The Plasma Disappearance of Radioactive Vitamin B_{12} in Myeloproliferative Diseases and Other Blood Disorders

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THE PLASMA vitamin B_{12} is abnormally high in chronic myelocytic leukemia.[1-8] It is sometimes increased above normal in polycythemia vera[3-5] and myeloid metaplasia,[3-5] but usually not as strikingly so. The plasma vitamin B_{12} is normal in chronic lymphocytic leukemia,[1,2,4,5,8] lymphoma,[4,5,8] myeloma,[3,4] and acute lymphoblastic leukemia.[1,6] It is usually normal in undifferentiated acute leukemia,[2,5] but high levels have been reported.[4] Many patients with acute or subacute myeloblastic leukemia have a normal plasma B_{12}, but in some cases it is markedly elevated.[3,6,8] It may be elevated in DiGuglielmo's syndrome.[9] This abnormality of plasma vitamin B_{12} appears to be found in the diseases often grouped as myeloproliferative disorders, but not in other neoplasms of the blood forming organs. Not only is the plasma B_{12} high in chronic myelocytic leukemia, but the capacity of plasma to bind added vitamin B_{12} is increased.[2,5-7] This high binding capacity probably is due to an increase in the normal B_{12} binding protein.[10,11] The exact nature of this protein is unknown, but it is in the seromucoid fraction of plasma protein.[10,11]

Four groups[12-15] have further studied the problem by determining the rate of plasma disappearance of a small intravenous dose of radioactive vitamin B_{12}. Although the dose ranged from 0.5 to 4.0 μg., all four groups demonstrated a distinct slowing of the disappearance of the B_{12} from the plasma in chronic myelocytic leukemia. Miller et al.[13] also found some slowing of disappearance in two patients with myeloid metaplasia, while a patient with chronic lymphocytic leukemia had a normal disappearance curve. Weinstein and Watkin[16] found slowing of plasma disappearance in chronic myelocytic leukemia and myeloid metaplasia after 0.5 μg. oral doses of radioactive B_{12}.

The present study was designed to determine whether the abnormality of plasma disappearance was common to all disorders of the myeloproliferative group, and whether it occurred in other blood diseases where there is hyperplasia or neoplasia of a particular cell series. In order to keep the intravenous dose as close to a tracer dose as possible, the usual dose was 0.04–0.05 μg. The normal total plasma B_{12} in this laboratory is of the order of 400 $\mu\mu$g./ml. and thus this test dose would be about 3.5 per cent of the total plasma content of a subject with a plasma volume of 2700 ml. Using the minimum value for total B_{12} binding capacity of 650 $\mu\mu$g./ml.,[11] a normal subject could completely bind the test dose in approximately 160 ml. of plasma.

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Methods

The technics of administration of the dose, sampling, vitamin B_{12} assay, measurement of radioactivity and calculation have been given in detail previously.[17] Deviations from former technics are given below. The dose was injected rapidly in 5 ml. of saline. $Co^{57}B_{12}$ as well as $Co^{58}B_{12}$* was used. The former was of lower specific activity but could be counted more efficiently. One ml. in the small well crystal gave 1.6 x 10^6 c/m/μc, and 10 ml. in the large well crystal gave 1.2 x 10^6 c/m/μc with backgrounds of 20–23 c/m and 28–31 c/m respectively. The plasma volume was measured by T-1824 in over two-thirds of the cases. In the remainder, it was estimated by the formula:

$$\text{Plasma volume (ml.)} = \frac{\text{Plasmacrit x 76.5 ml./Kg. x body wt. (Kg.)}}{100}$$

The control subjects were hospitalized patients who had recovered from their acute illness, and none had any disease known or thought likely to involve the intake or metabolism of vitamin B_{12}. The plasma B_{12} was normal in each. Usually the dose of radioactive vitamin B_{12} was 0.04–0.05 μg. A higher dose was necessary in some studies, particularly those of relative polycythemia, because of a low specific activity of the isotope. Doses of 0.14–0.18 μg. were used in a few patients with leukemia because the distribution of radioactivity was to be followed by means of body counting. The Co^{58} samples were usually counted to an accuracy of 4–7 per cent, but in some cases the accuracy was 7–10 per cent and in a few instances over 10 per cent. The Co^{57} samples, about one-half of the total, were easily counted to less than 4.5 per cent and usually to 3 per cent or less.

Results

The amounts of radioactivity remaining at various times in each case are given in table 1. Initially sampling was at 5, 15, 30, 60 minutes and 6 and 24 hours. Later the schedule was changed to 5, 25, 55, 90 minutes and 6 and 24 hours. When the latter was used, the 15, 30 and 60 minute points on table 1 were found by interpolation. The normal disappearance curve of this small dose was described previously,[17] and for present purposes it is sufficient to note that at one hour only 20 per cent or less of the radioactivity remained and at 24 hours usually less than 10 per cent.

Two abnormalities were seen in the patients with disease. The first was a slowing of plasma disappearance as illustrated in figure 1. This was found in some but not all patients with polycythemia vera, chronic myelocytic leukemia, myeloid metaplasia, acute myeloblastic leukemia and stem cell leukemia. Normal disappearance was found in secondary polycythemia, relative polycythemia, acute lymphoblastic leukemia and chronic lymphocytic leukemia. The results were borderline in the patient with thrombocythemia and in the patient with leukemoid reaction. The latter was difficult to classify. He had Hodgkin's disease with hypersplenism. The bone marrow was hypercellular and immature, and there were myeloblasts in the peripheral blood. Both Hodgkin's disease and extramedullary hematopoiesis were found in the spleen removed at splenectomy.

The second abnormality was a rise in plasma radioactivity at various times after the initial fall. This phenomenon was seen in five patients, four with polycythemia vera and one with myeloid metaplasia. The rise in plasma

*Both forms of labeled vitamin B_{12} were generously supplied by Dr. Nathaniel S. Ritter, Merck Sharp and Dohme Research Laboratories, Rahway, N. J.

radioactivity is not always indicated in table 1 because it sometimes occurred at times not given in the table or because the data in the table were taken from the curve best fitting the experiment data. The phenomenon is illustrated in figure 2, which clearly shows that the magnitude of fluctuations in plasma activity was too great for it to be attributed to counting error. Moreover, the samples of patient E. C. were counted twice with identical results. Patient R. R. was studied twice at an interval of one year and using two different cobalt isotopes of B_{12}. The irregular disappearance curve was seen both times. This abnormality was not found in the control subjects, in the lymphogenous leukemias, in relative or secondary polycythemia or in eleven subjects with liver disease not included in the present material.

The two abnormalities described could not be correlated with any other parameter such as leukocyte count or hematocrit. They were more common in subjects with a high plasma B_{12}, but some, such as W. S. and H. Q., had a high B_{12} and a normal disappearance curve. Others, such as E. C. and F. M. had a normal plasma B_{12} but abnormal plasma disappearance. Plasma disappearance abnormalities were greatest among untreated patients but were sometimes seen in those in remission.

Discussion

The previously described slow disappearance[12-15] of an intravenous dose of vitamin B_{12} in chronic myelocytic leukemia was again found in the present study. In addition, the same abnormality was seen in other disorders considered to be part of the myeloproliferative group. This is further evidence of a relationship among the diseases of the group. A test based on the present study might have some value in the separation of the types of leukemia and of the types of polycythemia, but many more cases must be studied before this could be attempted.

The re-entry of radioactivity into the plasma seen in the myeloproliferative disorders has been commented on previously only in the preliminary report of the present work[18] and by Weinstein and Watkin.[16] The latter used an oral dose instead of intravenous, and they were examining later processes than did the present study. However, it seems likely that both groups were observing the same fundamental abnormality. One should not conclude from the present study that the re-entry never occurs in normal subjects or that the true incidence in disease has been described. It was impossible to predict when a rise in radioactivity would take place. The most intensive searches and the best chances of detection of the rise were in patients with polycythemia where the frequent removal of 30 ml. samples was possible. However, the detection of the secondary rise in studies where sampling was routine indicates that it is at least more common in the myeloproliferative disorders.

The present study is primarily descriptive and gives no clue to the nature of the processes which were observed. Previously[17] we suggested that the B_{12} disappearance curve in normal subjects represented distribution into a multicompartment closed system. In such a system there may be many simultaneous processes moving B_{12} in and out of the sampled compartment, the plasma. The plasma concentration of the tracer would reflect only the net change

Table 1.—*The Plasma B_{12} Disappearance and Other Data on All Patients Studied*

Pt.	Dose μg.	Plasma volume ml.	Per cent 0 Concentration Remaining at: 5′	15′	30′	60′	90′	6h	24h	Hct.	WBC x 10^3	Plasma B_{12} μμg./ml.
Control subjects												
L. S.	0.04	2660*	46	30	25	19	--	12	7.6	48	11	411
C. T.	0.04	3090*	44	25	20	16	--	8.8	5.4	43	6.3	266
H. G.	0.04	2470*	36	23	18	12	—	7.4	3.5	48	4.6	413
F. S.	0.04	2580*	39	20	19	15	--	9.6	8.0	50	8.8	413
F. B.	0.04	3470	51	36	25	20	17	13	12	49	7.6	483
E. F.	0.04	2806	43	26	24	16	14	8.7	6.6	48	6.0	285
A. B.	0.04	2927	29	22	16	13	12	9.1	6.8	47	6.5	253
V. B.	0.04	2520	34	25	18	13	12	6.7	4.4	50	7.5	199
Mean—8 subjects (Plasma volume estimated)			42	28	22	17	16	10	7.3			
Mean—4 subjects (Plasma volume measured)			39	27	21	16	14	9.4	7.4			
Stem cell leukemia—possibly myeloblastic												
H. Q.	0.17	3710*	43	29	22	17	14	—	—	23	240	1344
G. J.	0.04	3340	55	48	40	34	31	25	21	31	230	1752
Acute lymphoblastic leukemia												
J. N.	0.04	2985	48	28	19	14	12	8.7	5.5	29	40	516
E. B.	0.04	3106	50	34	26	20	18	13	9.4	29	11	220
Chronic lymphocytic leukemia												
J. C.	0.18	2345	33	22	18	14	11	8.8	5.0	37	150	262
J. N.†	0.04	2760*	34	25	19	13	11	7.5	—	45	64	728
J. D.†	0.15	3265	35	19	15	11	8.5	—	—	40	86	269
Secondary polycythemia												
M. R.††	0.05	3940	48	30	20	12	9.4	7.7	8.5	53	7.3	337
J. C.	0.04	2587	33	23	17	14	12	9.0	5.7	58	7.2	265
Relative polycythemia												
R. C.	0.07	3488	52	28	17	11	9.2	6.6	4.8	62	6.8	221
S. W.	0.08	3806	38	27	20	15	12	6.9	4.7	55	5.8	232
J. S.	0.08	2266	34	22	18	14	12	7.9	4.8	54	13	354
W. B.	0.05	2700	36	26	18	14	13	8.6	2.6	59	9.0	259
C. S.††	0.08	2503	32	19	14	9.9	7.9	5.4	4.3	50	8.5	159

Leukemoid reaction—Hodgkins disease and hypersplenism												
F. M.	0.05	3439	57	37	29	25	23	17	14	31	7.2	1012
Hodgkins disease												
R. H.	0.04	3575	39	16	12	8.5	7.0	4.0	1.7	33	7.1	91
Myelofibrosis												
M. D.	0.04	3224	34	25	18	12	9.5	4.8	3.4	30	1.7	673
Polycythemia vera												
R. S.[tt]	0.06	3670	82	63	66	53	39	—	43	52	32	1319
R. G.[tt]	0.04	5020	71	59	55	51	48	39	25	28	34	397
E. C.[k,s]	0.04	1880*	35	25	16	21	14	12	7.5	53	9.7	548
T. F.[k]	0.04	3920*	43	32	27	24	—	—	—	51	12	490
P. M.[t]	0.04	3540	34	25	20	17	15	11	—	53	6.1	498
R. R.[t] (1959)	0.04	2460*	35	30	27	19	17	—	—	55	5.5	191
R. R.[t] (1960)	0.04	2812	37	29	23	19	17	14	—	50	6.2	154
Chronic myelocytic leukemia												
C. D.	0.04	3880	80	72	68	59	56	48	28	24	19	3296
T. C.	0.04	3621	66	50	46	42	40	35	27	28	10	667
P. B.	0.14	3229	83	71	62	56	53	46	36	50	23	923
R. J.[t]	0.04	3270*	33	26	25	19	18	15	10	46	7.8	813
Myeloid metaplasia												
J. H.[s]	0.04	2320*	54	52	48	37	—	39	31	58	94	2528
R. L.[s]	0.04	3290*	51	47	43	36	—	33	28	30	26	2183
O. S.	0.21	3280	46	36	31	27	27	25	18	32	11	751
Thrombocythemia												
A. C.	0.31	3561	44	35	27	23	19	15	12	44	20	419
Acute myeloblastic leukemia												
F. M.	0.18	3180*	69	62	53	43	39	31	25	32	140	355
W. S.	0.17	3500	36	27	20	17	16	12	8.7	19	40	3237
J. M.	0.04	2659	43	33	26	20	17	11	7.1	38	1.6	607
V. K.[t,m]	0.04	3485	31	21	13	9.7	8.0	5.7	—	47	7.3	321

*Plasma volume estimated.
†Receiving prednisone.
[tt]Post phlebotomy.
[k]Early in relapse.
[t]In remission.
[s]Classification difficult. Mixed myeloproliferative syndrome in which manifestations varied.
[m]On chemotherapy.

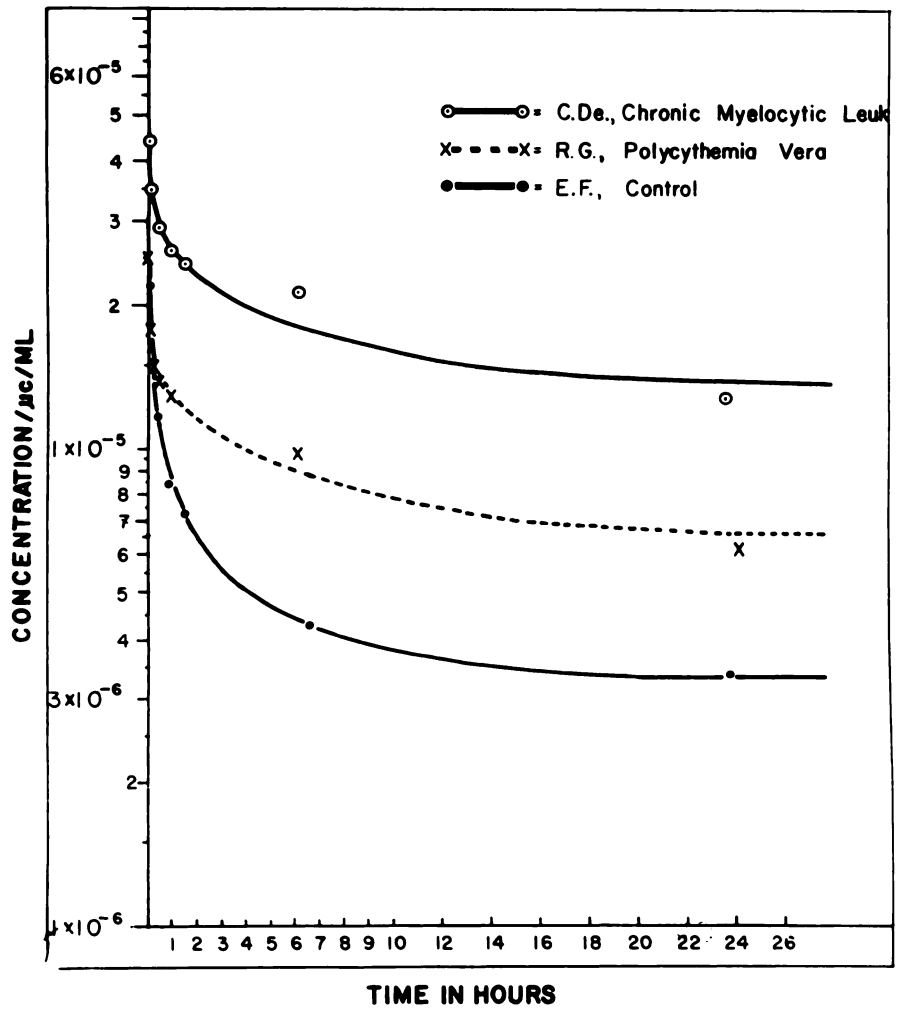

Fig. 1.—The plasma disappearance of an intravenous dose of 0.04 μg. of Co^{57} B_{12}.

of these processes, and an entrance process would be unobserved if the sum of the exit processes was greater. The rate of the initial rapid component of the disappearance curve was similar both in control subjects and in patients. This rapid loss of activity was halted sooner in the patients with myeloproliferative disease and was followed by a plateau, a rise, or a slow fall in radioactivity. These events may reflect a process of entry into plasma which is not present in normal subjects or is more active in myeloproliferative disease than in the normal state.

One may properly ask if the delayed plasma B_{12} removal in myeloproliferative disorders is not simply an indirect measurement of the known increase in plasma binding capacity. The in vitro plasma binding capacity as measured by current technics may or may not have any physiologic significance

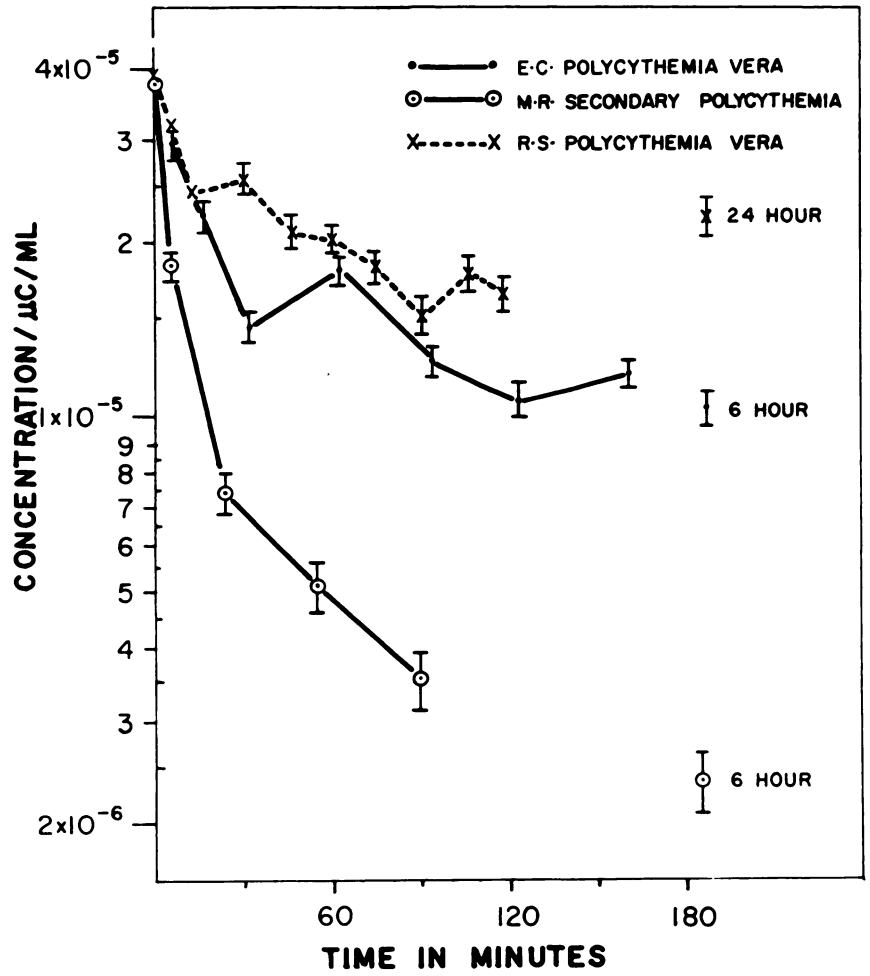

Fig. 2.—The plasma disappearance of 0.04-0.06 µg. of intravenous Co^{58} B_{12}. The counting accuracy is indicated for each point on the curves.

but it has been used widely in the interpretation of the behavior of vitamin B_{12} in the blood. If the fraction of an intravenous dose which remains in the plasma is directly related to the unsaturated binding capacity and the dose, the phenomenon of delayed disappearance in myeloproliferative disease could be reproduced in normal subjects by sufficiently lowering the dose. Such an experiment would be valid only if plasma binding capacities were not exceeded. Previous studies[17] showed that a dose of 0.008 µg. was removed at approximately the same rate as one of 0.04 µg. Five cases from the present study, selected to represent a wide range of binding capacities as measured by the charcoal extraction technic, are listed in table 2. Even with the greatest degree of retention, 94 per cent of the binding capacity remained unsaturated, and the amount retained appeared to be quite unrelated to the measured binding capacity. While the present study does not support the

Table 2.—*Comparison of B_{12} Retained in Plasma and the Total Plasma Binding Capacity*

Subject	Total plasma binding capacity μg.*	Retained in plasma μg. x 10^{-2} 5 min.	24 hr.	Per cent binding capacity utilized 5 min.	24 hr.
L. S.	1.1	1.8	0.34	1.6	0.3
P. B.	2.2	12.0	4.8	5.5	2.2
J. H.	3.3	2.2	1.2	0.6	0.4
R. S.	3.5	4.9	2.6	1.4	0.7
W. S.	4.1	6.1	1.5	1.5	0.4

*Plasma volume (ml.) x unsaturated binding capacity as measured by charcoal extraction method (μg./ml.).

theory that the plasma disappearance abnormalities of myeloproliferative disorders are produced by the increase in binding capacity, there is direct support for this theory. Brody et al.[14] showed that vitamin B_{12} mixed with serum from a patient with chronic myelogenous leukemia was removed at a slower than normal rate when given to a subject with an expected normal disappearance rate. Ritz and Meyer[15] demonstrated that the abnormal disappearance rate in chronic myelogenous leukemia could be returned to normal by giving large amounts of nonradioactive vitamin B_{12} prior to the study. The differences in the results and their interpretation among the various groups studying the problem simply points out our ignorance of the series of events which follow the injection of vitamin B_{12}.

Summary

1. The plasma disappearance of a small intravenous dose of radioactive vitamin B_{12} was determined in control subjects and in patients with various blood disorders.

2. A delayed, sometimes irregular, disappearance was observed in the majority of patients with acute and chronic myelogenous leukemia, myeloid metaplasia, and polycythemia vera.

3. Disappearance was normal in the lymphogenous leukemias, secondary polycythemia and relative polycythemia.

4. The abnormalities observed are believed to indicate an abnormality of vitamin B_{12} metabolism common to the diseases of the myeloproliferative group and are further evidence of the close relationship between these diseases.

Summario in Interlingua

1. Le disparition ab le plasma de un micre dose intravenose de vitamina B_{12} radioactive esseva determinate in subjectos de controlo e in patientes con varie disordines del sanguine.

2. Esseva observate un disparition retardate, a vices irregular, in le majoritate del patientes con acute e chronic leucemia myelogene, con metaplasia myeloide, e con polycythemia ver.

3. Le disparition esseva normal in casos de leucemia lymphogene, de polycythemia secundari, e de polycythemia relative.

4. Es opinate que le anormalitates observate reflecte un anormalitate in le metabolismo de vitamina B_{12} le qual es commun al disordines del gruppo myeloproliferative e le qual corrobora per consequente le concepto de un intime relation inter iste disordines.

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REFERENCES

1. Killander, A.: B_{12} vitaminhalt i serum vid akut och kronisk leukemi. Nord. med. 52:1513–1515, 1954.
2. Beard, M. F., Pitney, W. R., and Saneman, E. H., Jr.: Serum concentrations of vitamin B_{12} in patients suffering from leukemia. Blood 9:789–794, 1954.
3. Mollin, D. L., and Ross, G. I. M.: Serum vitamin B_{12} concentrations in leukaemia and in some other haematological conditions. Brit. J. Haemat. 1:155–172, 1955.
4. Erdmann-Oehlecker, S.: Der Vitamin B_{12}-Stoffwechsel bei Hämoblastosen. *In* Vitamin B_{12} und Intrinsic Factor, a Symposium, Heinrich, H. E., ed., Stuttgart, Ferdinand Enke, 1957.
5. Rachmilewitz, M., Izak, G., Hochman, A., Aronovitch, J., and Crossowicz, N.: Serum vitamin B_{12} in leukemias and malignant lymphomas. Blood 12: 804–813, 1957.
6. Raccuglia, G., and Sacks, M. S.: Vitamin B_{12} binding capacity of normal and leukemic sera. J. Lab. & Clin. Med. 50:69–77, 1957.
7. Miller, A.: The in vitro binding of cobalt 60 labeled vitamin B_{12} by normal and leukemic sera. J. Clin. Invest. 37:556–566, 1958.
8. Mendelsohn, R. S., and Watkin, D. M.: Serum vitamin B_{12} concentrations determined by l. leichmannii assay in patients with neoplastic disease. J. Lab. & Clin. Med. 51:860–866, 1958.
9. Baldini, M., Fudenberg, H. H., Fukutake, K., and Dameshek, W.: The anemia of the DiGuglielmo syndrome. Blood 14:334–363, 1959.
10. Weinstein, I. B., Weissman, S. M., and Watkin, D. M.: The plasma vitamin B_{12} binding substance: I. Its detection in the seromucoid fraction of plasma from normal subjects and patients with chronic myelocytic leukemia. J. Clin. Invest. 38:1904–1914, 1959.
11. Miller, A., and Sullivan, J. F.: Electrophoretic studies of the vitamin B_{12}-binding protein of normal and chronic myelogenous leukemia serum. J. Clin. Invest. 38:2135–2143, 1959.
12. Mollin, D. L., Pitney, W. R., Baker, S. J., and Bradley, J. E.: The plasma clearance and urinary excretion of parenterally administered cobalt 58 B_{12}. Blood 11:31–43, 1956.
13. Miller, A., Corbus, H. F., and Sullivan, J. F.: The plasma disappearance, excretion, and tissue distribution of cobalt 60 labelled vitamin B_{12} in normal subjects and patients with chronic myelogenous leukemia. J. Clin. Invest. 36:18–24, 1957.
14. Brody, E. A., Estren, S., and Wasserman, L. R.: The kinetics of intravenously injected radioactive vitamin B_{12}: Studies on normal subjects and patients with chronic myelocytic leukemia and pernicious anemia. Blood 15:646–661, 1960.
15. Ritz, N. D., and Meyer, L. M.: Clearance of intravenously injected radioactive cobalt-labeled vitamin B_{12} in chronic myeloid leukemia and other conditions. Cancer 13:1000–1007, 1960.

16. Weinstein, I. B., and Watkin, D. M.: Cobalt 58 B_{12} absorption, plasma transport, and excretion in patients with myeloproliferative disorders, solid tumors and non-neoplastic diseases. J. Clin. Invest. 39:1667–1674, 1960.
17. Hall, C. A.: The plasma disappearance of intravenously administered cobalt 58 vitamin B_{12}. J. Clin. Invest. 39: 1312–1319, 1960.
18. —: Plasma vitamin B_{12} removal in myeloproliferative disorders. Clin. Res. 8: 210, 1960.

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