Co\textsuperscript{60} Vitamin B\textsubscript{12} Binding Capacity of Human Leukocytes

By Leo M. Meyer, Eugene P. Cronkite, Inez F. Miller, Claire W. Mulzac and Irving Jones

It has been shown in this and other laboratories that normal human serum is capable of binding vitamin B\textsubscript{12} in vitro.\textsuperscript{1-8} In all patients with chronic myelocytic leukemia in relapse, and in some subjects with untreated polycythemia vera, this index is increased as much as fivefold.\textsuperscript{4,6-10} Following treatment, the sera of such leukemic patients show a reduction of B\textsubscript{12} binding capacity towards lower and even normal levels.\textsuperscript{6-10} In 1955 Mollin and Ross reported the B\textsubscript{12} content of lymphocytes from a patient with chronic lymphocytic leukemia to be higher than that of leukocytes from a person with chronic myelocytic leukemia.\textsuperscript{4} The differential count in the latter case was not given. They also reported that breakdown products of granulocytes from three patients with chronic myelocytic leukemia were capable of binding vitamin B\textsubscript{12} in vitro. No differential counts of these three patients were presented, and no studies were made on intact leukocytes. In 1956 Thomas and Anderson prepared homogenates of white blood cells from normal and leukemic subjects.\textsuperscript{11} After the addition of 2 \textmu g of crystalline vitamin B\textsubscript{12}, the homogenates were incubated at room temperature for four hours and then subjected to dialysis at 4 C. for 16 hours. The initial B\textsubscript{12} content of these cells was also measured. The authors found the B\textsubscript{12} content of myelocytic leukocytes and those from normal subjects to be essentially the same. Except for one case of myelocytic leukemia, leukocytes from other patients with chronic myelocytic leukemia, subacute myelocytic leukemia, chronic lymphocytic leukemia, acute blastic leukemia and from two normal subjects had approximately the same binding values. In one instance, leukocytes from a case of myelocytic leukemia showed increased binding capacity. There were no differential counts given in any of the patients described.

The following is a report of vitamin B\textsubscript{12} binding capacity of leukocytes obtained from three normal subjects and 19 persons with various hematologic disorders, consisting of acute and chronic lymphocytic and granulocytic leukemia, monoblastic leukemia, leukemoid reaction and idiopathic thrombocytopenic purpura.

The technic for harvesting leukocytes was as follows: Each subject was phlebotomized and 380 ml. of whole blood collected in two sterile glass bottles, each containing 20 ml of...
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1 per cent EDTA in 1.4 per cent saline solution, and an equal volume of 6 per cent Dextran solution. The blood was rotated gently for five minutes to insure complete mixing and prevent coagulation, and placed in a refrigerator at 4 C. for 45 minutes. Following this, the supernatant plasma containing leukocytes and platelets was aspirated and spun in a refrigerated centrifuge at 4 C. at 1000 rpm for 45 minutes. The clear supernatant was removed and sedimented leukocytes resuspended in about 20 ml. of the patient's own plasma. Ten ml. of resuspended cells were subjected to sonic oscillation for 45 minutes or longer in order to insure complete destruction of leukocytes. White blood cells and platelets were estimated in all three fractions using the Coulter counter. Blood smears were prepared for differential counts in order to calculate absolute numbers of leukocytes in the concentrates and to demonstrate destruction of cells. One ml. aliquots of these fractions were placed in Visking casing bags to which were added increasing amounts of Cobalt-60 vitamin B12 ranging from 1.0 to 100 mµg. These mixtures were allowed to incubate at room temperature for two hours and dialyzed against running tap water for 48 hours. Following this, each bag was placed in a 4 ml. vial, concentrated sulphuric acid added to disintegrate the bag, and radioactivity measured in a well-type scintillation counter. Bound B12 was calculated by comparing these values with standards prepared in an identical fashion and not subjected to dialysis. Absolute values for B12 binding were obtained by subtracting plasma binding levels from those observed for intact and disintegrated white blood cells. In the forthcoming figures the amount of radioactive B12 added per ml. leukocyte suspension is indicated along the abscissa, and mµg. of Co-60B12 bound per ml. leukocyte suspension appears on the ordinate. All values have been adjusted on the basis of a total leukocyte count of 300,000 per cu. mm. The solid line represents the binding capacity of intact white blood cells, and the broken line that of disintegrated cells.

RESULTS

Figures 1 and 2 show binding capacities of 1 ml. of resuspended leukocytes (adjusted to 300,000/cu.mm.) when 10 to 100 mµg. of Co-60 vitamin B12 were added. In the normal subject (1a) there were 65 per cent segmented neutrophils present. Values for intact and disintegrated cells were the same. In the patient with polycythemia vera (1b), 74 per cent of the leukocytes were segmented neutrophils. The values for binding capacity fell in the range observed for normal subjects. Disintegrated cells had a higher index when larger amounts of the labeled vitamin were added. Cases of acute leukemia of all varieties (figs. 1c, 1d, 2a) and chronic lymphocytic leukemia (2b) showed consistently low levels of B12 binding. In these subjects segmented neutrophils were 7 per cent, 3 per cent, 6 per cent and 9 per cent, respectively. Disintegrated cells disclosed no change in values over intact structures. In fig. 2c are results in a patient with chronic myelocytic leukemia with 34 per cent segmented neutrophils in the peripheral smears. The values for intact cells fall in the range of normal subjects. After sonic oscillation there is an apparent enhancement of this property. In a subject with marked leukocytosis of which 89 per cent were segmented neutrophils (probably a case of occult carcinoma), the highest values for binding capacity were observed (fig. 2d). There was little difference between intact and disintegrated cells. In figure 3 are shown results of plotting absolute numbers of leukocytes from the granulocytic series versus binding capacities of resuspended cells at the 100 mµg. level. Only in the instance of segmented neutrophils (3d) is there any apparent correlation.

In a patient with moderate leukocytosis with 60 per cent eosinophils and
**CO/ B/ B/ BINDING CAPACITY OF LEUKOCYTES**

**NORMAL (P.S.)**

![Graph](image1)

**POST-SONIC Mg Co^6^ added to leukocyte suspension**

**Acute monocytoid myeloblastic leukemia (CG)**

![Graph](image2)

**Acute lymphoblastic leukemia (Ke)**

![Graph](image3)

**POLYCYTHEMIA VERA (K.V.)**

![Graph](image4)

**RESUSPENDED CELLS (300,000 /cumm)**

**POST-SONIC**

**Fig. 1.—Co^{60} vitamin B\textsubscript{12} binding capacity of leukocytes in (a) normal subjects, (b) polycythemia vera, (c) acute monocytoid myeloblastic leukemia, (d) acute lymphoblastic leukemia.**

25 per cent neutrophils in the peripheral films, the binding capacity of re-suspended cells was low. This suggests that eosinophils contributed no binding substance, since the values obtained correlated well with the absolute number of neutrophils present.

The presence of erythrocytes or platelets in suspension of leukocytes did not affect B\textsubscript{12} binding capacity.

**DISCUSSION**

From the present studies it appears that segmented neutrophils have the greatest binding capacity of all leukocytes examined. Although in some instances disintegrated cells (polycythemia vera and chronic myelocytic leukemia (figs. 1b and 2c) had higher values, this was not a consistent finding. In all likelihood the vitamin is capable of diffusing through the cell membrane easily and is bound intracellularly, or possibly to the cell membrane itself.

Since lymphocytes have a life span of about two months, normal granulocytes nine to ten days, and leukemic leukocytes about three days, it is probable that elevated serum binding capacity seen in chronic myelocytic leukemia does not include these cells.
Fig. 2.—Co\textsuperscript{60} vitamin B\textsubscript{12} binding capacity of leukocytes in (a) acute myeloblastic leukemia, (b) chronic lymphocytic leukemia, (c) chronic myelocytic leukemia, (d) hyperleukocytosis.

and some cases of polycythemia vera is largely derived from material liberated from rapid turnover and destruction of neutrophilic leukocytes.\textsuperscript{12-16} With successful treatment of patients with chronic myelocytic leukemia and reduction of total number of cells produced and destroyed, a decrease of serum binding capacity follows.\textsuperscript{6-10}

In two subjects (figs. 1b and 2c) there are two phases noted in binding, a rapid ascent to the 25 or 50 \( \mu \text{g} \) level and then a less acute rise as more vitamin B\textsubscript{12} is added. This is similar to what has been found in serum binding studies, except that the “break” in the line takes place in normal subjects at a much lower level.\textsuperscript{6,10} In untreated chronic myelocytic leukemia with elevated binding capacity, the first phase of binding is shifted to the right, and moves to a lower level as binding capacity falls under treatment.\textsuperscript{6,10} In serum studies it has been suggested that the first phase is due to a specific binding protein, which when saturated is followed by nonspecific binding.\textsuperscript{5} The present data in patients with polycythemia vera and chronic myelocytic leukemia suggest that increased values in serum may be derived from substances present in neutrophilic leukocytes in these diseases. Studies are presently in
Fig. 3.—Co⁶⁰ vitamin B₁₂ binding capacity of absolute number of leukocytes per cu. mm. at 100 mµg. level, (a) myelocytes, (b) metamyelocytes, (c) non-segmented neutrophils, (d) segmented neutrophils. Data derived from all patients.

progress to determine whether the binding substances from mature neutrophilic leukocytes are identical with those found in serum.

The present investigation does not preclude the possibility that other leukocytes are capable of binding vitamin B₁₂. However non-granulocytic leukocytes obtained from patients with various acute leukemias and chronic lymphocytic leukemia showed little ability to bind the radiovitamin and this is reflected in the normal serum values in these subjects. Since many tissues in the body are capable of binding vitamin B₁₂, they undoubtedly contribute binding material during their metabolism to body fluids and so account for normal serum values obtained in patients with acute leukemia and chronic lymphocytic
leukemia. The failure of eosinophilic leukocytes to bind radioactive B₁₂ is a further example of the highly specific nature of this substance in neutrophilic white blood cells. These results are in marked variance with those reported by Thomas and Anderson who reported no difference in B₁₂ binding capacity of normal and leukemic leukocytes.⁷ They confirm the observations of Mollin and Ross that breakdown products of granulocytes (type of cell not reported) are capable of binding vitamin B₁₂.⁴ Our studies indicate that mature neutrophilic granulocytes possess the highest binding capacity and that other white blood cells have little or no such property.

CONCLUSIONS

1. Mature neutrophilic leukocytes show the highest Co⁶⁰B₁₂ binding capacity.
2. Less mature granulocytes, “blast” forms and eosinophils have little or no Co⁶⁰B₁₂ binding capacity.
3. Disintegrated mature leukocytes from chronic myelocytic leukemia and polycythemia vera show higher B₁₂ binding capacity than intact cells.
4. Mature leukocytes from patients with chronic myelocytic leukemia and polycythemia vera show a two-phase B₁₂ curve suggesting specific and non-specific binding, similar to that observed in human serum.
5. Disintegration products from mature neutrophilic leukocytes probably contribute largely to increased B₁₂ binding capacity of serum in chronic myelocytic leukemia and polycythemia vera.

SUMMARIO IN INTERLINGUA

1. Matur leucocytos neutrophilic monstrava le plus alte capacitate ligatori pro vitamina B₁₂ a Co⁶⁰.
2. Minus matur granulocytos, formas “blastic”, e eosinophilos ha pauc o nulle capacitate ligatori pro vitamina B₁₂ a Co⁶⁰.
3. Disintegrate leucocytos matur ab patientes con chronic leucenia myelocytic e polycythemia ver manifesta un plus alte capacitate ligatori pro vitamina B₁₂ que cellulas intacte.
4. Matur leucocytos ab patientes con chronic leucenia myelocytic e polycythemia ver manifesta un curva biphasic pro vitamina B₁₂, lo que suggere specific e non-specific potentias ligatori, simile a illo observate in sero normal.
5. Il es probable que productos de disintegration ab matur leucocytos neutrophilic contribue grandemente al augmentate capacitate ligatori pro vitamina B₁₂ in le sero de chronic leucenia myelocytic e de polycythemia ver.

ADDENDUM

Since this manuscript was accepted, the authors studied the Co⁶⁰ vitamin B₁₂ binding capacity of leukocytes from a case of chronic myelocytic leukemia with 38 per cent mature basophilic granulocytes. The results indicate a binding capacity of these cells equal to that of mature neutrophils.

REFERENCES

CO\textsuperscript{60} B\textsubscript{12} BINDING CAPACITY OF LEUKOCYTES

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