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Serum Vitamin B_{12}- Binding Proteins in Neutropenia[1,2] (38720)

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(Introduced by Ananda S. Prasad)

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Granulocytes appear to be a source of serum vitamin B_{12}-binding proteins (1, 2), particularly serum third binder or "R" binder (3) and probably transcobalamin (TC) I also (3), and high serum unsaturated B_{12}-binding capacity (UBBC) levels are usually found in states with increased granulocyte production (4–6). The use of UBBC as an index of total blood granulocyte pool (TBGP) was suggested because of the correlation of the two parameters in a study of patients with normal and high leukocyte levels (7). Whether UBBC can be used thus in evaluating neutropenic states, however, is not clear. High (4, 8), normal (9, 10), and low (5, 11) UBBC levels have been reported in a few cases of neutropenia. Therefore, the present study of neutropenic subjects was undertaken. Our findings suggest that a simple relationship does not hold in these subjects and that UBBC is affected by other factors in addition to TBGP.

Methods and Materials. Patients were considered neutropenic if neutrophils were less than 1800/μl. Subjects with leukemia or hepatitis were excluded, and no patients received vitamin B_{12} injections. Thirty-nine patients were studied. Serial observations were often made but were included only if significant changes occurred. Thus, 46 observations during neutropenia were recorded on the 39 subjects, and 11 observations were added after recovery. In one case of aplastic anemia, 29 determinations were done over 45 days but, since no significant change occurred, the results were averaged and presented as a single observation. In 20 cases simultaneous bone marrow evaluations were done. The subjects' ages ranged from 3 to 86 yr and there were 20 males and 19 females. Coexisting anemia or thrombocytopenia was present in 21 patients. The subjects studied included eight with malignant tumor, seven of whom were undergoing chemotherapy or radiotherapy at the time, seven with aplastic anemia, four with Felty's syndrome or hypersplenism, three with drug-induced leukopenia (propylthiouracil, chlorpromazine), two with chronic neutropenia of childhood, and one each with paroxysmal nocturnal hemoglobinuria, cyclic neutropenia just before neutrophil nadir, and Sjögren's disease, the rest having idiopathic neutropenia. Transient neutropenia, meaning neutropenia which spontaneously remitted, was present in 16 patients, in nine of whom serial studies were included. The rest had steady neutropenia, but in one of these, a patient with Felty's syndrome, recovery followed splenectomy at which time follow-up studies were obtained.

Leukocyte and differential counts were done by standard visual methods. Neutrophil kinetic studies using $DF^{32}P$ were performed by the standard method (12, 13) 10 times on seven of the subjects. TBGP was calculated in each case by the standard isotope dilution method (14) using the specific activity of blood at the earliest sampling time, 10–15 min after the end of labeled blood reinfusion. Several of these patients had extreme neutropenia (less than 250 neutrophils per μl) and monocyte counts higher than the neutrophil counts. Monocytes are known to take up some $DF^{32}P$, and

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their radioactivity might interfere with determination of the neutrophil lifespan by the standard technique (15). To avoid this source of error as well as to obtain larger numbers of neutrophils to study, we utilized a leukapheresis and white cell separation technique (15) to obtain samples at 10–15 min and 3–4 hr after reinfusion of the labeled blood. This was done in seven of the 10 total studies on five of the seven total patients in addition to the standard technique for isolating mixed leukocytes. The other patients could not be so studied because of lack of adequate-sized veins for leukapheresis. In the patients studied by this new technique, the survival ($T_{1/2}$) of separated pure neutrophils could be determined and this value was used to calculate the granulocyte turnover rate (GTR) by the standard calculation (14). In patients studied only by the standard $DF^{32}P$ technique who had a type B specific-activity curve (16) (two components) the calculation of GTR was done in two ways: (1) utilizing the $T_{1/2}$ value of the first component (steep slope) of the survival curve, and (2) utilizing the $T_{1/2}$ of the over-all curve, as if the points were best described by a single straight line on the semilogarithmic plot. Standard line-fitting methods by least squares were utilized in either case. Such estimate of GTR by methods (1) and (2) was different in one case and is noted in Table I. Statistical tests for correlation with vitamin B_{12} parameters were done using results of each method of calculation.

Sera for B_{12} studies were obtained, usually in the morning, and stored at −20° until used. Serum vitamin B_{12} (17) and UBBC (18) levels were assayed by coated charcoal radioisotope methods. UBBC was fractionated in all cases by the DEAE-cellulose batch technique (19) and in 21 instances by 2 *M* ammonium sulfate precipitation (20) as well. The former technique separates UBBC into "α-globulin," containing TC I, and "β-globulin," containing TC II and third binder or "R" binder, while the latter technique precipitates only TC II and produces fractionation results identical to those obtained by Sephadex gel chromatography (20). Use of both methods thus allows quantitation of all three serum B_{12} binders (20).

Results. Serum vitamin B_{12} levels (Fig. 1). The neutropenic subjects had a wide range of serum B_{12} levels with a mean of 525 ± 373 (1 SD) pg/ml. This was not significantly higher than normal (465 ± 174 pg/ml). Levels did not correlate with neutrophil count or bone marrow picture. In those tested, correlation was poor with GTR and TBGP (Table I). In almost half the cases B_{12} levels did not change when neutropenia disappeared, change being variable in the others.

Higher than normal B_{12} levels were seen in (1) both children with chronic neutropenia of childhood, although one of them subse-

TABLE I. NEUTROPHIL KINETIC AND SERUM B_{12} AND UBBC RESULTS IN PATIENTS WITH NEUTROPENIA.

Patient	Neutrophil/μl	TBGP× 10^7/kg	GTR[a] × 10^7/kg/day	B_{12} (pg/ml)	UBBC (pg/ml)	Diagnosis
T.G.	70	4.5	95	934	1258	Idiopathic neutropenia
	90	1.6	24	457	1854	(after splenectomy)
L. D.	100	3.5	21	509	1875	Cyclic neutropenia
K. G.	100	45	722[b]	493	1307	Lupus erythematosus
	250	48	1500	462	1572	
M. M.	120	35	72	544	2097	Felty's syndrome
A. M.	490	36	120	407	2229	Felty's syndrome
	2260	34	123	615	2694	(after splenectomy)
G. C.	1080	62	84	445	2319	Lymphoma
R. M.	1700	16	84	574	2136	Hodgkin's disease
Normal	>1800	27–138	62–400	465 ± 174	1759 ± 269	

[a] Calculated from the first component of the disappearance curve if type B curve is present.

[b] If calculated by constructing a single straight line on the semilogarithmic plot of this patient's type B curve instead of using first component, 164 × 10^7/kg/day.

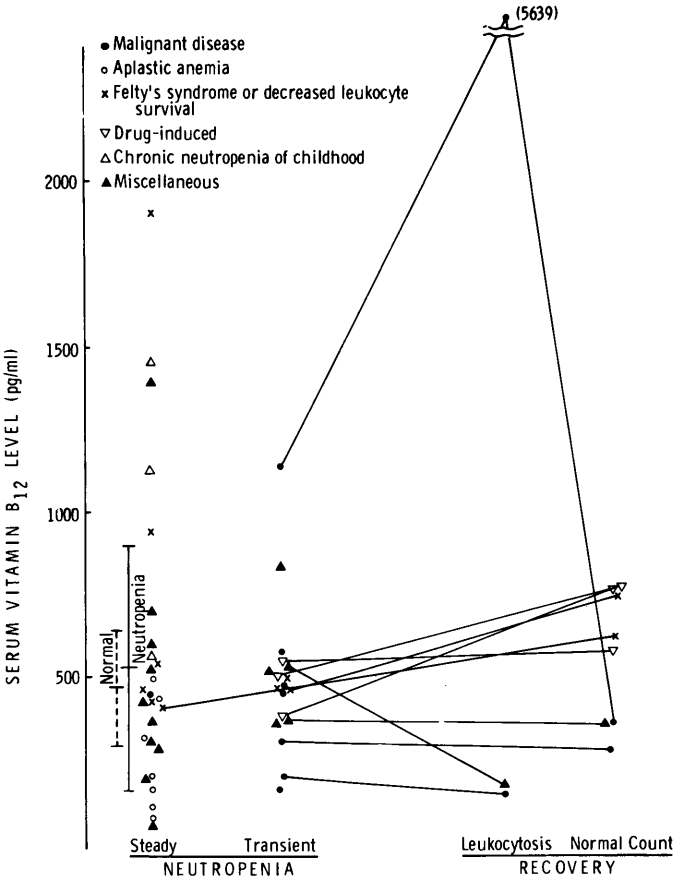

FIG. 1. Serum B_{12} levels in neutropenia and with recovery. Lines connect points of a given subject after recovery. (One recovery value is connected to a patient with steady neutropenia whose neutrophil count became normal after splenectomy.) Some subjects are represented more than once when neutropenic, due to changes in some clinical or B_{12} parameter. The vertical line on the right (—) represents mean ± 1 SD of all the neutropenic subjects, and the line on the left (---) represents mean ± 1 SD of 32 normal subjects.

quently had a normal level without any recognizable change in his status, (2) on two occasions in a patient with idiopathically shortened neutrophil half-life whose B_{12} level became normal after splenectomy although neutropenia persisted, (3) a patient with paroxysmal nocturnal hemoglobinuria, and (4) a patient with chemotherapeutic myelosuppression.

Subnormal levels occurred in (1) three of of the seven patients with aplastic anemia, the entire group of seven having lower levels (252 ± 164 pg/ml) than the other neutropenics, (2) a patient with multiple myeloma on chemotherapy, and (3) a patient with idiopathic pancytopenia and a hypercellular bone marrow. None of these patients had evidence of B_{12} malabsorption or deficiency and none responded to B_{12} therapy.

Serum UBBC (Fig. 2). There was a wide scatter of UBBC levels among the neutropenic subjects, the mean being significantly greater than normal (2012 ± 795 pg/ml vs 1759 ± 269 pg/ml, $P < 0.05$). As with serum B_{12} and total B_{12}-binding capacity (B_{12} + UBBC), UBBC did not correlate with GTR, TBGP, neutrophil count, or bone marrow picture. However, as shown in Fig. 2, UBBC rose with recovery from neutropenia except in four cases where UBBC was initially greatly elevated during neutropenia.

No significant correlation of UBBC with

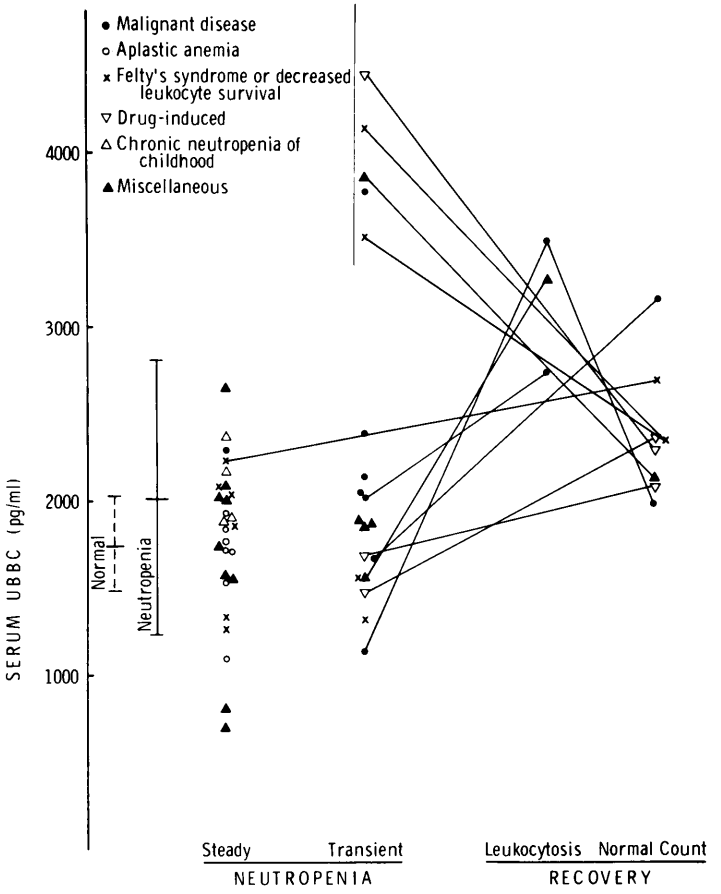

FIG. 2. Serum UBBC levels in neutropenia and with recovery. (See Fig. 1 for other explanatory comments.)

diagnosis was found. However, the transiently neutropenic patients, whether drug-induced or idiopathic, had significantly higher UBBC than did the patients with steady neutropenia (2331 ± 1047 pg/ml vs 1787 ± 456 pg/ml, $P < 0.02$). In contrast, B_{12} levels during transient neutropenia were generally lower but not significantly so.

Elevated UBBC (>2 SD above normal mean) occurred in (1) a patient with thorazine-induced neutropenia whose UBBC fell with recovery, (2) five patients with malignant tumor, three of whom were receiving myelosuppressive drugs, (3) a patient with Felty's syndrome, whose UBBC fell to normal after splenectomy but rebounded when neutropenia recurred; it should be noted that in another similar patient UBBC was initially normal and rose with remission after splenectomy, (4) two patients with idiopathic, transient neutropenia, and (5) one patient each with paroxysmal nocturnal hemoglobinuria and chronic neutropenia of childhood.

Subnormal levels occurred only in (1) a patient with aplastic anemia, (2) a patient with cancer receiving myelosuppressive drugs who at the same time had a high serum B_{12} level, so that her total B_{12}-binding capacity was normal, and (3) twice in a patient with multiple congenital anomalies and idiopathic neutropenia with a normal bone marrow.

TC levels. There was no significant difference from normal in absolute or relative levels of TC I, TC II, or serum third binder, nor did the levels correlate with disease, marrow pattern, neutrophil count, GTR, or TBGP. However, TC I levels were significantly higher in transiently neutropenic sub-

jects than in those with steady neutropenia (647 ± 427 pg/ml vs 422 ± 127 pg/ml, $P < 0.02$). The patients with aplastic anemia had normal TC I levels as did some of the subjects with abnormal UBBC levels mentioned above. TC II levels were higher in the transient leukopenia group, but not significantly so. Serum third binder levels were elevated in a few patients, but no consistent pattern was seen.

Discussion. A relationship exists between neutrophils and serum vitamin B_{12}-binding proteins, particularly TC I and third binder. However, the nature of the relationship is not fully clear and its clarification could prove useful in the evaluation of neutrophil disorders.

Elevation of serum third binder or of TC I levels in various neutrophilias (4–6) and correlation of UBBC with TBGP (7) is consistent with current knowledge of B_{12} binders. However, past reports on neutropenic states have been conflicting, and examination of the low end of the neutrophil spectrum may help to shed light on the nature of the relationship between B_{12}-binding proteins and neutrophil status.

In the present report 46 observations on 39 patients with various types of neutropenia showed that mean UBBC paradoxically was above normal, but with a wide range of values. In this regard, it should be noted that in a previous report which found low mean UBBC (5), the total B_{12}-binding capacity of the four undefined subjects was normal and TC I actually elevated. The other such report (11) noted low UBBC in four of six subjects with aplastic anemia. Our patients with aplastic marrows tended to have among the lower values in the study, but not significantly so nor, with one exception, did they have subnormal levels (Fig. 2). They also had a tendency to subnormal serum B_{12} levels (Fig. 1) without evidence of vitamin B_{12} deficiency.

The chief observation, the explanation for which is not clear, was that significantly high UBBC in our subjects occurred almost entirely in transiently neutropenic subjects, TC I being significantly elevated. Patients with steady neutropenia tended to have normal UBBC.

It has been reported that serum UBBC may be elevated due to release *in vitro* of third binder from granulocytes if blood is collected without sodium fluoride anticoagulant (21). Such would not apply to our data since serum third binder levels were generally unremarkable in our group. Furthermore, our patients were all neutropenic and, therefore, had less circulating granulocytes to release B_{12} binder *in vitro* than did our normal controls. As shown in Table I, TBGP was normal in many neutropenics. Thus, any increased release of B_{12} binder occurring would have had to take place *in vivo*. In this regard, it should be emphasized that UBBC did not correlate with TBGP or GTR, and that UBBC was not lower in neutropenic subjects with subnormal TBGP than in those with normal TBGP.

Within the neutropenic group there was no correlation of B_{12} or any B_{12}-binding parameter, including UBBC, with neutrophil count, bone marrow picture, TBGP, or GTR. Thus, UBBC cannot be used to indicate TBGP, contrary to a suggestion made from a study of patients with only normal or elevated leukocyte counts (7). It should be noted, however, that in any given subject UBBC generally rose when neutropenia cleared. The exceptions were limited to four patients with high UBBC initially which fell with recovery (Fig. 2). Transiently elevated UBBC has been observed in agranulocytosis in rabbits given nitrogen mustard (10).

The explanation for normal or high, rather than low, UBBC in neutropenia is not clear. A minimal "baseline" serum UBBC may exist below which levels rarely fall. Furthermore, it may be that in neutropenia leukocytes contain more B_{12}-binding protein or secrete it more actively. Although leukocyte UBBC content has not been found to vary in several disorders (3, 22) the content in neutropenia is unknown. Another possibility relates to the proposal, as yet unsubstantiated, that there is a relationship between vitamin B_{12}-binding protein and colony-stimulating factor (23). If correct, this might explain the unexpected pattern found in our study. It is also possible that nonneutrophil factors contribute to the normal and high UBBC levels in neutropenia, although such

factors affecting serum UBBC are not well defined. The absence of low UBBC in most neutropenic patients and particularly the significantly elevated serum UBBC observed in transient neutropenias may result from the interplay of one or more of the possible mechanisms mentioned. Such mechanisms may be superimposed to varying degrees on the relationship between TBGP and serum B_{12}-binding proteins.

Summary. Serum unsaturated vitamin B_{12}-binding capacity (UBBC) has been shown to fluctuate with neutrophil levels and has been reported to correlate with TBGP in normal and hyperleukocytic states. However, the present report demonstrates that the above relationship is not present in neutropenia, suggesting that some of our concepts regarding UBBC may have to be reexamined, since factors other than TBGP appear to be operative

There was a wide scatter of UBBC values among the 39 neutropenic subjects studied, the mean being significantly above normal. There were few low values. High UBBC was primarily confined to subjects with transient neutropenia. Normal values were generally seen in steady neutropenia. The difference in UBBC was primarily due to Transcobalamin I, the other serum binders being similar in both groups. No other significant diagnostic pattern of UBBC was found. Recovery from neutropenia was accompanied by a rise in UBBC in all cases except in four patients whose UBBC was initially very high and fell with recovery. No discernible pattern of serum B_{12} levels existed, although subnormal levels without evidence of B_{12} deficiency were found in three of the seven patients with aplastic anemia. Serum B_{12} levels did not change with recovery in approximately half of the neutropenic subjects, change being variable in the others.

Neither serum B_{12}, UBBC, total B_{12}-binding capacity, or any of the three serum B_{12} binders correlated with neutrophil count, bone marrow findings, TBGP, or granulocyte turnover rate.

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