1 month prior to the study. All but 3 patients were studied during residency on the metabolism unit of the National Cancer Institute where they were fed diets of known composition. Their stool and urine collections were rigidly controlled according to previously described procedures (10). Each patient was ambulatory during the study.

Subjects have been classified according to the following diagnoses: non-neoplastic diseases, solid tumors, myeloid metaplasia, chronic myelocytic leukemia, hypersplenism and multiple myeloma. For the most part, subjects with non-neoplastic diseases and solid tumors displayed findings similar to those noted by other investigators in normal subjects given a 0.5 μg oral dose of $Co^{58}B_{12}$ and therefore in the discussion are referred to as control subjects.

RESULTS

Serum B_{12} concentrations (Table I)

Four of the five patients with non-neoplastic disease had serum B_{12} concentrations within 2 SD of the normal mean. The fifth patient, whose diagnosis was primary pulmonary hypertension had a moderately increased serum B_{12} concentration of 1,213 $\mu\mu$g per ml.

The five patients with solid neoplasms had a mean serum B_{12} concentration of 577 $\mu\mu$g per ml and a range of 330 to 890 $\mu\mu$g per ml. Two of the four patients with myeloid metaplasia had moderately increased serum B_{12} concentrations. The other two had normal concentrations. The mean concentration for this group was 1,129 $\mu\mu$g per ml with a range of 420 to 2,100.

The seven patients with CML (including one diagnosed as eosinophilic leukemia) demonstrated striking increases in serum B_{12} concentration, the mean concentration being 8,550 $\mu\mu$g per ml with a range of 1,800 to 17,700 $\mu\mu$g per ml. Patient

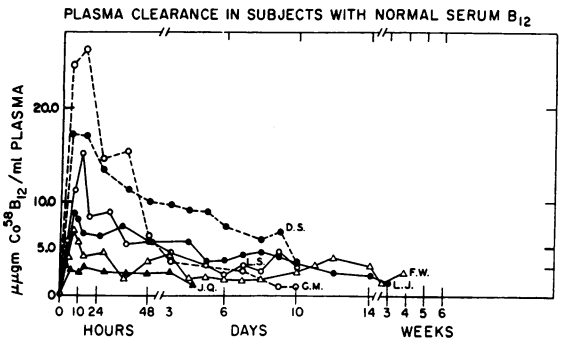

FIG. 1. PLASMA $Co^{58}B_{12}$ CONCENTRATIONS FOLLOWING ORAL ADMINISTRATION TO SUBJECTS WITH A NORMAL SERUM B_{12}. J.Q., L.S., G.M. and F.W., non-neoplastic diseases. D.S. and L.J., embryonal sarcoma.

D.L. was in clinical and hematological remission and Patients J.L. and P.P. were in partial relapse at the time they were studied. The two patients with hypersplenism and pancytopenia had serum B_{12} concentrations at the lower limits of normal, 195 and 200 $\mu\mu$g per ml. The one subject with multiple myeloma had a normal serum B_{12} concentration of 625 $\mu\mu$g per ml.

Gastrointestinal absorption

Since all of the patients but J.G. received a dose of $Co^{58}B_{12}$ in the 0.5 μg range, the percentage of oral dose absorbed has been used as a basis for comparisons between patients. The percentage absorbed by each of the 24 subjects is listed in Table I. The mean for all of the 24 subjects was 66.8 per cent with a range of 30 to 81 per cent. No significant difference between the various disease groups studied was noted in terms of the percentage of dose absorbed. No correlation between a patient's serum B_{12} concentration and the percentage of dose absorbed could be made.

J.G., who received a dose of 2.73 μg, had an absorption of 71 per cent of the administered dose, a value not significantly different from that noted in the others.

Plasma concentrations of $Co^{58}B_{12}$

Curves of plasma radioactivity were obtained in 19 of the 24 patients. For the purpose of this study, the rate of plasma disappearance has been evaluated by noting the $T_{\frac{1}{2}}$.[3]

[3] Since plasma curves had both an appearance phase as well as a disappearance phase, and since the disappearance phase was not exponential, the use of this term

TABLE I

Clinical data on 24 subjects given an oral dose of $Co^{58}B_{12}$; data obtained on gastrointestinal absorption and plasma concentration

Subject	Sex	Age	Diagnosis	Clinical status		Serum B_{12}	Dose of $Co^{58}B_{12}$	Absorbed	Plasma $Co^{58}B_{12}$ Peak concentration	Plasma $Co^{58}B_{12}$ Time of peak	"$T_{1/2}$"*
					WBC/mm³	*μμg/ml*	*μg*	*%*	*μμg/ml*	*hrs*	*days*
J.Q.	F	33	Partial resection of ileum	Stable	6,000	800	0.37	60	3	12	4
L.S.	M	22	Primary pulmonary hypertension	Stable	7,500	1,213	0.49	86	15	10	1.2
S.M.	F	32	Rheumatic heart disease	Stable	10,200	550	0.49	69			
F.W.	F	68	Subtotal gastrectomy	Weight loss	6,000	465	0.49	76	7	8	4
G.M.	F	42	Anorexia nervosa	Weight loss	8,200	790	0.56	76	27	12	2
L.J.	F	16	Embryonal sarcoma	Stable	7,500	725	0.49	80	9	7	5
R.L.	M	20	Hepatoma	Partially resected	9,000	462	0.49	62	13	35	>9
H.C.	M	52	Prostatic carcinoma	Bone metastases	3,000	330	0.48	75			
D.S.	M	15	Embryonal sarcoma	Metastases	5,400	478	0.56	41	18	5	7
W.P.	M	54	Pancreatic carcinoma	Untreated	7,600	890	0.56	68			
W.J.	M	61	Myeloid metaplasia	Untreated	4,000	2,100	0.33	80	15	48	9
S.P.	M	65	Myeloid metaplasia	Untreated	8,000	1,215	0.48	30	28	10d	?
J.G.	M	50	Myeloid metaplasia	Untreated	9,000	780	2.73	71	16	9	?
M.G.	F	64	Myeloid metaplasia	Untreated	3,000	420	0.48	52	20	6d	?
D.L.	M	8	Chronic myelocytic leukemia	Remission	8,000	17,700	0.48	81	71	48	13
J.L.	F	23	Chronic myelocytic leukemia	Partial relapse	5,600	8,700	0.49	76	54	72	4
O.F.	M	48	Chronic myelocytic leukemia	Untreated	200,000	6,800	0.48	55	8	72	8
P.P.	M	36	Chronic myelocytic leukemia	Partial relapse	40,000	6,700	0.48	78	18	24	11
M.B.	F	37	Chronic myelocytic leukemia	Untreated	194,000	6,150	0.48	48	30	36	13
R.F.	M	52	Chronic myelocytic leukemia	Untreated	190,000	1,800	0.48	78			
L.E.	M	25	Eosinophilic leukemia	Untreated	17,000	12,000	0.49	79	22	36	15
C.F.	M	40	Hypersplenism with pancytopenia	Untreated	2,500	195	0.48	81	14	72	?
P.K.	M	55	Hypersplenism with pancytopenia	Untreated	3,000	200	0.49	50	8	6	0.5
J.M.	F	60	Multiple myeloma	Untreated	6,000	625	0.48	51			

* See Footnote 3 in text.

Non-neoplastic disease and solid tumor groups. A grossly similar pattern of plasma radioactivity was noted in four patients with non-neoplastic diseases and in two patients with solid tumors (Figure 1). Significant plasma radioactivity was noted within the first 3 to 4.5 hours after administration of $Co^{58}B_{12}$. Thereafter, plasma radioactivity increased in an approximately linear manner until a peak level was attained at a mean of 9 hours (range, 5 to 12 hours). The peak radioactivity

in the present context is somewhat unconventional. In the present paper "$T_{\frac{1}{2}}$" has been arbitrarily defined as the time interval between peak plasma radioactivity and the time when plasma concentration of $Co^{58}B_{12}$ falls to half of the peak concentration.

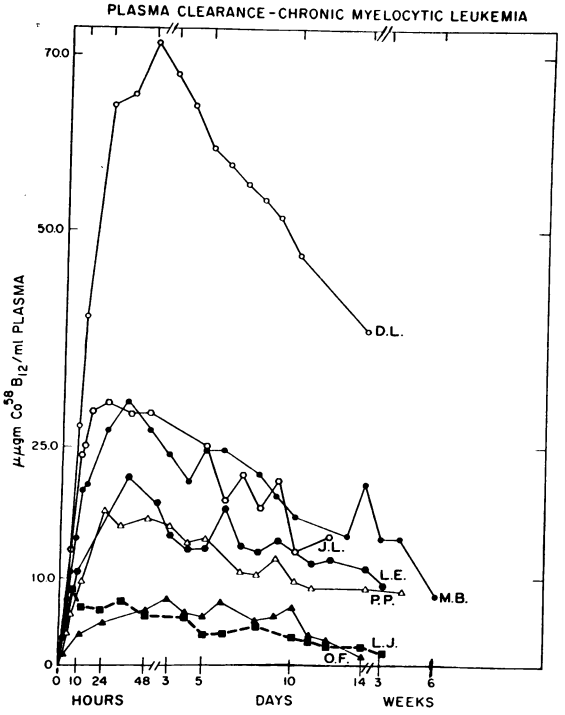

FIG. 2. PLASMA $Co^{58}B_{12}$ CONCENTRATIONS FOLLOWING ORAL ADMINISTRATION OF 0.5 μG TO SUBJECTS WITH CHRONIC MYELOCYTIC LEUKEMIA. L.E., eosinophilic leukemia. L.J., embryonal sarcoma, a characteristic pattern of subjects with a normal serum B_{12} included for comparison.

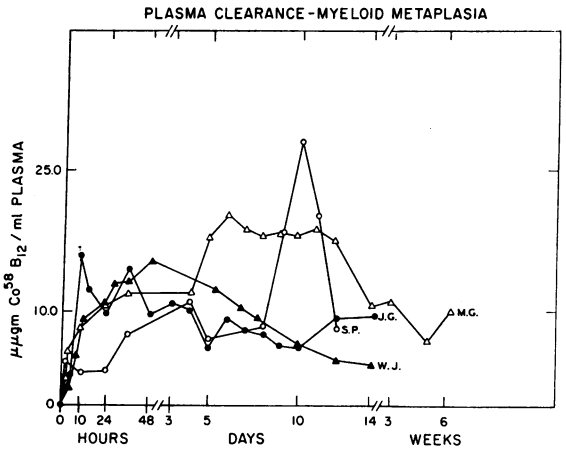

FIG. 3. PLASMA $Co^{58}B_{12}$ CONCENTRATIONS FOLLOWING ORAL ADMINISTRATION OF 0.5 μG TO FOUR SUBJECTS WITH MYELOID METAPLASIA.

was equivalent to a mean of 13 μμg of $Co^{58}B_{12}$ per ml of plasma (range, 3 to 27 μμg per ml). Subsequently, there was a gradual and quite irregular decline in plasma radioactivity during the next 10 to 14 days. The $T_{\frac{1}{2}}$ of plasma disappearance had a mean value of 4 days (range, 1.5 to 7 days). Three of these six patients had significant radioactivity in their plasma for as long as 10 days after administration of the $Co^{58}B_{12}$.

A seventh patient, R.L., whose diagnosis was hepatoma, had a peak concentration of 13 μμg $Co^{58}B_{12}$ per ml of plasma. The peak, however, occurred at 35 hours. The plasma disappearance was also delayed with a $T_{\frac{1}{2}}$ of > 9 days.

Chronic myelocytic leukemia. Figure 2 summarizes the plasma $Co^{58}B_{12}$ concentrations observed in seven patients with chronic myelocytic leukemia. (Subject L.E. with eosinophilic leukemia is included in this group because of his similar pattern.) The initial portion of their plasma *appearance* curves was approximately linear and had a slope similar to that of the previous group. At from 10 to 14 hours, however, instead of reaching a peak concentration, serial plasma samples continued to rise more slowly, resulting in a delay in reaching peak concentrations. The latter did not occur until a mean of 2 days (range, 1 to 3 days). In general, plasma radioactivity also reached much higher concentrations, being equivalent to a mean of 34 μμg $Co^{58}B_{12}$ per ml of plasma (range, 8 to 71 μμg per ml). In addition, there was delayed plasma disappearance with a mean $T_{\frac{1}{2}}$ of 10.7 days (range, 4 to 15 days). At the end of 10 days, four of the six patients retained in their plasma 56 to 88 per cent of their peak plasma concentration. A plasma sample drawn on M.B. at 6 weeks demonstrated significant residual radioactivity.

Myeloid metaplasia. Inspection of the plasma radioactivity curves for the four patients with myeloid metaplasia (Figure 3) reveals that, except for one subject, W.J., whose curve has the features noted for patients with chronic myelocytic leukemia, these patients had bizarre and irregular *appearance* and *disappearance* curves. They had a mean peak plasma concentration of 20 μμg $Co^{58}B_{12}$ per ml of plasma (range, 15 to 28 μμg per ml). Because of the very irregular plasma disappearance, $T_{\frac{1}{2}}$ could not be evaluated. In general, however, these patients demonstrated more residual $Co^{58}B_{12}$ in their plasma at the end of 10 days than did the non-neoplastic disease-solid tumor group.

Others. P.K., a patient with hypersplenism and pancytopenia, had a plasma $Co^{58}B_{12}$ curve similar to the non-neoplastic disease and solid tumor groups. C.F., on the other hand, with hypersplenism and pancytopenia, had a delayed plasma peak occurring at 3 days. Plasma activity was not studied in the single case of multiple myeloma.

Urinary excretion of $Co^{58}B_{12}$

There was no detectable urinary excretion of radioactive material in the unconcentrated urine specimens of any of the subjects studied. Calculations indicate that the method used in the present study would have detected excretion of more than 1 per cent of the absorbed dose per 24 hour urine specimen.

DISCUSSION

The studies indicate that gastrointestinal absorption of B_{12}, as measured by the fecal excretion technique, is not increased in the patient with CML. The percentage of administered $Co^{58}B_{12}$ absorbed had an over-all mean of 66.8, with a range of 30 to 81 for the 24 subjects studied. These figures are in good agreement with those reported by Halsted and colleagues (11) who found that with a 0.5 μg dose of radioactive B_{12}, normal subjects absorbed 43 to 95 per cent of the dose, with a mean of 66 and a maximum variation within the same patient of 18 per cent. The absorption in seven subjects with CML in the present study did not differ from this range, nor was the amount absorbed abnormal in the two subjects with myeloid metaplasia and a high serum B_{12}. Heinrich and Erdmann-Oehlecker (12) have also found normal gastrointestinal absorption of B_{12} in one patient with CML given 1 μg of B_{12}.

A corollary to these findings is that the serum concentration of B_{12} per se does not control the amount of B_{12} absorbed. Both Patient L.J., with a serum B_{12} of 725 $\mu\mu$g per ml and Patient D.L., with a concentration of 17,700 $\mu\mu$g per ml, absorbed approximately 80 per cent of the test dose. The data also indicate that the plasma concentration of "unsaturated B_{12}-binding protein" does not greatly influence the amount of B_{12} absorbed, since plasma from patients with CML has a markedly increased unsaturated B_{12}-binding capacity (13–16).

It would appear, therefore, that the high plasma B_{12} concentrations seen in CML are not due to excessive gastrointestinal absorption of the vitamin. In addition, it is unlikely that the abnormality is caused by impaired renal excretion, since the total daily urinary excretion of vitamin B_{12} is normally very small, viz., about 0.1 μg per day (17), and $Co^{58}B_{12}$ was undetectable in the urine of both control and CML patients in the present study.

We believe that the high B_{12} content of CML plasma is due to a relative shift of B_{12} from tissue sites to plasma. It can be calculated from known data (18) that the transfer of only 3 per cent of the normal liver's content of B_{12} to the plasma compartment would be sufficient to produce the tenfold increase in plasma B_{12} seen in CML. This possibility would, of course, be excluded should studies reveal that CML is associated with a high tissue content as well as high plasma concentration of the vitamin. Data on tissue concentrations of B_{12} in CML are limited. In the single autopsied case studied by Mollin and Ross (2) the total B_{12} content of liver, spleen and muscle was within the normal range. Nelson has studied the B_{12} content of liver tissue obtained by needle biopsy in two cases of myelocytic leukemia associated with a high serum B_{12} and found it to be approximately 30 per cent below that of the average normal control (19).

The marked differences between control and CML subjects in plasma radioactivity curves following oral administration of $Co^{58}B_{12}$ provide evidence for a disturbed kinetics of B_{12} in CML. An analysis of these curves necessitates a consideration of the factor involved in the absorption, plasma transport and distribution of B_{12}. These are summarized in schematic form in Figure 4. Because of gaps in our present knowledge the scheme is tentative.

The delay in peak plasma concentration (9 hours in control patients) following an oral dose of $Co^{58}B_{12}$ has been noted by other investigators (20, 21) and appears to be due to a delay in the movement of B_{12} across the small bowel mucosa (21–23). The initial slope of the plasma *appearance* curve for the CML patients appears to be comparable with that of the control patients (Figure 2) suggesting a normal rate of entry into the

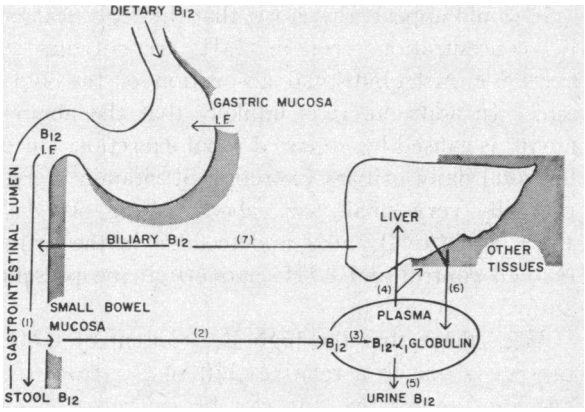

FIG. 4. A SCHEMATIC MODEL OF VITAMIN B_{12} METABOLISM. Reaction rates, (1 through 7). I.F. is intrinsic factor. It is postulated that in chronic myelocytic leukemia there is an increased plasma B_{12} concentration because of a relative shift of tissue stores of B_{12} to the plasma via 6 as well as increased retention of exogenous B_{12} within the plasma compartment via 3. References and further details are given in the Discussion.

plasma compartment. A greater than normal delay in peak plasma concentration (24 hours) was noted in the CML patients and is discussed below.

In both the control and CML subjects the plasma *disappearance* curves had an irregular downward slope. We believe that this is due to a complex recycling of B_{12} through the plasma compartment. This is supported by the accumulating evidence for an enterohepatic circulation of B_{12} (24, 25) as well as by the studies of Miller, Corbus and Sullivan (8) and Glass and Schaffer (22) which suggest that there is a transfer of absorbed B_{12} from plasma to unidentified compartment(s) prior to its eventual deposition in the liver.

Because of the complex nature of the plasma radioactivity curves, in the present study $T_{\frac{1}{2}}$ is defined as the time following peak radioactivity when plasma radioactivity falls to and remains below half the peak concentration. The mean value for control and CML patients was 4 and 11 days, respectively. Employing an intravenous dose of 1.5 or 4 μg of radioactive B_{12} Mollin, Pitney, Baker and Bradley (7) and Miller and colleagues (8) have demonstrated that in patients with CML there is a diminished plasma disappearance rate for injected B_{12}. The rates reported were much more rapid than those obtained in the present study and are probably due to the unphysiologic effects of administering a large dose of free B_{12} intravenously. This is especially true in normal subjects who have a relatively limited plasma unsaturated B_{12}-binding capacity (see below) and, therefore, might be expected to have a rapid loss of free B_{12} from the plasma.

The slower plasma disappearance rate combined with the prolonged influx at a normal rate into the plasma from the small bowel of the tracer dose of $Co^{58}B_{12}$ could, by a cumulative effect, account for the higher than normal but more delayed peak plasma concentration of $Co^{58}B_{12}$ seen in the patients with CML in the present study. In patients with CML, a given dose of $Co^{58}B_{12}$ enters a plasma B_{12} pool which is approximately 10 times normal size. Therefore, the delayed plasma disappearance of the labeled vitamin may merely represent an isotope dilution effect. Since CML plasma has a high unsaturated B_{12}-binding capacity (13–16), an additional factor may be that as the tracer dose enters the plasma compartment a greater than normal fraction of it is tightly bound to plasma protein.

In both normal subjects and patients with CML, 80 to 90 per cent of total plasma B_{12} is tightly bound to an α_1-globulin present in the seromucoid fraction of plasma (16). Since, within a few hours following an oral dose of $Co^{58}B_{12}$, the radioactive B_{12} appearing in plasma is also in this form (13) it seems reasonable to assume that, once absorbed, an orally administered dose of $Co^{58}B_{12}$ rapidly equilibrates with the total plasma B_{12}. Using the previously mentioned disappearance rates it seems possible, therefore, to calculate the daily plasma turnover of vitamin B_{12}. For a control subject with a plasma B_{12} of 0.5 mμg per ml, a plasma volume of 2,500 ml and a $T_{\frac{1}{2}}$ of four days, the plasma turnover would be 0.16 μg per day. Similar calculations for a patient with CML, assuming a plasma B_{12} of 5 mμg per ml, a plasma volume of 2,500 ml and a $T_{\frac{1}{2}}$ of 11 days, give a plasma turnover of 0.57 μg per day.

If plasma turnover of B_{12} is indeed increased in CML, the abnormality in B_{12} metabolism may be more fundamental than merely a shift of tissue stores of B_{12} to the plasma compartment resulting from an excess of plasma B_{12}-binding protein. Clearly, the assumptions underlying the calculations and the validity of the disappearance rates obtained bear further investigation.

SUMMARY

1. Gastrointestinal absorption, plasma transport and urinary excretion of radioactivity following oral administration of 0.5 μg of $Co^{58}B_{12}$ have been evaluated in 24 patients, 7 of whom had chronic myelocytic leukemia.

2. The amount absorbed by all subjects, as measured by stool excretion, had a mean of 67 per cent. There was no correlation between diagnosis or serum B_{12} concentration and percentage of dose absorbed.

3. Subjects with non-neoplastic diseases as well as patients with solid tumors, all of whom had a normal serum B_{12} concentration, had a similar pattern of plasma clearance. $Co^{58}B_{12}$ reached a mean peak concentration of 13 $\mu\mu$g per ml of plasma at 9 hours and fell to half the peak concentration at a mean of 4 days. In seven patients with chronic myelocytic leukemia, $Co^{58}B_{12}$ reached a mean peak plasma concentration of 33 $\mu\mu$g per ml at 2 days and fell to half the peak concentration at a mean of 11 days.

4. No detectable urinary excretion of radioactive material in unconcentrated urine specimens was noted in any of the subjects studied.

5. The increased serum concentration of vitamin B_{12} seen in patients with chronic myelocytic leukemia does not appear to be due to increased gastrointestinal absorption or decreased urinary excretion of the vitamin. A relative shift of B_{12} from tissue sites to plasma appears to be the most likely mechanism.

6. Calculations based upon the plasma disappearance rates obtained in the present study suggest that, in addition to the increased concentration of B_{12} and B_{12}-binding protein found in plasma of patients with chronic myelocytic leukemia, there is an increased plasma turnover of the vitamin in this disease.

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