

Serum Concentrations of Vitamin B₁₂ in Patients Suffering from Leukemia

By MARION F. BEARD, W. R. PITNEY and E. H. SANNEMAN

DURING the course of an investigation into the manner of binding of vitamin B₁₂ to serum protein,¹ it was observed that the vitamin B₁₂ serum concentration in some cases of leukemia was greatly above the normal range. This has also been noted by Ross.² Because the abnormally high serum concentrations have been so consistent in certain types of leukemia, it was decided to investigate the phenomenon more completely. The ability of these sera to bind added crystalline vitamin B₁₂ in vitro and the location of the vitamin in sera fractionated by paper electrophoresis were studied.

METHODS

Vitamin B₁₂ serum concentrations have been assayed by a microbiologic technic, using *Euglena gracilis*³ as test organism. The technic used was identical with that described by Ross.⁴ By this method, vitamin B₁₂ bound to serum protein and free vitamin B₁₂ in serum can be differentiated. Methods used to test the ability of serum to bind added vitamin B₁₂ in vitro and to establish the location of the vitamin in electrophoretic fractions of serum have already been described.¹

RESULTS

Total Vitamin B₁₂ Serum Concentrations in Leukemia

In table 1 are shown the total vitamin B₁₂ serum concentrations in a group of normal individuals and in patients suffering from various types of leukemia.

In twenty-two normal individuals a range of concentration from 86 to 460 $\mu\mu\text{g./ml.}$ was found with a mean of 184 $\mu\mu\text{g./ml.}$ In eighteen cases of chronic lymphatic leukemia, the range was slightly greater, from 40 to 540 $\mu\mu\text{g./ml.}$ but the mean of 209 $\mu\mu\text{g./ml.}$ approximated to that of the normal group. In fourteen of these eighteen cases, all the vitamin B₁₂ was estimated to be in the bound form. In the remaining four, levels of free vitamin of 40, 44, 122, and 136 $\mu\mu\text{g./ml.}$ were recorded. Three of the eighteen were considered cases of lymphosarcoma with low total white cell counts but showing marked infiltration of bone marrow and lymph nodes with lymphocytes. The remaining fifteen were of the usual type of chronic lymphatic leukemia. At one time or another, all of these had shown high total white cell counts with a greatly increased

From the Section of Hematology, Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky.

Submitted October 2, 1953; accepted for publication November 19, 1953.

This investigation was supported in part by a grant from the National Institutes of Health, Bethesda, Md. and in part by a grant from the Squibb Institute for Medical Research, New York, N.Y.

Vitamin B₁₂ used in these studies furnished by E. R. Squibb and Sons, New York, N. Y.

The authors wish to acknowledge the valuable assistance of Dr. E. J. VanLoon of the Medical Research Laboratory, Veteran's Administration Hospital, Louisville, Ky., who performed the electrophoretic fractionation of all sera studied in this investigation.

TABLE 1.—Total Serum Concentrations of Vitamin B₁₂ ($\mu\mu\text{g./ml.}$) from Normal Individuals and Patients with Various Types of Leukemia

Number of sera tested	Normals	Chr. lymphatic leukemia	Chr. myeloid leukemia	Acute leukemia
1	132	162	768	630
2	280	106	1504	840
3	460	172	540	776
4	210	355	3200	376
5	166	460	2000	356
6	122	272	1220	1000
7	224	154	4672	216
8	148	140	1176	
9	130	540	6500	
10	148	340	3040	
11	146	66	4800	
12	200	104	3360	
13	124	56		
14	124	86		
15	196	278		
16	188	216		
17	86	40		
18	140	212		
19	94			
20	228			
21	180			
22	320			
Range.....	86-460	40-540	540-6500	216-1000
Mean.....	184	209	2732	599

percentage of mature lymphocytes. At the time these serum levels were assayed, some cases were in remission following x-ray therapy. The white cell counts of the whole group ranged from 3000 to 342,000 per cu.mm. with a mean of 82,350 per cu. mm.

In contrast to the above normal findings in chronic lymphatic leukemia, the serum vitamin B₁₂ concentration was uniformly elevated in the cases of chronic myeloid leukemia studied. In twelve patients with chronic myeloid leukemia, the range of concentration was found to be from 540 to 6500 $\mu\mu\text{g./ml.}$ with a mean of 2732 $\mu\mu\text{g./ml.}$, approximately fifteen times the mean of the normal group and thirteen times that of the group with chronic lymphatic leukemia (table 1). In all cases the vitamin was assayed to be totally in the bound form. These cases will be analyzed in greater detail below.

The concentrations in seven cases of acute leukemia were variable. An attempt has not been made to differentiate between cell types in this group. In three cases the vitamin B₁₂ concentration was in the normal range. In the remaining four, there was a moderate elevation in concentration. The range for the seven cases was from 216 to 1000 $\mu\mu\text{g./ml.}$ with a mean of 599 $\mu\mu\text{g./ml.}$

As the concentrations of vitamin B₁₂ were so markedly elevated in chronic myeloid leukemia, it was decided to investigate these sera in more detail.

TABLE 2.—*Serum Vitamin B₁₂ Concentration and Total White Cell Count in Twelve Cases of Chronic Myeloid Leukemia*

Serum case number	Vitamin B ₁₂ conc. ($\mu\mu\text{g./ml.}$)	Total white cell count/cu. mm.
1	768	12,800
3	540	20,900
8	1176	22,500
6	1220	30,000
10	3040	31,600
4	3200	77,200
2	1504	79,200
12	3360	94,200
11	4800	183,000
5	2000	231,000
7	4672	283,000
9	6500	326,000

Correlation of Serum Vitamin B₁₂ Concentration and Total White Cell Count in Chronic Myeloid Leukemia

In general, there was a correlation between the total white cell count and the vitamin B₁₂ serum concentration in chronic myeloid leukemia (table 2). Thus cases 1, 3, 6, and 8 all showed white cell counts not above 30,000 per cu. mm. and B₁₂ serum concentrations of 1220 $\mu\mu\text{g./ml.}$ or less. Case 9 showed the highest serum concentration and the highest white cell count. The correlation was not absolute however. Case 10 showed a white cell count of 31,600 per cu.mm. and a serum concentration of vitamin B₁₂ of 3040 $\mu\mu\text{g./ml.}$ Case 5 showed a white cell count of 231,000 per cu.mm., and a serum concentration of B₁₂ of 2000 $\mu\mu\text{g./ml.}$

Some of these cases were already under treatment when the original serum concentration determination was made. In six cases, multiple serum concentrations have been performed at varying stages of remission or relapse. The results are shown in table 3. In case 4, two periods of relapse and remission were followed. With each remission there was a fall in the serum vitamin B₁₂ concentration. However, although the white cell count returned to the normal range, the serum B₁₂ concentration remained well beyond the upper limit of normal. In three other remissions (cases 6, 9, 11), a similar trend was seen. In cases 2 and 7 the patients relapsed while under observation. Coincident with the rise in white cell count, there was an increase in the serum vitamin B₁₂ concentration.

The Binding Capacity for Vitamin B₁₂ of Sera from Cases of Chronic Myeloid Leukemia

In four cases, 1000 $\mu\mu\text{g.}$ of crystalline vitamin B₁₂ were added to 1 ml. volumes of serum. Mixture was allowed to occur at 37 C. for 2 hours and the sera then reassayed for their content of bound and free vitamin (table 4). In these sera, before the addition of the crystalline vitamin, the content of vitamin B₁₂ already present, was estimated to be wholly in the bound form. When the mix-

TABLE 3.—*Correlation Between Serum Vitamin B₁₂ Concentration and Total White Cell Count in Six Cases of Chronic Myeloid Leukemia While Receiving Treatment with Either Urethane or X-ray Therapy. Serum Case Number Refers to Table 2*

Serum case number	Date	Vitamin B ₁₂ conc. (μg./ml.)	Total white cell count/cu. mm.
2	1/ 9/53	1000	40,200
	3/10/53	1504	79,200
4	2/13/53	2000	28,600
	3/26/53	1240	6,500
	4/29/53	3200	77,200
6	5/25/53	912	5,150
	2/ 9/53	1220	30,000
7	3/ 6/53	930	11,300
	2/ 1/53	2000	32,200
9	6/15/53	4672	283,000
	3/24/53	6500	326,000
11	6/ 6/53	880	9,000
	4/27/53	4800	183,000
	6/24/53	960	23,750

TABLE 4.—*In Vitro Binding Capacity of Four Sera from Individuals with Chronic Myeloid Leukemia, After the Addition of 1000 μg. Crystalline B₁₂ to 1 ml. Serum. Serum Case Number Refers to Table 2*

Serum case number	Vitamin B ₁₂ conc. (μg./ml.)			Vit. B ₁₂ conc. (μg./ml.) after 1000 μg. crystalline B ₁₂ added			Added B ₁₂ recovered (μg./ml.)	% recovery added B ₁₂
	Total	Bound	Free	Total	Bound	Free		
1	768	768	0	1296	1296	0	528	52.8
2	1504	1504	0	2016	2016	0	512	51.2
9	6500	6500	0	6848	6848	0	348	34.8
10	3040	3040	0	3424	3424	0	384	38.4

tures were assayed, all the vitamin B₁₂ recovered was also in the bound form, and no detectable free vitamin was present. This is contrary to similar experiments with normal sera, where free vitamin of a mean value of 399 μg./ml. was present in the mixtures.¹ This indicates an abnormal binding capacity for vitamin B₁₂ of chronic myeloid leukemic sera. Recovery of added vitamin B₁₂ was imperfect, ranging from 34.8 per cent to 52.8 per cent. This phenomenon of poor recovery of added B₁₂ has been also constantly observed with normal sera, and may be associated with the present method of Euglena assay, in which heat is used to denature the serum proteins and presumably free all the available B₁₂.

The Location of the Vitamin in Electrophoretic Separation of Sera from Cases of Chronic Myeloid Leukemia

In three cases, electrophoretic separation of serum on paper and assay of the strips for their vitamin B₁₂ content was performed by an already described technic.¹ The results are shown in table 5. Serum from case 11 was investigated during relapse and remission (11a and 11b, table 5). As with normal sera, the alpha globulin fractions are seen to be the primary source of the bound

TABLE 5.—Vitamin B₁₂ Concentrations of Electrophoretic Fractions of Sera from Three Cases of Chronic Myeloid Leukemia

Serum Case Number	Whole Serum Conc. (Bound)	Albumin	Alpha 1 Globulin	Alpha 2 Globulin	Beta Globulin	Gamma Globulin
11a	4800	520	2080	1320	132	0
		400	2240	1600	124	0
11b	960	0	512	320	0	0
		0	480	320	0	0
12	3360	100	680	1520	112	0
		0	576	1664	0	0
5	2000	0	1904		224	132

The concentrations present in whole sera have been estimated at the same time. Serum case number refers to table 2. Case 11 was tested while in relapse (a) and remission (b) following x-ray therapy. Most of the estimations have been performed in duplicate.

vitamin. In most sera, there was some detectable vitamin B₁₂ activity in albumin and beta globulin fractions. This was considered probably due to technical imperfections in the method of separating a large quantity of serum (0.1 ml.) on paper. The amount of vitamin recovered from the strips was in fair agreement with the assayed concentration of vitamin in the whole serum.

DISCUSSION

The finding of hypervitaminosis without superalimentation has not hitherto been reported. Elevation of the folic acid content of leukemic cells has been observed,⁵ but increased total body folic acid content as evidenced by increased serum concentration has not been recorded. The present studies indicate that the average serum vitamin B₁₂ concentration in chronic myeloid leukemia is approximately 15 times the normal mean. The serum concentration in chronic lymphatic leukemia, however, is in the normal range, in spite of higher total white cell counts in the latter. This would indicate that the high B₁₂ concentrations are associated in some way with myeloid activity. This is further substantiated by the approximate correlation of the total white cell count and serum B₁₂ concentration in chronic myeloid leukemia. That myeloid activity is not wholly responsible however, is shown by the fact that three patients in remission with normal total white cell counts still showed about 4 times the normal vitamin B₁₂ serum concentration.

In the sera of chronic myeloid leukemia, as in normal sera, binding is maximal in the alpha globulin fractions. In contrast to normal serum however, these sera can bind, in vitro, large amounts of added vitamin B₁₂. In three such sera with a high vitamin B₁₂ concentration, electrophoretic patterns revealed quantitatively normal alpha globulin fractions. These results would suggest that in chronic myeloid leukemia there exists a qualitative change in the alpha globulin in so far as its capacity to bind vitamin B₁₂ is concerned.

The possibility that the high vitamin B₁₂ concentrations may be the result of decreased erythroid activity has been considered. In other conditions associated with decreased erythroid activity, such as aplastic anemia, serum concentrations have been found to be normal however.

These high serum levels are not due solely to increased absorption of the vitamin, since an abnormal binding capacity of the serum has been demonstrated. When 1000 $\mu\mu\text{g}$. of B₁₂ is added to 1 ml. amounts of normal sera, considerable free vitamin is recoverable upon assay of the mixtures.¹ In the mixtures of leukemic sera and vitamin B₁₂, no free vitamin was recovered. In patients receiving large doses of vitamin B₁₂ therapeutically, similar high concentrations of bound vitamin have not been found. Whether the high serum B₁₂ concentration plays any role in the leukemic process is as yet unknown.

SUMMARY

1. Vitamin B₁₂ serum concentrations have been investigated in various types of leukemia.
2. The concentration in chronic myeloid leukemia is approximately 15 times normal. In chronic lymphatic leukemia it is normal. In acute leukemia it is variable.
3. In chronic myeloid leukemia, the serum vitamin B₁₂ exists in the bound form and the binding capacity for added B₁₂ is increased.
4. Electrophoretic studies indicate that serum vitamin B₁₂ in chronic myeloid leukemia is bound in the alpha globulin fractions.
5. Some correlation was found between serum vitamin B₁₂ concentrations and total white cell counts in chronic myeloid leukemia. The possible significance of this is discussed.

SUMMARIO IN INTERLINGUA

1. Ha essite investigate le concentration de vitamina B₁₂ in le sero de patientes con varie typos de leucemia.
2. In chronic leucemia myeloide le concentration es circa 15 vices plus que normal. In chronic leucemia lymphatic illo es normal. In leucemia acute illo es variabile.
3. In chronic leucemia myeloide, le vitamina B₁₂ del sero existe in forma ligate e le capacitate a ligar B₁₂ additional es augmentate.
4. Studios electrophoretic monstra que in chronic leucemia myeloide le vitamina B₁₂ es ligate al fractiones de globulina alpha.
5. In chronic leucemia myeloide un certe correlation ha essite constatate inter le concentration de vitamina B₁₂ in le sero e le conto total del leucocytos. Le signification possibile de iste facto es discutite.

REFERENCES

- ¹ PITNEY, W. R., BEARD, M. F., AND VANLOON, E. J.: Observations on the bound form of vitamin B₁₂ in human serum. *J. Biol. Chem.* 207: 143, 1954.
- ² ROSS, G. I. M.: Personal communication.
- ³ HUTNER, S. H., PROVASOLI, L., STOKSTAD, E. L. R., HOFFMAN, C. E., BELT, M., FRANKLIN, A. L., AND JUKES, T. H.: Assay of anti-pernicious anemia factor with euglena. *Proc. Soc. Exper. Biol. & Med.* 70: 118, 1949.
- ⁴ ROSS, G. I. M.: Vitamin B₁₂ assay in body fluids using *Euglena Gracilis*. *J. Clin. Path.* 5: 250, 1952.
- ⁵ SWENSEID, M. E., BETHELL, F. H., AND BIRD, O. D.: The concentration of folic acid in leukocytes. Observations on normal subjects and persons with leukemia. *Cancer Research* 11: 864, 1951.