Serum Vitamin B_{12} in Leukemias and Malignant Lymphomas

By M. Rachmilewitz, G. Izak, A. Hochman, J. Aronovitch and N. Grossowicz

RECENTLY developed microbiologic methods for the determination of vitamin B_{12} concentrations in body fluids[1,2] have established that the vitamin B_{12} levels in the serum are low in patients with pernicious anemia in relapse and other macrocytic anemias.[1,3] Normal blood levels of vitamin B_{12} were found in a wide variety of diseases including the sera of patients with lymphatic leukemia, undifferentiated stem cell leukemia and some types of malignant lymphomas[4,5] while extremely high vitamin B_{12} values were reported in the sera of patients with myeloid leukemia.[4-7]

In this communication our findings on B_{12} concentrations in various types of leukemias and malignant lymphomas are reported. An attempt has been made to use the serum B_{12} concentrations as additional means of differentiation between the various types of malignant conditions of the blood forming organs. In addition the effect of treatment in chronic leukemia on serum vitamin B_{12} concentrations was studied.

Methods and Material

The modified *E. coli* assay described previously[2] was employed. Serum vitamin B_{12} values ranged from 200–500 $\mu\mu$g/ml. with a mean of 340 $\mu\mu$g/ml. in 25 healthy individuals. The serum samples were diluted 1:8 with distilled water. For estimation of the total vitamin B_{12} content the diluted serum (1:8) was heated in a boiling water bath for 30 minutes and added aseptically to the medium. In an alternative procedure the vitamin B_{12} was liberated from the serum proteins by precipitation with acetate buffer (pH 5.5), according to Spray;[8] nearly the same results were obtained as without precipitation. The free vitamin B_{12} was determined in the unheated sample. The bound vitamin was calculated as the difference between the total and free values.

To determine the maximum binding capacity of serum for B_{12} (M.B.C.) 1 ml. of serum was mixed with 0.5 ml. of distilled water containing known amounts of crystalline vitamin B_{12} and incubated at 4 C. for 24 hours. The serum was then re-assayed for bound and free vitamin B_{12}. The M.B.C. was determined using at least two different concentrations of vitamin B_{12}, usually 1,500 and 3,000 $\mu\mu$g/ml. In 10 normal individuals in whom the total B_{12} ranged from 200–500 $\mu\mu$g/ml., the M.B.C. was in the range of 1,200–2,000 $\mu\mu$g/ml.; the recovery was 80–110 per cent of the added vitamin. When the serum with added B_{12} was incubated for one hour instead of twenty-four hours, the M.B.C. was found to be lower, approximately 1,000 $\mu\mu$g/ml.

Since the *E. coli* responds to methionine as well as to vitamin B_{12}, control assays were done on serum treated with sodium hydroxide (final concentration 0.4N), to destroy the B_{12}. Thereafter the samples were steamed for one hour, neutralized and re-assayed. Practically no growth promoting activity was found in the sera after this treatment.

Sixty-seven patients are included in this study.

From the Departments of Internal Medicine 'B' and Bacteriology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

This study was aided by grants from the Research Fund of the Hadassah Medical Organization, Jerusalem, and the Hematology Research Foundation, Chicago, U.S.A.

Submitted Oct. 14, 1956; accepted for publication Feb. 24, 1957.

	Patients
Acute, subacute and chronic myeloid leukemia	20
Chronic lymphatic leukemia	8
Undifferentiated leukemia	9
Hodgkin's disease	11
Generalized and localized lymphosarcoma	6
Myelosclerosis	3
Primary polycythemia	8
Multiple myeloma	2

In some of the patients with myeloid leukemia who showed elevated serum levels of vitamin B_{12}, repeated determinations were performed after various therapeutic procedures.

Results

Table 1 shows the vitamin B_{12} concentrations in patients with myeloid leukemia. It may be seen that all but one patient had very high serum vitamin B_{12} values ranging from 1,100 to 25,000 $\mu\mu$g/ml. This group includes one patient with subacute (case 1, table 1) and another with acute (case 6, table 1) myeloid leukemia, with myeloid differentiation; in both these cases the serum B_{12} was high. In the one patient with chronic myeloid leukemia with normal B_{12} concentrations the assay was performed shortly before death at the time when the disease was complicated by erysipelas and septicemia for which large amounts of antibiotics had been administered.

Generally, there was no correlation between the total W.B.C. count and the serum vitamin B_{12} concentrations; the highest B_{12} value of about 25,000 $\mu\mu$g/ml. was found in a case where at the time the W.B.C. was 14,000/cu.mm. On the other hand, the serum of a patient with 166,000 W.B.C./cu.mm. had a serum B_{12} level of 2,500 $\mu\mu$g/ml.

There also appeared to be no correlation between the duration of the illness, the extent of hepato-splenomegaly and the serum vitamin B_{12} concentrations.

Serial examinations of B_{12} in 4 cases (1, 2, 5, 19, table 1) of myeloid leukemia illustrate the effect of treatment on the B_{12} level. In case no. 2 the initial B_{12} level of 3,350 $\mu\mu$g/ml. fell to 2,750 $\mu\mu$g/ml. within 10 days following x-ray therapy and then dropped to 720 $\mu\mu$g/ml. 4 weeks later. At the same time the leukocyte count had dropped gradually from 135,000/cu.mm. to 68,000/cu.mm. after 10 days and down to 5,900/cu.mm. after 4 weeks. Two months later, the B_{12} rose slightly to a level of 1,120 $\mu\mu$g/ml., while the leukocyte count was 23,400/cu.mm. At this time x-ray therapy was reinstituted, following which the B_{12} level dropped to 680 $\mu\mu$g/ml. in spite of a high leukocyte count (45,300) (fig. 1).

In another patient (case 1, table 1) a woman aged 32, with subacute myeloid leukemia and a W.B.C. count of 3,350/cu.mm., the B_{12} value was found to be 2,700 $\mu\mu$g/ml. After cortisone therapy and several blood transfusions the patient's condition improved, the vitamin B_{12} concentrations fell to 950 $\mu\mu$g/ml. 2 weeks later, but it soon rose to the pre-treatment level in spite of continuous therapy (fig. 2). In 2 other cases of chronic myeloid leukemia (cases 5 and 19) the drop in white cell count following x-ray therapy preceded the decrease in the serum B_{12} concentration (fig. 3).

In a case of myeloid leukemia which developed in the course of a long-standing

TABLE 1.—*Myeloid Leukemia*

Case no.	Date	Hb Gm%	W.B.C./cu.mm.	Serum B_{12} $\mu\mu$g/ml.	Treatment
1	11.18.54	5.0	3,350	2,700	ACTH + Blood
	12. 3.54	14.3	5,350	950	ACTH
	12.19.54		15,800	3,000	ACTH
	1.13.55	5.3	21,000	2,400	ACTH + Blood
	1.27.55	6.6	19,400	1,120	ACTH + Blood
2	4.21.55	11.0	135,000	3,350	X-ray
	5. 2.55	10.5	68,000	2,750	X-ray
	6.29.55	12.0	5,900	720	
	8.24.55	12.6	23,400	1,120	X-ray
	11.10.55		45,300	680	
3	11.16.55	11.7	22,900	4,500	X-ray
	12. 8.55	10.0	29,600	1,650	X-ray
4	3.24.55	12.8	126,000	4,600	Myleran
	10.10.55	13.6	80,000	3,000	X-ray
	4.13.56	9.8	108,000	6,000	Mercaptopurine
5	12.28.54	12.4	166,200	2,500	Mercaptopurine +
	6.26.56	11.5	38,200	6,590	X-ray
	7. 1.56	11.2	21,000	6,400	
	7. 8.56	12.0	9,700	6,270	Mercaptopurine +
	7.28.56	10.6	7,600	3,850	X-ray
	8. 2.56		7,500	2,100	
6	7.15.55	4.8	87,000	2,270	Mercaptopurine
7	8. 7.55	9.0	32,000	1,600	ACTH Mercaptopurine
8	3.29.55	10.0	64,000	1,100	X-ray
9	4.12.55	13.8	42,000	1,920	X-ray
10	10.12.55	9.8	131,000	1,100	Cortisone
11	12.28.54	7.3	61,500	160	X-ray
12	1.12.56	8.0	14,000	26,500	Meticorten
13	4.21.56	12.0	18,500	2,000	Mercaptopurine
14	5.18.56	12.2	97,000	1,400	
15	6. 8.55	5.2	52,000	1,700	ACTH + Blood Radium
16	2. 3.55	10.0	28,000	1,100	Urethan
17	2.22.56	8.0	27,000	14,600	Mercaptopurine
18	3. 4.56	7.6	97,000	1,000	Urethan
19	7. 5.56		116,000	5,200	X-ray
	7.10.56		75,400	—	X-ray
	7.19.56		31,300	5,350	X-ray
	8. 6.56			2,400	X-ray
20	6.20.56	7.0	45,000	6,000	

polycythemia (case 7, table 3) x-ray treatment had no effect on the W.B.C. and on the size of liver and spleen; the B_{12} level was very high (6,250 $\mu\mu$g/ml.) and remained so despite treatment.

Acute and Chronic Undifferentiated Leukemia

This group includes 9 patients of whom 7 were suffering from acute stem cell leukemia, 1 from chronic stem cell leukemia and 1 from chronic reticulum cell leukemia. The diagnosis in these cases was established on the basis of the morphology of the peripheral blood and bone marrow. In 4 of the cases post mortem

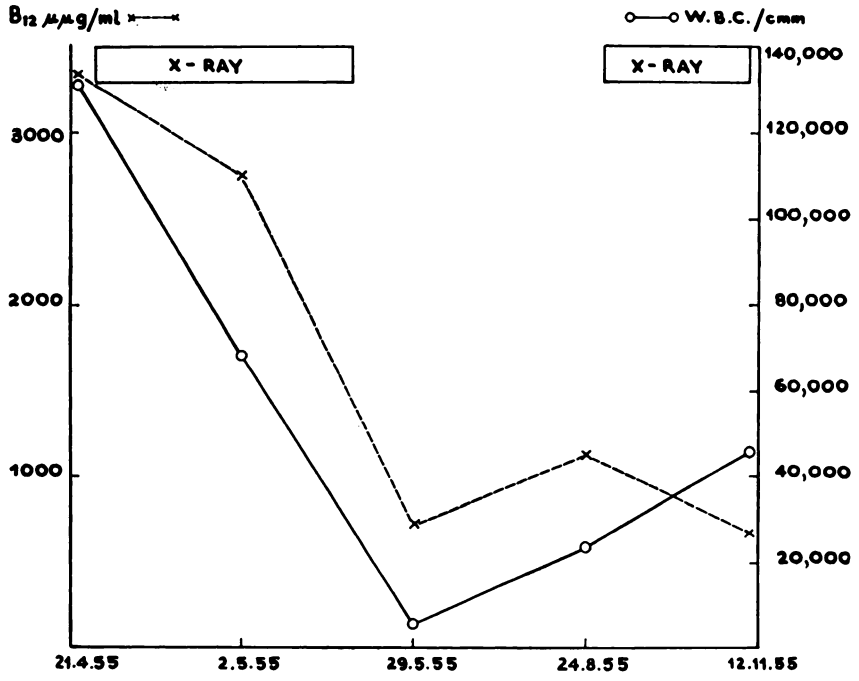

FIG. 1

examinations confirmed the clinical diagnosis. In all of these patients the serum vitamin B_{12} levels were normal and ranged from 130 to 600 μμg/ml. (table 2.)

In 8 patients with chronic lymphatic leukemia the serum vitamin B_{12} fell within the normal range (from 50 to 650 μμg/ml.).

Hodgkin's Disease

In 11 cases the serum vitamin B_{12} examined at different stages of the disease showed normal values (from 120 to 650 μμg/ml.) (table 2).

Lymphosarcoma

Four cases with generalized lymphosarcoma with systemic lymph node involvement, without abnormal cells in the peripheral blood and bone marrow were studied. The diagnosis in these cases was established by biopsy. In 2 of them the serum B_{12} was normal (380, 450 μμg/ml.) while in the other 2 cases the serum B_{12} was increased (790 and 1,450 μμg/ml.). Two cases with localized lymphosarcoma were examined. In one case with round cell sarcoma involving the spleen and abdominal lymph nodes, which was verified at autopsy, the serum B_{12} value was moderately increased (780 μμg/ml.). In the sixth case with lymphosarcoma of the spleen infiltrating the surrounding tissue, the B_{12} was normal (table 2).

Myeloproliferative Disorders: Polycythemia Vera

Eight patients were examined and the results are given in table 3. One patient was found to have very high B_{12} serum levels (case 7, table 3) at the time when she developed clinical and hematologic signs of myeloid leukemia. In this patient

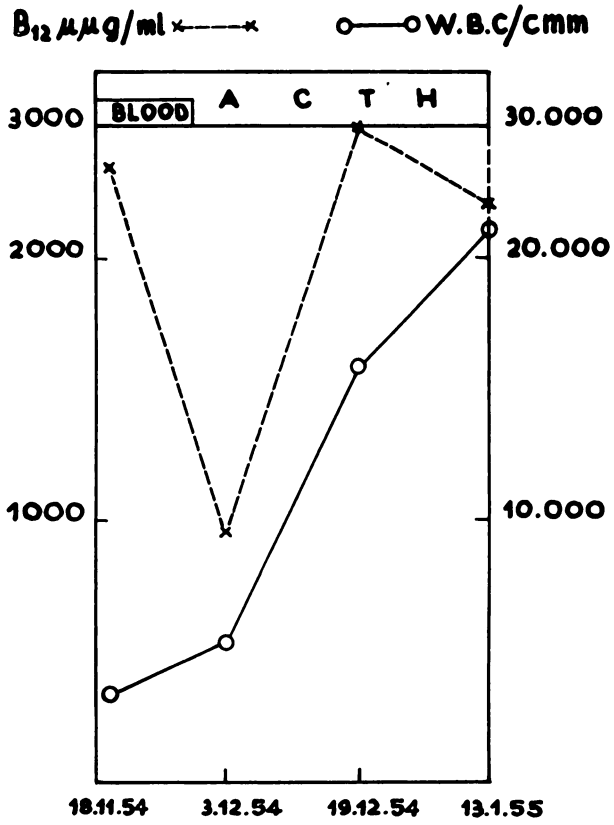

FIG. 2

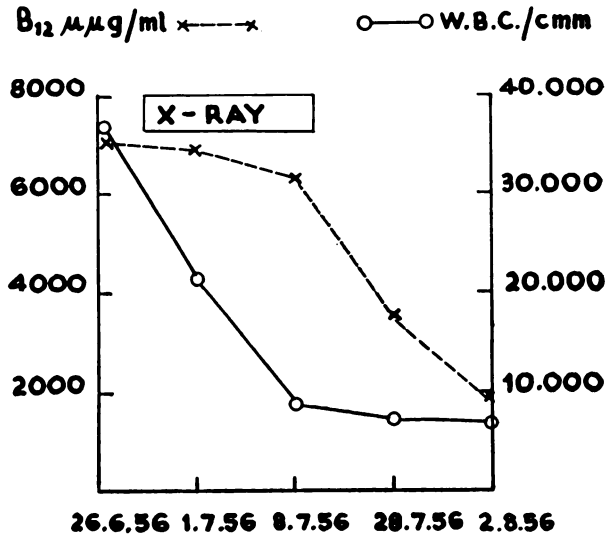

FIG. 3

the serum B_{12} was found to be high on three occasions during x-ray therapy and remained high following the treatment. The remaining 7 patients had serum vitamin B_{12} concentrations within the normal range with leukocyte counts as high as 60,000/cu.mm., but with a normal differential count.

In another patient (case 8, table 3) with polycythemia of 5 years duration which had been treated by venesection, at the time of the serum vitamin B_{12} examination, the leukocyte count was 36,000 with a differential count of 1 per cent blasts, 3 per cent myelocytes, 29 per cent metamyelocytes, 54 per cent polymorphonuclears, thus suggesting myeloid leukemia. The serum vitamin B_{12} was in the upper range of normal.

Myelosclerosis

Three patients were examined. In one case of myelosclerosis (case 1, table 4) with myeloid infiltration of kidneys, liver and spleen, which was later verified at autopsy, the total serum vitamin B_{12} was at first slightly increased (970 $\mu\mu$g/ml.). The patient had marked leukopenia without abnormal cells in the peripheral blood. Repeated blood transfusions were followed by the lowering of the serum vitamin B_{12} level (480 and 300 $\mu\mu$g/ml.). Shortly before death the serum vitamin

TABLE 2

Diagnosis	Number of cases	Serum B_{12} $\mu\mu$g/ml	
		Minimum	Maximum
Lymphosarcoma			
(a) Generalized	4	340	1,450
(b) Localized	2	360	780
Multiple myeloma	2	250	280
Stem cell leukemia	9	130	600
Lymphatic leukemia	8	50	650
Hodgkin's disease	11	120	650

TABLE 3.—*Polycythemia Vera*

No.	Date	Hb Gm%	R.B.C./cu.mm. (million)	W.B.C./cu.mm.	Thromb./cu.mm.	Serum B_{12} $\mu\mu$g/ml
1	12.13.54	17.1	6.06	11,000	320,000	150
2	3. 6.55	18.0	7.5	60,000	280,000	260
3	5. 4.55	16.8	5.6	15,700	288,000	400
4	12.18.54	16.7	5.6	11,600	350,000	190
5	12.16.54	17.0	6.2	14,000	280,000	350
6	5. 9.55	21.7	7.1	10,300	168,000	320
7	1.18.56	9.5	3.2	10,500	160,000	3,000
	3. 4.56	9.5	2.36	9,150	188,000	3,000
	6.10.56	8.5		20,000	—	2,800
	7.15.56	8.0	2.4	65,200	120,000	6,250
	7.30.56					6,000
8	4.26.56	17.2	7.65	36,000		600

TABLE 4.—*Myelosclerosis*

No.	Date	Hb Gm%	R.B.C./cu.mm. (million)	W.B.C./cu.mm.	Serum B_{12} $\mu\mu$g/ml
1	12.16.54	8.4	3.3	2,400	970
	12.28.54	10.5	4.0	3,700	480
	1.18.55	9.0	3.5	3,600	300
	2.23.55	5.2	2.1	3,150	400
	5. 2.55	4.8	1.6	3,300	400
	5.22.55	5.4	—	21,000	2,000
2	5.31.55	—	—	—	700
	6. 6.55	6.2	2.2	3,100	400
	11.11.55	7.4	2.8	3,700	900
3	6.18.56	—	—	16,500	300
	7.24.56	—	—	15,400	400

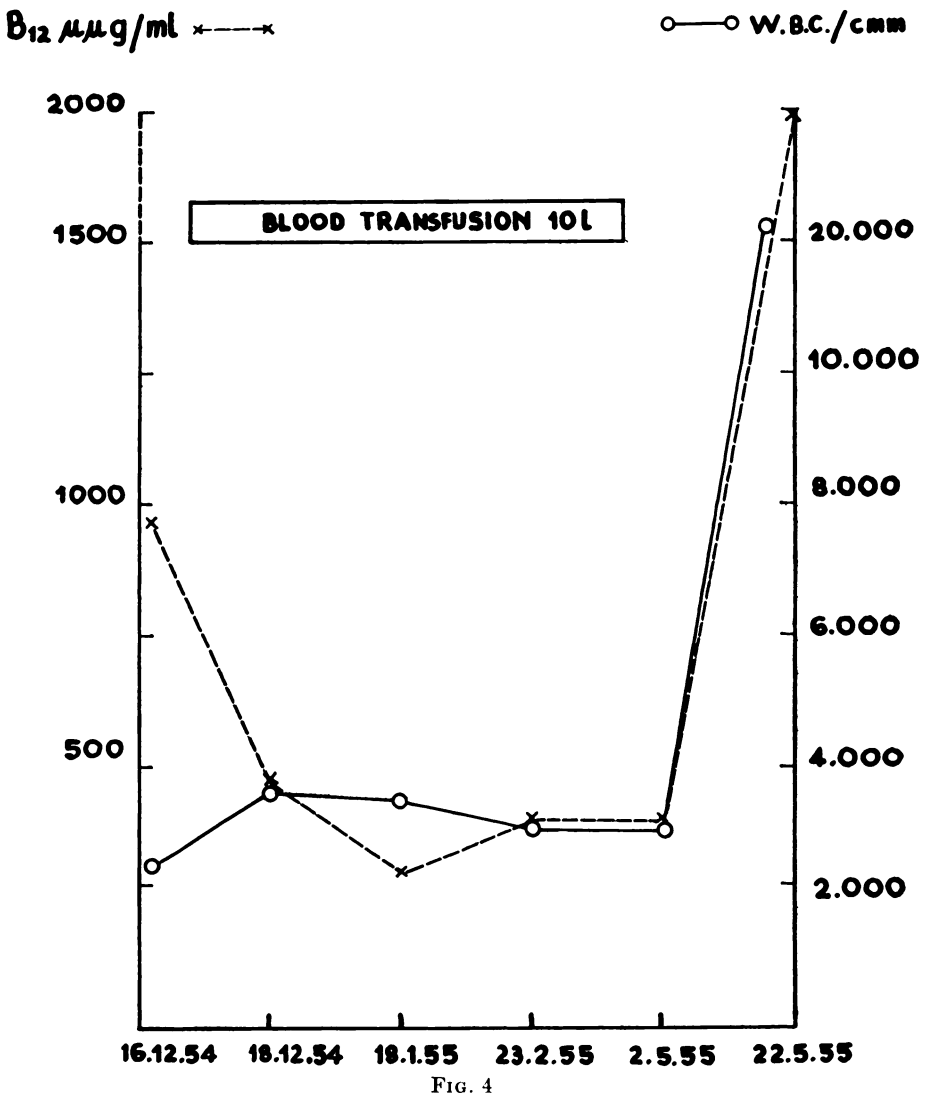

FIG. 4

B_{12} concentration suddenly rose to 2,000 $\mu\mu$g/ml., together with the appearance of leukocytosis and young myeloid cells in the peripheral blood (fig. 4).

In the second case, which ran a chronic course, the vitamin B_{12} concentration in the serum was slightly elevated on two occasions (700, 900 $\mu\mu$g/ml.). Seven months later, the patient developed a full-blown picture of myeloid leukemia and died shortly afterwards. In the third case the serum vitamin B_{12} was normal.

In 2 cases of multiple myeloma the serum vitamin B_{12} concentrations were normal (table 2).

Serum Binding Capacity of Vitamin B_{12}

In 6 patients with chronic myeloid leukemia with high serum vitamin B_{12} concentrations, free and total B_{12} were examined. In 5 of them practically no free B_{12} was present and all the B_{12} was bound to the proteins of the serum. In the sixth patient, free vitamin B_{12} constituted 20 per cent of the total. The B_{12} binding capacity was examined in 5 patients of this group. The values found were rather high: 3,500, 4,300, 5,000, 5000 and 8,000 $\mu\mu$g/ml. respectively.

In 6 patients with Hodgkin's disease, with normal vitamin B_{12} concentrations, the binding capacity was within the normal range (900–1,400 $\mu\mu$g/ml.).

In 2 cases with acute undifferentiated leukemia and in 1 case of lymphosarcoma, the binding capacity was normal (750, 750, and 1,050 $\mu\mu$g/ml. respectively).

In 1 case of myelosclerosis, with normal serum vitamin B_{12}, the binding capacity of the serum was increased (1,900 and 3,100 $\mu\mu$g/ml.). When the serum vitamin B_{12} increased to 2,000 $\mu\mu$g/ml. the binding capacity of the serum rose to 6,000 $\mu\mu$g/ml.

Discussion

Our findings of high serum vitamin B_{12} concentrations in chronic myeloid leukemia are in agreement with those reported by Beard et al.[5, 6] Killander[7] and Mollin and Ross.[4] In 2 cases of acute myeloid leukemia with differentiation beyond the myeloblast stage, high serum vitamin B_{12} values were found. On the other hand, acute and chronic stem cell leukemia and lymphatic leukemia were associated with normal serum vitamin B_{12} levels. These results indicate that the serum vitamin B_{12} determinations may be used as an additional means of differentiation between the various types of leukemia, particularly in those cases where the classification on a morphologic basis is difficult or impossible.

It seems that the myeloid proliferation is responsible for the elevation of serum vitamin B_{12}. There is, however, no correlation between the number of leukocytes in the peripheral blood and serum vitamin B_{12} concentrations. It was also impossible to correlate the differences in the serum vitamin B_{12} concentration with differences in the numbers of mature or immature cells in the peripheral blood and bone marrow, an observation made also by Mollin and Ross.[4]

It might be concluded, therefore that the degree of increase of serum vitamin B_{12} is dependent upon the extent of the myeloid proliferation in the bone marrow and in the extramedullary sites of hematopoiesis, and not on the degree of release of myeloid cells into the peripheral blood. This point is also borne out by the observation in myelosclerosis, where the serum B_{12} level seems to rise with

myeloid proliferation in the extramedullary organs, in the absence of abnormal cells in the peripheral blood.

The cause of the increase in serum vitamin B_{12} is not known. The increased binding capacity of serum protein for vitamin B_{12} reported,[6] and confirmed in this study, suggests that in the process of myeloid proliferation vitamin B_{12} binding proteins are liberated in large amounts. In view of our findings of increased serum vitamin B_{12} values in liver disease as well as in experimental liver damage[9, 10] the possibility of liver cell disintegration in myeloid leukemia as the cause of the high serum vitamin B_{12} concentrations must be considered, more so since in liver cell necrosis the binding capacity of serum for vitamin B_{12} is also increased, together with elevation of serum vitamin B_{12}. However, it is questionable whether the extent of liver cell injury in myeloid leukemia could account for the increase of serum vitamin B_{12}.

It is possible that the moderate elevation of serum vitamin B_{12} in 2 cases of generalized lymphosarcoma was due to invasion of the liver. This assumption gains support by the findings of high B_{12} values in the sera of patients with malignant tumors with extensive metastases to the liver.[11] The role of the liver as a possible cause of elevation of serum vitamin B_{12} in myeloproliferative diseases needs further elucidation.

Our findings in polycythemia suggest that leukocytosis alone is not associated with elevated serum vitamin B_{12}. In 1 case with a leukocyte count as high as 60,000 the serum vitamin B_{12} concentration was found to be normal. In another case, however, in which the full-blown clinical and hematologic picture of leukemia developed in spite of only a moderate leukocytosis, a very high B_{12} concentration was present and the values increased steadily despite x-ray therapy. Thus the serum vitamin B_{12} determination may possibly help to differentiate between leukemoid reactions and real leukemia in the course of polycythemia.

It is also possible that the serum vitamin B_{12} may be used in evaluating the extent of myeloid metaplasisa in myelosclerosis.

Summary

High serum vitamin B_{12} levels were found in chronic myeloid leukemia and in acute leukemia with myeloid differentiation. Following x-ray therapy and repeated blood transfusions, a drop of serum vitamin B_{12} was found.

In chronic lymphatic leukemia, undifferentiated stem cell leukemia, Hodgkin's disease, and multiple myeloma, the serum vitamin B_{12} concentrations were normal.

In polycythemia with marked leukocytosis the serum B_{12} was normal.

In myelosclerosis high B_{12} values may be found.

The serum vitamin B_{12} in chronic myeloid leukemia is in a bound form and the binding capacity for added B_{12} is increased.

Serum vitamin B_{12} determination may be of some value in differentiating various types of leukemia and other myelo-proliferative disorders.

Summario in Interlingua

Alte nivellos seral de vitamina B_{12} esseva trovate in chronic leucemia myeloide e in acute leucemia con differentiation myeloide. Post roentgenotherapia e

repetite transfusiones de sanguine, un reduction del nive'lo seral de vitamina B_{12} esseva constatate.

In chronic leucemia lymphatic, leucemia a cellulas indifferentiate, morbo de Hodgkin, e myeloma multiple, le concentrationes seral de vitamina B_{12} esseva normal.

In polycythemia con marcate grados de leucocytosis le nivello seral de vitamina B_{12} esseva normal.

In myelosclerosis, alte nivellos seral de vitamina B_{12} esseva trovate.

In leucemia myeloide, le vitamina B_{12} del sero es presente in forma ligate, e le capacitate ligatori pro B_{12} additional es augmentate.

Determinationes del nivellos seral de vitamina B_{12} va possibilemente esser de valor in le differentiation de varie typos de leucemia e de altere disordines myelo-proliferative.

REFERENCES

[1] MOLLIN, D. L. AND ROSS G. I. M.: Vitamin B_{12} concentrations of serum and urine of normals and of patients with megaloblastic anaemias and other diseases. J. Clin. Path. *5:* 129, 1952.

[2] GROSSOWICZ, N., ARONOVITCH, J. AND RACHMILEWITZ, M.: Determination of vitamin B_{12} in human serum by a mutant strain of Escherichia Coli. Proc. Soc. Exper. Biol. & Med. *87:* 513, 1954.

[3] IZAK, G., RACHMILEWITZ, M., STEIN, Y., BERCOVICI, B., SADOWSKY, A., ARONOVITCH, J. AND GROSSOWICZ, N.: Vitamin B_{12} and iron deficiency anemia in pregnancy and puerperium. Arch. Int. Med. *99:* 346, 1957.

[4] MOLLIN, D. L. AND ROSS, G. I. M.: Serum vitamin B_{12} concentrations in leukemia and in some other hematological conditions. Brit. J. Haemat. *1:* 155, 1955.

[5] BEARD, M. F., PITNEY, W. R., SANNEMAN, E. H., SAKOL, M. J. AND MOORHEAD, H. H.: Serum concentrations of vitamin B_{12} in acute leukemia. Ann. Int. Med. *41:* 323, 1954.

[6] ——, —— AND ——: Serum concentrations of vitamin B_{12} in patients suffering from leukemia. Blood *9:* 789, 1954.

[7] KILLANDER, A.: B_{12}-vitaminhalt I serum vid akut och kronisk leukemi. Nord. Med. *52:* 1513, 1954.

[8] SPRAY, G. H.: An improved method for the rapid estimation of vitamin B_{12} in serum. Clin. Sc. *14:* 661, 1955.

[9] RACHMILEWITZ, M., ARONOVITCH, J., AND GROSSOWICZ, N.: Serum concentrations of vitamin B_{12} in acute and chronic liver disease. J. Lab. & Clin. Med. In press.

[10] STEIN, Y., STEIN, O., ARONOVITCH, J., GROSSOWICZ, N. AND RACHMILEWITZ, M.: Serum vitamin B_{12} in experimental liver injury. Bull. of the Res. Council of Israel, Section E. Exper. Med. 6E, 5, 1956.

[11] GROSSOWICZ, N., ARONOVITCH, J., IZAK, G., HOCHMAN, A. AND RACHMILEWITZ, M.: Serum vitamin B_{12} in patients with malignant tumors with liver metastases. Unpublished observations.