THE PLASMA DISAPPEARANCE, EXCRETION, AND TISSUE DISTRIBUTION OF COBALT^{60} LABELLED VITAMIN B_{12} IN NORMAL SUBJECTS AND PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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The serum concentration of vitamin B_{12} is increased in chronic myelogenous leukemia, and there is an associated increase in the *in vitro* binding of vitamin B_{12} by such sera (1, 2). On the other hand, the vitamin B_{12} concentration in the white cells and tissues in chronic myelogenous leukemia is not elevated (2). Mollin, Pitney, Baker, and Bradley (3) have demonstrated a delayed disappearance of cobalt^{58} labelled vitamin B_{12} from the plasma of patients with chronic myelogenous leukemia using an intravenous dose of 1.5 micrograms. It was the purpose of this study to measure plasma disappearance, tissue distribution, and excretion of a 4-microgram intravenous dose of cobalt^{60} labelled vitamin B_{12} in normal subjects and patients with chronic myelogenous leukemia. *In vitro* experiments would indicate that this dose exceeds the binding capacity of normal serum by about four fold, but is within the total binding capacity of chronic myelogenous leukemia serum (1, 2, 4).

Using a 4-microgram dose, a clear-cut differentiation could be made between normal controls and patients with chronic myelogenous leukemia.

METHODS

A. *Clinical material*

The subjects chosen for normal controls in this study were hospitalized patients convalescing from cerebrovascular accidents. Two to four months had elapsed since the onset of hemiparesis. All were asymptomatic save for residual paralysis, and none had evidence of anemia, renal disease, liver disease, congestive heart failure or infection. The diagnosis of chronic myelogenous leukemia was well established in eight patients. At the time of study, four patients had received no anti-leukemic therapy, and four had received some form of anti-leukemic therapy from three weeks to six months previously. Also studied were two patients with myeloid metaplasia, one patient with chronic lymphatic leukemia, one patient with Laënnec's cirrhosis with ascites and two postoperative patients with common bile duct drainage.

B. *Experimental plan*

1. *Plasma disappearance.* Four micrograms of cobalt^{60} labelled vitamin B_{12} was injected intravenously and its disappearance from the plasma determined by serum sampling and measurement.

2. *Tissue distribution.* Following injection, external monitoring of organ sites was carried out for periods up to 33 days. The concentration of radioactivity in the organs of two patients with leukemia was determined at post mortem. The concentration of radioactivity in red cells and white cells during the first twenty-four hours after injection was measured after separation from the plasma.

3. *Excretion.* Excretion of radioactivity in urine and stools was determined by measuring radioactivity in 24-hour urine and 7 to 10-day stool collections.

4. *Miscellaneous.* Two postoperative patients with T-tube drainage of the biliary tract were given cobalt^{60} labelled B_{12} intravenously and the radioactivity in 10-day bile collections determined. One patient with Laënnec's cirrhosis with massive ascites underwent paracentesis over a 2-hour period following the injection of cobalt^{60} labelled vitamin B_{12} and the radioactivity in the ascitic fluid was determined.

C. *Procedures*

1. *Plasma disappearance.* For intravenous injection, a suitable dilution of cobalt^{60} labelled vitamin B_{12} was made so that 5 ml. contained 4 micrograms of vitamin B_{12} and 3.0 microcuries of radioactivity. This was injected from a calibrated syringe into an antecubital vein. Ten ml. of blood was withdrawn with added heparin from the opposite arm at frequent intervals during the first two hours, and at 1 to 3 days thereafter for the duration of the study (up to 33 days). The percentage of the administered dose which remained in the plasma at any given time was determined by the formula:

$$\% \frac{\text{Counts per min./ml. plasma} \times \text{plasma vol. (ml.)}}{\text{Total counts per min. injected}} \times 100$$

2. *Radioactivity measurements.*

Scintillation well counting: Three-ml. plasma samples were counted in a thallium-activated sodium iodide well-

type scintillation counter. White cells were separated by the method of Buckley, Powell, and Gibson (5), and made up to 3-ml. volume and counted in the same manner. Washed red cells were also counted in 3-ml. volume. A standard was prepared by making a 1:500 dilution of the original injected sample, and duplicate 3-ml. aliquots were counted. Total injected radioactivity was calculated from the standard. All plasma samples collected during the first twenty-four hours after injection were counted with a counting error of less than 3 per cent. Samples collected after twenty-four hours were counted with a counting error of less than 5 per cent. Red cells and white cells were counted for 10 minutes to demonstrate negligible radioactivity.

Geiger-Müller well counting: Seventy-five-ml. aliquots of urine, bile, or concentrated ascitic fluid, and 75-gram aliquots of stool homogenate or organs were counted in a Texas-Allyn well-type GM counter. Seventy-five ml. of a 1:500 dilution of the injected material was used as a standard. Urine from normal patients and organ samples were counted with a counting error of less than 5 per cent. All other samples contained very little radioactivity, and were counted for 10 minutes.

External monitoring: External monitoring was done with a solid one-inch thallium-activated sodium iodide probe counter. A cobalt^{60} standard was counted each morning to correct for daily variations in the counter. Counts were taken over liver, spleen, precordium, and thigh. Several areas over the liver and spleen were counted, and the area giving the highest counting rate was used.

3. *Serum vitamin B_{12}.* The concentration of vitamin B_{12} was determined by the Euglena gracilis method of Ross (6) as modified by Lear, Harris, Castle, and Fleming(7). The normal range reported by the latter group is 292 to 856 micromicrograms per ml.

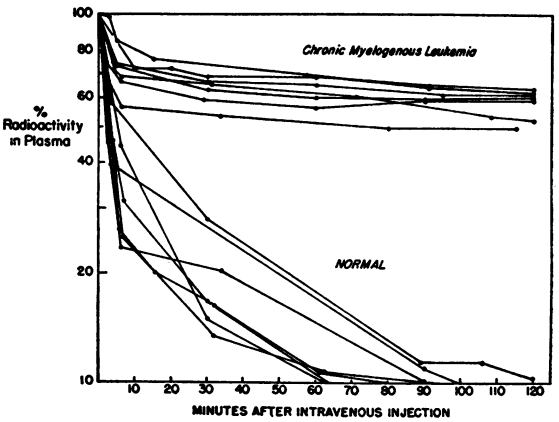

FIG. 1. DISAPPEARANCE OF COBALT^{60} VITAMIN B_{12} FROM THE PLASMA OF NORMAL SUBJECTS AND PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

4. *Plasma volume.* Plasma volume was determined by the Evans blue technique (8) in half of the patients, and estimated from the body weight in the remainder of the subjects using the formula:

$$\frac{\text{Plasmatocrit}}{100} \times 69 \text{ ml.} \times \text{body weight (kg.)}$$

RESULTS

A. *The disappearance of cobalt^{60} labelled vitamin B_{12} from the plasma*

1. *Normal subjects.* The plasma radioactivity declined rapidly after injection; 35 to 55 per cent of the injected dose remained at the end of 5 min-

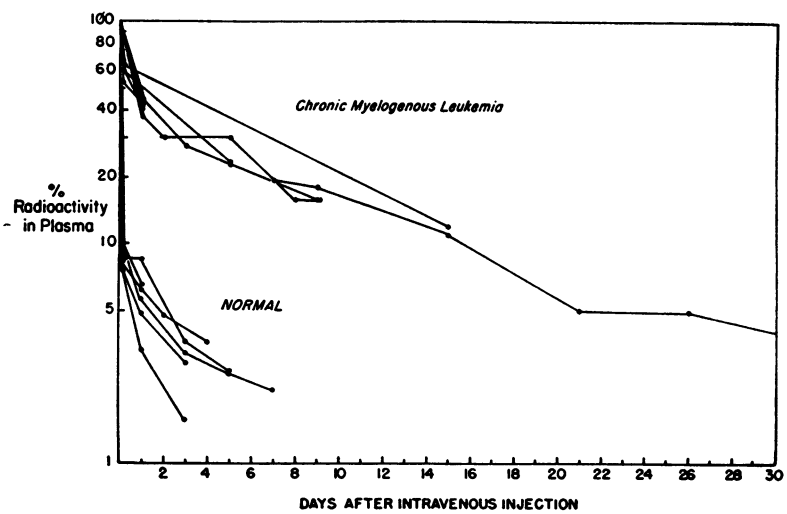

FIG. 2. DISAPPEARANCE OF COBALT^{60} VITAMIN B_{12} FROM THE PLASMA OF NORMAL SUBJECTS AND PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

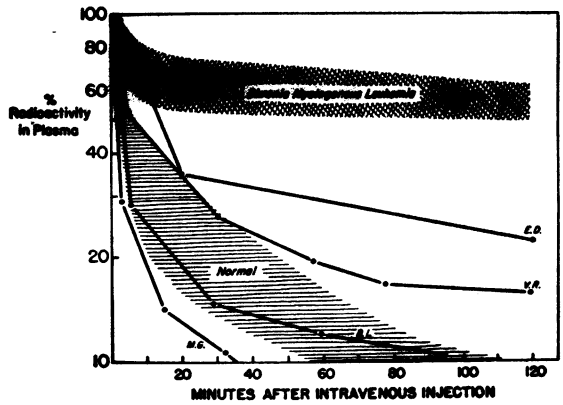

FIG. 3. DISAPPEARANCE OF COBALT^{60} VITAMIN B_{12} FROM THE PLASMA OF PATIENTS WITH VARIOUS DISORDERS

E. D. and V. R.—myeloid metaplasia.
M. G.—chronic lymphocytic leukemia.
B. L.—chronic myelogenous leukemia, treated and in remission.

utes and 8 to 10 per cent at two hours (Figure 1). Thereafter, the disappearance rate was slower (Figure 2). Because of the small amount of radioactivity present in the plasma after 24 hours, it was not possible to determine the slope of the disappearance curve accurately.

2. *Chronic myelogenous leukemia.* In seven out of eight patients with chronic myelogenous leukemia, plasma radioactivity disappeared more slowly: 50 to 63 per cent of the dose remained at two hours, and 38 to 42 per cent at 24 hours. After 24 hours, sufficient radioactivity remained in the plasma to determine the slope of the curve. This was approximately exponential, with a half time of 5 days. After 33 days, the plasma from one patient still contained 4 per cent of the administered dose. All seven of these patients showed evidence of activity of their disease (anemia, fever, splenomegaly), but not necessarily a high white count. Patient B. L., who was in complete clinical and hematological remission, showed a normal plasma disappearance (Figure 3).

3. *Other.* One patient with chronic lymphocytic leukemia showed normal plasma disappearance (Figure 3). Both patients with myeloid metaplasia showed a plasma disappearance which was intermediate between that of the normal subjects and the patients with chronic myelogenous leukemia (Figure 3).

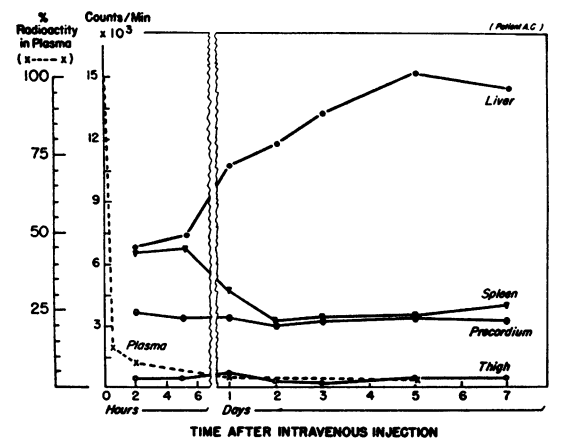

FIG. 4. DISTRIBUTION OF RADIOACTIVITY AFTER INTRAVENOUS COBALT^{60} VITAMIN B_{12} IN A NORMAL SUBJECT

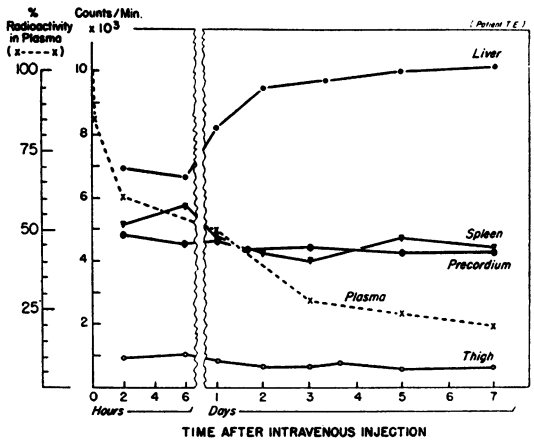

FIG. 5. DISTRIBUTION OF RADIOACTIVITY AFTER INTRAVENOUS COBALT^{60} VITAMIN B_{12} IN A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA

B. *Tissue distribution*

1. *External monitoring.* External monitoring of normal subjects showed an increase in liver radioactivity throughout the period of observation. During the first 5 hours after injection, there was very little increase in counting rate over the liver, despite the fact that during this interval 90 per cent of the administered dose had left the plasma. From 5 to 48 hours, the counts over the precordium were constant, counts over the liver rose, and there was a consistent *fall* in counts over the spleen (Figure 4). Patients with chronic myelogenous leukemia, having a slower plasma disappearance, had a smaller rise in counting rate over the liver (Figure 5). No increase in concentration of radioactivity over enlarged spleens was found. Counting rates over organ sites in the patient with chronic lymphocytic leukemia with

normal plasma disappearance were the same as in the normal subjects.

2. *Red and white cells.* Red and white cells from both normal subjects and patients with leukemia contained no radioactivity.

3. *Post mortem.* The radioactivity in the organs of two patients with chronic myelogenous leukemia were determined at post mortem. Patient T. L. was injected 9 days and patient E. M. 23 days before death. The liver contained 39 to 42 per cent of the administered dose, the spleen 8 to 11 per cent and the other viscera less than one per cent (Table II). The concentration of radioactivity in the liver (counts per gram) was five to seven times greater than in spleen and other viscera.

C. *Excretion*

In the twenty-four hours after injection, normal subjects excreted 1 to 4 per cent of the administered dose in the urine, and chronic myelogenous leukemia patients, 0 to 2 per cent (Table I). Seven to ten-day stool collections were made in two normal subjects and contained no radioactivity. Ten-day bile collections contained less than 2 per cent of the administered dose. Ascitic fluid from a patient with cirrhosis (E. M.) was collected at intervals up to two hours during the measurement

TABLE I

Summary of clinical and laboratory data

Patient	Age	Serum vit. B_{12} concentration *μμg./ml.*	Wbc *per cu. mm.*	Plasma vol. *ml.*	Per cent radioactivity in plasma at 2 hrs.	Per cent radioactivity in plasma at 24 hrs.	24-Hour urinary excretion %
Normals							
1) N. G.	54		8,300	3,300	8	6	2
2) A. C.	65		5,800	3,000*	9	5	3
3) J. S.	63	530	8,500	3,200*	10	7	4
4) T. D.	64		5,000	3,000*	8	5	4
5) N. G.	55	364	9,600	3,400	8	3	4
6) V. K.	65	504	8,700	2,700*	10	6	1
7) H. M.	59		10,300	3,400*	8	4	2
Range					8–10	3–7	1–4
Mean					9	5	3
Chronic myelogenous leukemia							
1) T. L.	62		363,000	3,800	62	38	0
2) J. P.	57		91,100	3,600	63		
3) E. M.	32	9,266	12,600	3,800*	59		0
4) M. O.	53		118,000	3,300	53	42	
5) T. E.	62	2,046	313,000	3,900*	60	43	1
6) E. C.	52	7,020	71,000	4,000*	50		
7) W. S.	29	4,874	102,000	3,300*	59	42	2
Range					50–63	38–42	0–2
Mean					58	41	1
Miscellaneous							
Chr. myel. leukemia (In remission)							
1) B. L.	55		8,900	3,200	10		
Chr. lymphatic leukemia							
1) M. G.	62		160,000	3,200	7	3	2
Myeloid metaplasia							
1) E. D.	64	704	102,000	3,900	22		
2) V. R.	49		81,000	4,000	16	8	
Laënnec's cirrhosis							
1) E. M.	53	2,104	11,100	3,200*	10	3	2

* Plasma volume was determined by the Evans blue technique.

TABLE II

The tissue distribution of radioactivity in two autopsied patients with chronic myelogenous leukemia after intravenous Co^{60} vitamin B_{12}

Organ	Per cent injected radioactivity in total organ		Counts per sec. 100 gm. (wet weight)	
	T. L.	E. M.	T. L.	E. M.
Liver	42	39	54.9	63.1
Spleen	11	8	12.5	10.4
Kidney	1	1	14.3	11.3
Lung	1	1	10.3	7.0
Stomach	1		15.8	
Pancreas	1		7.3	

of the plasma disappearance. The entire 11 liters of fluid contained no radioactivity despite the fact that 90 per cent of the cobalt^{60} labelled vitamin B_{12} had disappeared from the plasma within the two-hour interval.

D. *Serum vitamin B_{12} concentration*

In three normal subjects, the concentration of vitamin B_{12} in the serum ranged from 364 to 530 micromicrograms per ml. Serum vitamin B_{12} levels were elevated in four patients with chronic myelogenous leukemia ranging from 2046 to 9266 micromicrograms per ml. No correlation was found between the leukocyte count and the vitamin B_{12} level. Thus, patient E. M., with a normal white cell count, had the highest serum concentration of vitamin B_{12}. The serum concentration of vitamin B_{12} was 704 micromicrograms per ml. in E. D., a patient with myeloid metaplasia, and 2104 in E. M., a patient with cirrhosis.

DISCUSSION

The 4-microgram dose used in this study exceeds the total plasma vitamin B_{12} in the normal by about three fold. Because of the specific activity of the cobalt^{60} labelled vitamin B_{12} available, the administration of a dose which would not significantly elevate the plasma concentration, *i.e.*, a tracer amount, would result in plasma counting rates not measurable by current techniques. Although the 4-microgram dose approaches a tracer amount in some of the chronic myelogenous leukemic patients, in the normal subjects the results may not be truly physiological because of the dosage employed.

In the normal subjects, intravenous vitamin B_{12} in the dosage used disappeared rapidly from the plasma in spite of minimal excretion in urine, stool, and bile. This decline in plasma concentration of cobalt^{60} labelled vitamin B_{12} during the first two hours after injection is too rapid to be explained solely by mixing in extracellular fluid. Also, no radioactivity was recovered in ascitic fluid, although the patient with impaired liver function may not be completely comparable. The liver is thought to be the chief storage site of vitamin B_{12} (9, 10) and one might expect that the material would be taken up by the liver as it leaves the plasma. However, we found no concentration of radioactivity over this or any other organ site during the first five hours as measured by external monitoring. During this same period, direct sampling of erythrocytes and leukocytes revealed no measurable radioactivity. It is apparent that during the first five hours after injection, when virtually all of the material had left the plasma, no site of localization of the injected material was found with the techniques employed. A diffuse cellular uptake during this period could account for these observations.

However, external monitoring data indicate that the liver concentrates cobalt^{60} labelled vitamin B_{12} and/or related compounds containing cobalt^{60} beginning five hours after injection and continuing throughout the period of observation. This increase would seem too large to be accounted for by the small amount of material remaining in the plasma. Furthermore, during this period (five hours to two days) counting rates over the spleen continued to fall at a time when plasma and precordium counts were stable, suggesting cellular release of the vitamin or a cobalt-containing intermediate. Continued accumulation of the radioactivity in the liver was not due to an enterohepatic circulation, since negligible radioactivity was recovered in the bile.

The slow disappearance of vitamin B_{12} from the plasma of patients with chronic myelogenous leukemia probably reflects increased binding of this vitamin by the plasma (1, 2). This increased binding capacity correlated with the clinical state of the disease. Since serum concentrations decrease towards normal following successful therapy (1, 2), it is likely that this plasma abnormality also may disappear during remission (patient B. L.). Increased binding capacity did not correlate with the level of the white count, consistent with

the reports that increases in serum concentration of B_{12} do not correlate with the level of the white count (1, 2). The plasma disappearance curve can be used to estimate the daily plasma turnover of vitamin B_{12} in the leukemic patient. This is based on the assumption that the disappearance curve beginning at 24 hours, with a half time of 5 days, represents the physiological turnover of plasma vitamin B_{12}. This is likely, since this part of the curve is exponential. Also, the added radio-vitamin elevated the original serum concentration of vitamin B_{12} by less than 20 per cent, 24 hours after injection. Using this half time of five days, and knowing the original serum concentration of B_{12} and the plasma volume, the daily turnover rates in four leukemic patients were calculated, and ranged from 1.1 to 4.8 micrograms per 24 hours. This cannot be related to the normal, since the small amount of radioactivity in the normal plasma precludes calculation of daily turnover.

Although the serum from patients with chronic myelogenous leukemia binds increased amounts of vitamin B_{12}, the leukemic tissues from such patients do not appear to do so. No radioactivity was found in the leukocytes of leukemic patients at any time External monitoring over leukemic spleens revealed no concentration of radioactivity during the entire experiment (1 to 33 days), despite the disappearance of the radio-vitamin from the plasma. In two patients, the absence of splenic concentration of radioactivity as measured by external monitoring was corroborated by actual measurement of organ radioactivity at post mortem.

One patient with chronic lymphocytic leukemia was studied. Serum concentrations of vitamin B_{12} in this disease have been reported to be normal (1, 2). *In vivo* binding, as demonstrated by plasma disappearance, was normal in this patient. The serum concentration of vitamin B_{12} in patients with myeloid metaplasia has been reported to be somewhat elevated, although not as high as in chronic myelogenous leukemia (2). Although both patients with myeloid metaplasia were found to have a delayed disappearance of $cobalt^{60}$ labelled vitamin B_{12} from the plasma, patient V. R. closely approximated the normal. In this patient histochemical staining of the polymorphonuclear leukocytes showed a decrease in the alkaline phosphatase as found in chronic myelogenous leukemia (11). However, by clinical criteria, this patient did not appear to have leukemia. It seems likely that these biochemical abnormalities may exist in varying degrees in this group of patients. It is of interest that the other patient with myeloid metaplasia, E. D., with a more delayed plasma disappearance, had a normal serum vitamin B_{12} level. Consistent with other reports (7) patient E. M. with cirrhosis had a high serum vitamin B_{12} level. However, this patient had a normal plasma disappearance. There have been reports of elevated serum concentrations of vitamin B_{12} in acute leukemia (12); unfortunately no such patients were available for the present study.

Mollin, Pitney, Baker, and Bradley (3) have recently described delayed plasma disappearance of an intravenous dose of 1.5 micrograms of $cobalt^{58}$ labelled vitamin B_{12} in two patients with chronic myelogenous leukemia. It is difficult to compare our results because of the difference in the doses used. It is of interest that they have taken the 6-minute plasma sample as representative of 100 per cent of the injected dose. Our studies using both 1.5 (13) and 4 micrograms showed that 6 minutes following injection, 45 to 65 per cent of the dose had left the plasma in normal subjects and 15 to 34 per cent in the leukemic patients. These factors may explain the greater differences between the two groups of patients found in the present study.

The mechanism of the increased binding of vitamin B_{12} in the serum of chronic myelogenous leukemic patients is not known. Vitamin B_{12} is bound to the alpha fraction of serum globulin in both normal subjects and leukemic patients (1, 4). Increases in the concentration of this protein have been reported in many diseases not accompanied by an elevation of serum vitamin B_{12} concentration (14–16). Material liberated by chronic myelogenous leukemic white cells will bind vitamin B_{12} *in vitro,* and it has been suggested that this is the *in vivo* mechanism (2). However, the vitamin can be bound to normal white blood cells (17) as well as to other protein fractions. Further work is needed to clarify this problem.

In any case, the present study demonstrates a biochemical *plasma* abnormality in a disease which chiefly affects white cells. Other biochemical changes which have been described in leukemia, such as alterations in the concentrations of alkaline phosphatase (11) and histamine (18), have

been intracellular. This relatively simple method of demonstrating increased *in vivo* binding of vitamin B_{12} may be useful clinically in differentiating myelogenous leukemia from leukemoid states.

SUMMARY AND CONCLUSIONS

1. Four micrograms of cobalt^{60} labelled vitamin B_{12} were injected intravenously into normal subjects and patients with chronic myelogenous leukemia, and its plasma disappearance, tissue distribution and excretion were determined.

2. A rapid decline in plasma radioactivity occurred in normal subjects, contrasted with a slow decline in patients with clinically active chronic myelogenous leukemia. Excretion in urine and stools was negligible.

3. External monitoring of normal subjects, showed an increase in liver radioactivity throughout the period of observation, which was not associated with a comparable fall in plasma radioactivity. No concentration of radioactivity was found in spleen or white cells of patients with leukemia.

4. The plasma of patients with active chronic myelogenous leukemia can bind increased amounts of vitamin B_{12} *in vivo*. This may aid in differentiating chronic myelogenous leukemia from leukemoid states.

5. At the dosage level used, an increased binding of cobalt^{60} labelled vitamin B_{12} by chronic myelogenous leukemic tissue was not found.

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