Serum Co^{60} Vitamin B_{12} Binding Capacity in Some Hematologic Disorders.*† (23477)

LEO M. MEYER, ROBERT W. BERTCHER AND EUGENE P. CRONKITE
Department of Laboratories, South Nassau Communities Hospital, Oceanside, N. Y., and the Medical Department, Brookhaven National Laboratory, Upton, N. Y.

In normal human subjects, vit. B_{12} is bound to serum protein, probably alpha globulin(1). The serum of these individuals has an additional capacity to bind vit. B_{12} which can be estimated *in vitro* using the radioactive vitamin(2). By microbiological assay, both serum content and binding capacity have been found elevated in chronic myelocytic leukemia, and some cases of polycythemia vera and acute leukemia(3,4). In the present study, the unsaturated binding capacity of serum has been measured in some hematological disorders using Co^{60} vit. B_{12} by a method recently reported from this laboratory (2).

Method. Co^{60} vit. B_{12} having a specific activity of 968 μc/mg was used. To 1 ml aliquots of serum in a Visking bag were added 1, 2.5, 5, 10, 25, 50, or 100 mμg of the radioactive vitamin. After incubating 2 hours at room temperature, the mixture was subjected to exhaustive dialysis in cold, running tap water for 48 hours. "Bound" vit. B_{12} was then measured in terms of residual radioactivity.

Results. Normals. A range of unsaturated binding capacity was derived from studies on 6 normal adults. This was similar to, but generally lower than the binding capacity in normal subjects previously reported(2). In the present inquiry, a single fresh batch of Co^{60} vit. B_{12} was used, while originally the material used was several years old.

Chronic myelocytic leukemia. Sera from 14 patients were examined. Ten showed unequivocal elevation of vit. B_{12} binding capacity, 2 were borderline, and 2 fell into the normal range (Table I). Of the ten, 3 showed marked and 7 moderate elevation (Fig. 1a, b).

Using the binding capacity of 1 ml of serum for 25 mμg of vit. B_{12} as a reference point, the correlation was fair with total leukocyte count, and less good with the absolute myelocyte-myeloblast count (Fig. 1c, d).

Two patients were studied before and after chemotherapy. In patient K, when hematological and clinical remission ensued, Co^{60} vit. B_{12} binding fell to the normal range. When partial remission occurred in patient S, the binding capacity was insignificantly reduced (Fig. 2a). Two patients, C and F, who showed normal binding capacity were in clinical remission, although the former still had a leukocyte count elevated to 41,000/cmm.

Patient J was studied terminally at which time he showed morphological evidence of acute disease. His serum had the highest vit. B_{12} binding capacity in this survey.

Chronic lymphocytic leukemia. Previous reports indicated no significant increase in serum content of vit. B_{12} in this disease(3,4). Of 6 patients studied, serum of one showed moderate, and 2 slight increase in vit. B_{12} binding capacity. The other 3 were in the normal range (Fig. 2b). The binding capacity when abnormal was less elevated than in myelocytic leukemia. Correlation between binding capacity of 1 ml of serum at the 10 mμg level and total leukocyte count was poor.

Acute leukemia. Eight adults in relapse were studied. In 7 the cell type was identified as myeloblastic. Two sera showed an increased vit. B_{12} binding capacity (Fig. 2c). In patient McC the course was subacute, of one year's duration. One person who had acute stem cell leukemia showed a normal serum binding capacity. *Polycythemia vera.* Two patients were studied. One showed mild elevation of serum binding capacity for vit. B_{12} while the other was within the normal

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TABLE I. Co^{60}Vit. B_{12} Binding Capacity of Serum in Chronic Myelocytic Leukemia.

Patient	mμg $Co^{60}B_{12}$ bound at 25 mμg/ml	Current therapy	Total leukocytes per mm^3	Total myelocytes-myeloblasts
Normal avg + 2 S.D.	3.51			
P	6.95	Prednisone	18,900	764
K (pre-therapy)	12.40	6 M-P	117,000	24,570
K (post-therapy)	3.45	6 M-P	5,050	101
Me	3.78	Myleran	73,000	14,600
Mu	4.78	"	22,400	1,568
C	3.10	"	41,000	1,640
M	5.15	"	39,000	3,540
Pa	3.80	None	21,000	6,930
Wi	4.40	"	376,000	92,120
F	2.68	"	11,650	0
Ma	5.60	Myleran	87,000	31,500
S (pre-therapy)	11.83	"	95,000	17,100
S (post-therapy)	10.08	"	47,000	5,640
R	4.23	None	16,500	165
Po	7.40	6 M-P	30,000	3,600
J	20.25	None	73,000	38,690

range. The patient with elevated binding capacity had a persistent leukocytosis of 15-25,000/cmm, while the other patient's leukocyte count remained about 10,000/cmm. *Pernicious anemia.* Serum binding capacity for vit. B_{12} of 3 patients in relapse was normal.

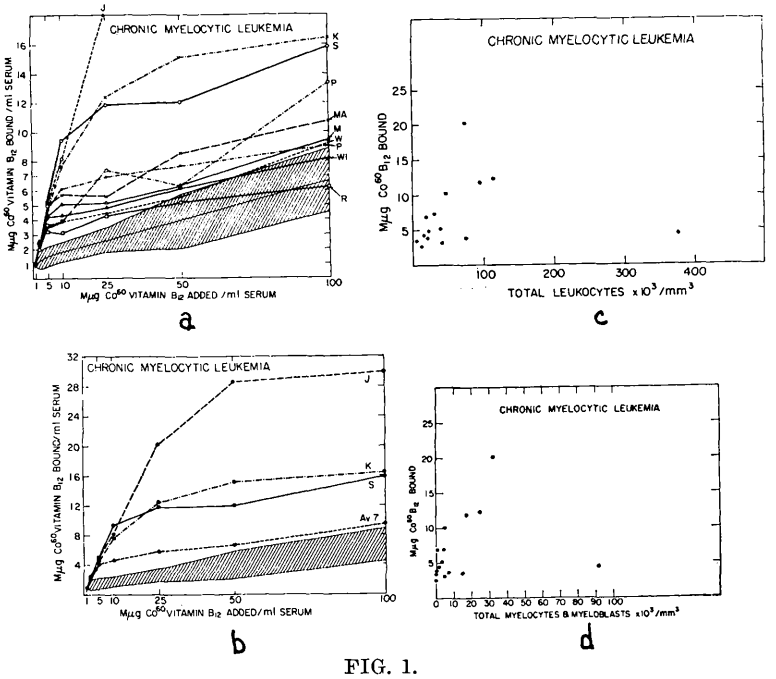

FIG. 1.

(a) Binding capacity for Co^{60} vit. B_{12} of sera from 10 patients with chronic myelocytic leukemia. Shaded area represents normal avg binding capacity $\pm$ 2 S.D.

(b) Serum binding capacity for Co^{60} vit. B_{12} in 3 cases of chronic myelocytic leukemia showing the greatest elevation. Avg of binding capacity in 7 other patients is shown.

(c) Correlation of serum binding capacity for Co^{60} vit. B_{12} in chronic myelocytic leukemia with total leukocyte count.

(d) Correlation of serum binding capacity for Co^{60} vit. B_{12} in chronic myelocytic leukemia with absolute myeloblast-myelocyte count.

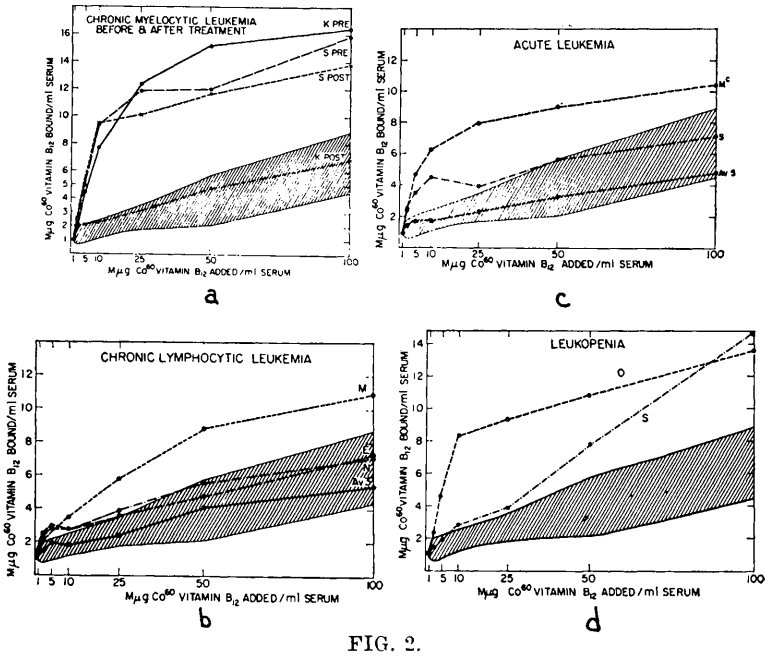

FIG. 2.

(a) Serum binding capacity for Co^{60} vit. B_{12} in chronic myelocytic leukemia before and after therapy.

(b) Serum binding capacity for Co^{60} vit. B_{12} in 3 cases of chronic lymphocytic leukemia showing an elevation. Avg of 3 cases falling within normal range is shown.

(c) Serum binding capacity for Co^{60} vit. B_{12} in 2 cases of acute leukemia showing an elevation and 5 cases (avg) falling within normal range.

(d) Serum binding capacity for Co^{60} vit. B_{12} in 2 cases of idiopathic neutropenia.

Leukopenia. Two adults with chronic, idiopathic neutropenia were studied. The bone marrow of each was normal. Each exhibited abnormally elevated serum binding capacity for vit. B_{12} (Fig. 2d). It will be noted that the pattern of binding of patient O resembles that seen in chronic myelocytic leukemia. In patient S, the abnormal binding is manifest only when the larger amounts of vit. B_{12} are used.

Discussion. Several observers, using microbiological estimation, have noted an increased binding capacity for vit. B_{12} in sera of patients with chronic and acute myelocytic leukemia(3,5). Others, employing radioactive vitamin, have noted a decreased rate of its plasma clearance in chronic myelocytic leukemia(6,7). This may be considered an equivalent *in vivo* phenomenon, because with increased binding capacity a larger part of the administered vitamin is bound to serum protein and consequently lost from plasma more slowly than in normals. In general, the *in vitro* or *in vivo* abnormality showed a mildly positive or no relationship with total leukocyte count and severity of disease. These findings are in harmony with the results of the present study. We have also confirmed (though in one case only) the increased binding capacity which may be present in polycythemia vera.

Inspection of the graph (Fig. 1 a, b) of vit. B_{12} binding in patients with chronic myelocytic leukemia shows a rapidly ascending essentially straight segment followed by a portion which parallels the normal. This suggests a specific binding mechanism which becomes saturated, following which non-specific binding—as in normals—becomes evident. If the straight portions of the ascending and horizontal limbs of the graphs were extended to an intersecting point, this point might be a measure of total binding capacity. We have arbitrarily used the amount of vitamin bound when 10 or 25 mμg were added per ml serum for correlation with leukocyte count.

Because previous investigation had shown increased serum binding capacity for vit. B_{12}

only in those hematological disorders characterized by increased granulopoiesis, Mollin (3) proposed that disintegrating granulocytes might be the source of an increased binding substance in the serum. He was able to demonstrate this *in vitro* by mechanically injuring granulocytes. In the present report, we have shown that the serum of patients with chronic lymphocytic leukemia and idiopathic granulocytopenia may have an increased binding capacity for vit. B_{12}. This suggests an alternative source for binding substance. However, if the rate of granulocyte production were masked by an excessive rate of destruction, these cells might represent the source of binding substance in all instances. Leukocyte agglutinins, which have been reported to be only infrequently present in chronic lymphocytic leukemia and idiopathic neutropenia(8), were not sought in these cases.

Other investigators have noted serum content of vit. B_{12} to be elevated always in chronic myelocytic leukemia, even during remission(3,4,7). Our method does not permit accurate measurement of less than 1 mμg of vitamin. With this reservation, 2 of our patients with this disease showed normal, 2 borderline elevation of vit. B_{12} binding capacity. All 4 were under treatment and in various degrees of clinical and hematological remission. A fifth patient's serum fell to normal in binding capacity following treatment. Patient W, who had received no specific therapy and who had the highest leukocyte count in this study, showed only a moderate elevation of binding capacity. However, she had been treated with parenteral vit. B_{12} for several months before she came to our attention. This may have saturated her binding capacity, as Mollin has shown(3), thus accounting for the relatively small degree of unsaturation demonstrated.

The findings in acute myelocytic leukemia are in accord with previous demonstrations that serum content of and binding capacity for vit. B_{12} in this disease may be elevated, though usually to a lower level than in the chronic form of the disease.

Summary and conclusions. 1. A survey of *in vitro* Co^{60} vit. B_{12} binding capacity of serum from patients with various hematological diseases is presented. Results are compared with previous studies employing microbiological assay and with measurements of serum content as well as binding capacity for the vitamin. 2. These data confirm earlier findings of elevated binding capacity in acute and chronic myelocytic leukemia and in polycythemia vera. 3. An increased binding capacity in some cases of chronic lymphocytic leukemia and idiopathic neutropenia is noted for the first time.

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