Tumor Res. 5 (1970) 53 - 66

CLINICAL STUDIES ON VITAMIN B_{12} BINDING PROTEINS

I. Transcobalamin Levels in Normal Subjects and Patients with Various Diseases

Morimichi Fukuda, Atsuko Yamamoto, Hiroshi Natori, Satoshi Katayama and Ichiro Urushizaki

Department of Medicine, Cancer Research Institute,

Sapporo Medical College

The vitamin B_{12} in blood in man is virtually bound to serum protein(1). The total binding capacity of the B_{12} transport protein, transcobalamin I and II, is approximately 2 to 4 times of the serum vitamin B_{12} concentration. While there are a number of reports on the transcobalamin levels in western countries (2, 3, 4, 5, 6), no quantitative studies on the Japanese, in which the incidence of Addison-Biermer type megaloblastic anemia is markedly lower than the Caucassian populations, are available at present.

Thus, this paper describes the transcobalamin levels in a small portion of Japan measured on normal control sera obtained from the blood donors of blood banks and healthy hospital personnel, and on a number of pathological sera obtained from hospitalized patients with established diagnoses. Statistical analyses were also carried out on the correlation between transcobalamin levels and certain parameters determined simultaneously on the same sera in parallel.

Materials and Methods

Fresh serum samples were collected from 138 normal subjects ranging from 15 to 60 years of age including healthy hospital personnel and blood donors of the Red Cross Blood Bank in Sapporo. 238 pathological sera were obtained from hospitalized patients with established diagnoses at the Sapporo Medical College Hospital and consisting of 40 cases of carcinoma, 56 cases of various hematologic disorders, 46 cases of liver diseases, 24 cases of tuberculosis, 12 cases of gastric ulcer, 11 pregnancies and 49 cases of miscellaneous diseases. All specimens were immediately frozen after separation of the serum and stored at $-20^{\circ}C$ until used.

^{57}Co-CN B_{12} with a specific activity of 112$\mu Ci/\mu g$ was purchased from The Radiochemical Centre, Amersham, England. Bovine serum albumin and intrinsic factor concentrate were obtained from Armour pharmaceutical Co. and Squibb pharmaceutical Co., respectively. Norit A charcoal was purchased from Kishida Chemicals, Tokyo.

The serum vitamin B_{12}, and unsaturated B_{12} binding capacity ($UB_{12}BC$) were determined according to the albuminated charcoal adsorption methods of Lau et al.(7), and Gottlieb et al.(8), respectively. Total B_{12} binding capacity ($TB_{12}BC$) was expressed as the sum of B_{12} and $UB_{12}BC$ of the individual serum examined. On sera with B_{12} levels below 100 pg/ml, B_{12} concentrations were determined by the standard microbiological method which employed Lactobacillus Leichmanii as the test organism by courtesy of Dr. T. Abe at the 1st Department of Medicine, Tokyo Medical and Dental College, Tokyo.

The serum iron content and total iron binding capacity were determined by the modified automated method of Clarke and Nichlas (9) using a Technicon autoanalyser. Cellulose acetate electrophoresis was carried out using Separax membrane (Fuji Chemicals, Tokyo) in a veronal buffer solution of pH 8.6, μ=0.05, at 300 v, 0.5mA/cm, for one hour. The stained electrophoregram was scanned by a standard densitometer and absolute fractional concentrations were calculated from the total protein concentration.

Statistical analyses were carried out using Olivetti- Underwood desk top computor, Programma 101.

Results

Serum B_{12} concentrations and $UB_{12}BC$ of 138 normal subjects ranged from 126 - 1260 pg/ml with a mean of 517.9 pg/ml, and 508 - 1484 pg/ml, with a mean of 883.6 pg/ml, respectively. As is apparent from the values listed in Table 1, B_{12}, $UB_{12}BC$, $TB_{12}BC$ and ratios of B_{12} to $TB_{12}BC$ in each age groups were reasonably close to the overall mean values of normal subjects, although considerable variations were seen among individual samples. The only difference noted is a slightly low level of B_{12} in a group younger than 20 years of age ($0.05 < p < 0.1$), as compared with the other age groups.

As shown in Fig. 1 a, b, c, and d, certain characteristic features can be observed in pathological sera. In patients with neoplastic disease, the B_{12} levels were within a normal range of 130 - 1339 pg/ml, with a mean of 604.2 pg/ml, while $UB_{12}BC$ increased markedly to 1446 pg/ml, of

Table 1. Vitamin B_{12} and Transcobalamin Levels in Normal Subjects.

Age group	No.	V.B_{12}(pg/ml)	$UB_{12}BC$(pg/ml)	$TB_{12}BC$(pg/ml)	$B_{12}/TB_{12}BC$
15-20	28	429.5 ± 210.5	899.4 ± 217.9	1336.8 ± 275.6	0.31 ± 0.12
		(130-924)	(668-1392)	(968-1721)	(0.14-0.52)
21-30	55	508.8 ± 301.9	893.4 ± 251.4	1427.4 ± 447.9	0.35 ± 0.16
		(126-1260)	(529-1360)	(665-2619)	(0.10-0.64)
31-40	36	543.2 ± 302.9	859.4 ± 179.8	1392.4 ± 346.5	0.37 ± 0.14
		(130-975)	(576-1180)	(932-2334)	(0.12-0.60)
41-60	19	565.3 ± 202.4	913.7 ± 224.6	1477.7 ± 315.3	0.37 ± 0.14
		(223-896)	(508-1370)	(1053-2196)	(0.16-0.52)
51-60	138	517.9 ± 292.3	883.6 ± 223.5	1403.6 ± 361.3	0.36 ± 0.13
		(126-1260)	(508-1392)	(655-2619)	(0.10-0.64)

* Mean ± standard deviation

() denotes range of values.

which the range was from 490 to 2851 pg/ml ($p<0.005$). In cases with inflammatory diseases, which consisted mostly of the active tuberculosis of various grades of severity, B_{12} concentrations were subnormal with a mean of 427 pg/ml whereas $UB_{12}BC$ levels were significantly higher than the normal controls with a mean of 1321 pg/ml ($p<0.005$). Among patients suffering from various kinds of anemia, a marked decrease of B_{12} levels were noted only in cases with megaloblastic anemia, in which $UB_{12}BC$ were within a normal range.

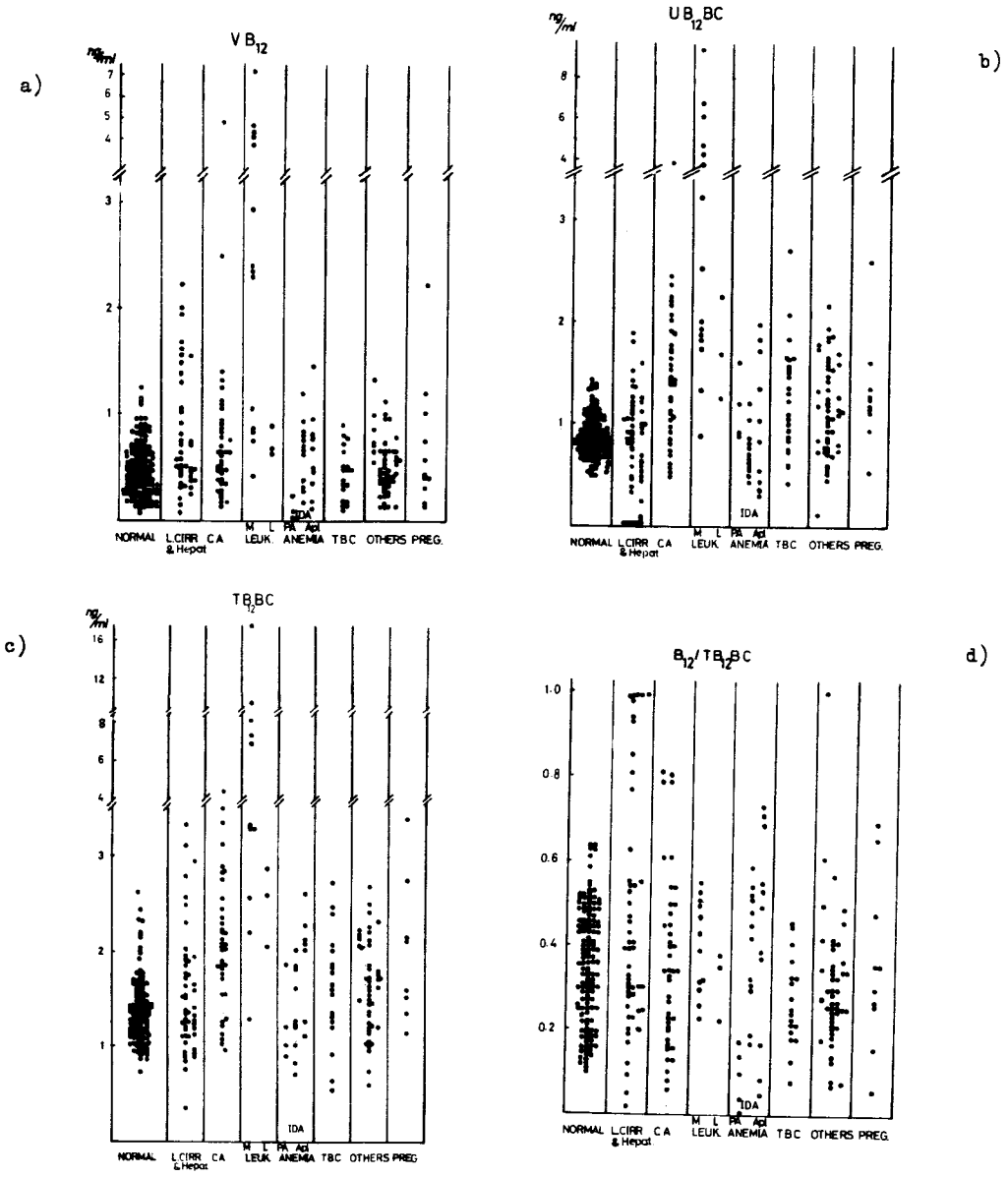


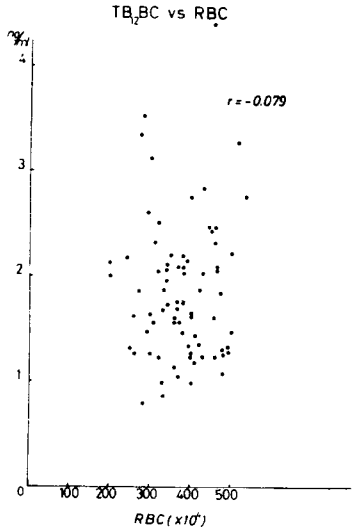
Fig. 1. The vitamin B_{12} and transcobalamin levels in the normal subjects and in patients with various diseases. a) the vitamin B_{12}, b) the unsaturated B_{12} binding capacity, c) the total B_{12} binding capacity, and d) ratio of the B_{12} to total B_{12} binding capacity of serum.

In cases with hepatic cirrhosis without prior administration of B_{12}, B_{12} concentrations were generally subnormal or normal, and $UB_{12}BC$ were normal or slightly increased.

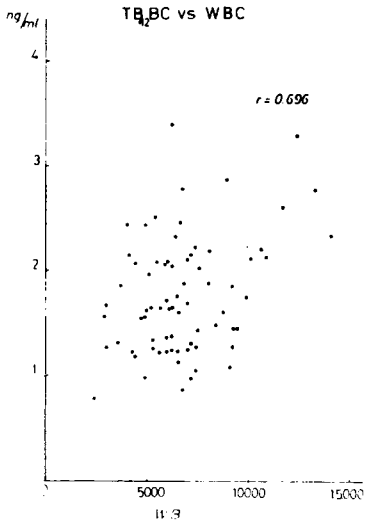
In cases with previous medication of large doses of intramuscular B_{12} injection, serum B_{12} were invariably higher than 1000 pg/ml and completely devoid of $UB_{12}BC$ in serum. This tendency was particularly noted in cases with advanced liver cirrhosis.

As already pointed out by the previous workers, serum B_{12} and $UB_{12}BC$ were abnormally elevated in untreated chronic myelogenous leukemia ranging from 2300 - 7000 pg/ml for B_{12} and 2540 - 9300 pg/ml for $UB_{12}BC$. Slight to moderate increases of the B_{12} and $UB_{12}BC$ were demonstrated in therapeutically controlled chronic myelogenous leukemia and acute myelogenous leukemia.

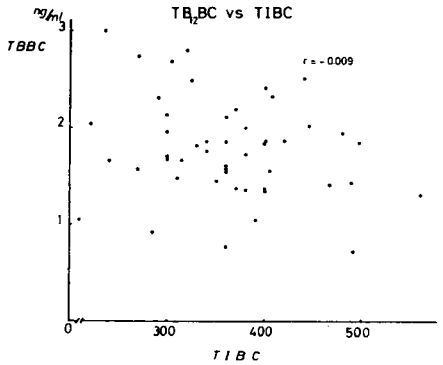
In order to assess the possible cause of elevation of serum transcobalamin levels in these diseased states, $TB_{12}BC$ values were plotted against a number of parameters determined simultaneously with the transcobalamin determination. (Fig. 2, a, b, c, d, e, f, g,), and the following correlation coefficients were obtained: erythrocyts count, r=-0.079 (n=83) leucocyte count, r=0.696, (n=83), total protein r=0.162 (n=97) albumin, r=-0.060 (n=97), alpha-1-globulin, r=0.336 (n=97), alpha-2 globulin, r=0.275 (n=97), beta-globulin, r=-0.033 (n=97), gamma-globulin, r=0.174 (n=97), and total iron binding capacity, r=-0.009 (n=49). The coefficient of correlation of $UB_{12}BC$ versus B_{12} was r=-0.196 (n=152).



a)

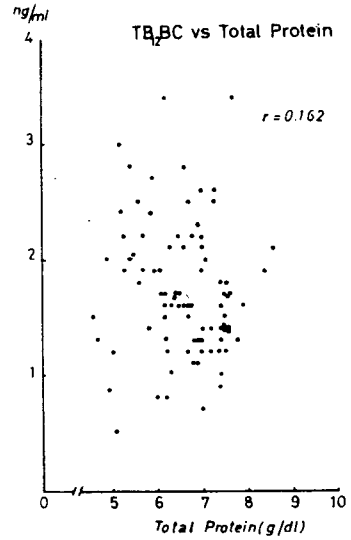


b)

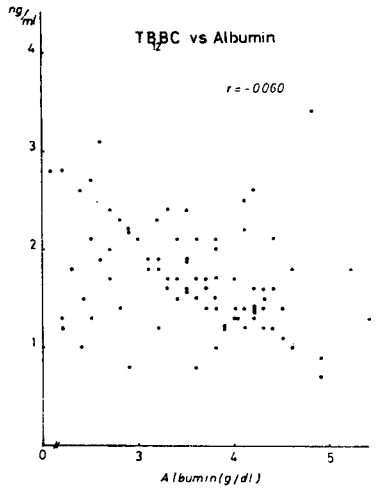


c)

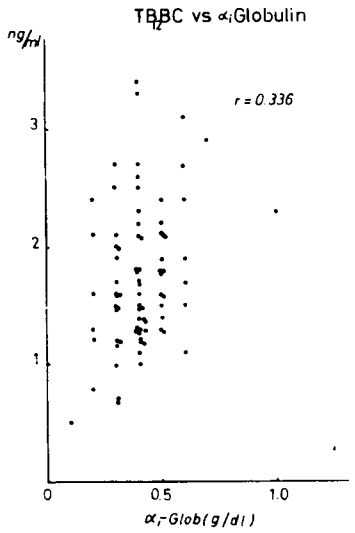
Fig.2. Comparison of serum transcobalamin and a) the erythrocyte count, b) the leucocyte count, c) the total iron binding capacity, d) the total protein content, e) the albumin concentration, f) the alpha-1 globulin concentration, and g) the alpha-2 globulin concentration.



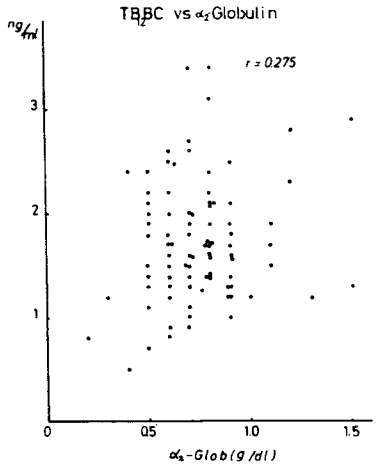
d)



e)



f)



g)

Fig. 2 cont'd

Discussion

Serum B_{12} concentrations of normal subjects obtained in the present survey employing charcoal adsorption technique coincided well with the values reported by previous investigators(5, 10,11, 12). The fact that the decrease of B_{12} levels with aging was not apparent in the present series may depend on the paucity of the normal senile population in the present investigation. In the younger population of the age group of 21 to 30 some high levels of B_{12} were observed The amounts of $UB_{12}BC$ in the present series were slightly lower than the values reported by Gottlieb at al. (5), in which normal level of $UB_{12}BC$ for American population was 1180 pg/ml, and also slightly lower than the data reported by Jørgenson (13) et al., and Olesen et al.(6), of a Scandinavian population of 1388 and 1337 pg/ml. The reason for this difference is not known at present and further studies will be required to elucidate the possible presence of racial difference in the oriental races. The marked increase of $UB_{12}BC$ and $TB_{12}BC$ in neoplastic diseases may very well be nonspecific as postulated by Olesen et al. (6), the fact that a significant correlation was observed between the leucocyte count and transcobalamin level in the present study which corresponded with those of Gottlieb et al. (5), may be worthy of comment. A preliminary study (14) employing modified DEAE cellulose column chromatography of Retief et al. (15), disclosed that the increase of $UB_{12}BC$ in neoplastic as well as inflammatory diseases were due mostly to the increase of TC-I and much less of TC-II. As well known, and confirmed in this laboratory also, the abnormal increase of

transcobalamin level in chronic myelogenous leukemia (5, 16, 17, 18, 19) may be attributed to an increase of TC-I alone or an increase in the so-called alpha binder in serum.

Comparative studies on serum TC-I and leucocyte binder conducted in this laboratory (19, 20, 21) using ion exchanger cellulose chromatography, gel filtration on Sephadex G-200, electrophoresis combined with auto-radiography and isoelectric fractionation has disclosed close similarities of these binders suggesting a possible origin of TC-I in the circulating granulocytes.

Another study (22) to be reported elsewhere, in which the separation of segmented leucocyte and mononuclear cell were carried out from circulating blood of normal and leukemic patients, has disclosed that recovered B_{12} binding capacities per cell were higher in normal leucocytes (5.85 pg/10^4 cells) than leukemic cells (2.90 pg/10^4 cells) harvested from the blood of chronic myelogenous leukemia. However, the correlation coefficients of cell numbers and B_{12} binding capacities of the lysate of the segmented neutrophils were the highest ($r=0.98$) among cell compartments studied and no difference was noted between normal and leukemic patients. Olesen et al. (6) reported recently that serum transcobalamin levels correlate rather well with the erythrocyte sedimentation rates and postulated that the increase of transcobalamin in neoplastic as well as other inflammatory diseases may be a part of the reactions of the host similar to the mobilization of the acute phase reactants in these diseased states.

Further studies on the quantitation of transcobalamin subtypes are in progress employing automated DEAE cellulose chromatography and will be reported shortly.

Summary

Serum transcobalamin determinations were made on 138 normal control sera, and on 238 various pathologic sera obtained from patients with carcinoma, cirrhosis of the liver, leukemia, anemia, and miscellaneous diseases. Serum B_{12} concentrations and unsaturated B_{12} binding capacities were determined by the method of Lau et al., and Gottlieb et al., respectively. Determination of serum iron concentration, total iron binding capacity and electrophoresis of the serum protein fractions were carried out in parallel.

The normal ranges of B_{12}, $UB_{12}BC$, $TB_{12}BC$ were as follows; B_{12} 126-1260 pg/ml, with a mean of 518 pg/ml, $UB_{12}BC$ 508-1484 pg/ml with a mean of 884 pg/ml, $TB_{12}BC$, 655-2619 pg/ml with a mean of 1408 pg/ml, respectively.

A significant increase of $UB_{12}BC$ was noted in cases with carcinoma, and a moderate increase was observed in cases with infectious disease. In chronic myelogenous leukemia, serum transcobalamin level increased to 2 to 5 fold of the normal controls. $TB_{12}BC$ values were compared with various parameters and the following coefficients of correlations were obtained; RBC ($r=-0.08$), WBC ($r=0.696$), total protein ($r=0.162$), albumin ($r=-0.060$), alpha-1 globulin ($r=0.336$), alpha-2 globulin ($r=0.275$),

beta-globulin (r=-0.033), gamma-globulin (r=0.174), total iron binding capacity (r=-0.079), and B_{12} vs $UB_{12}BC$ (r=-0.196), respectively.

(Received Feb. 16, 1970)

References

1. Beal, R. W., Gizis, E. and Meyer, L. M.: Serum protein binding of vitamin B_{12}, Scand. J. Haemat. Suppl. No. 10, 3-39 (1969).

2. Rosenthal, H. L. and Sarrett, H. P.: The determination of vitamin B_{12} activity in human serum, J. Biol. Chem., 199, 433-442 (1952).

3. Pitney, W. R., Beard, M. F. and van Loon, E. J.: Observations on the bound form of vitamin B_{12} in human serum. J. Biol. Chem., 207, 143-152 (1954).

4. Heller, P., Epstein, R., Cunningham, B., Henderson, W. and Yakulis, V.: Vitamin B_{12} binding capacity of serum in B_{12} deficiency, Proc. Soc. Exp. Biol. Med., 115, 342-346 (1964).

5. Gottlieb, C. W., Retief, F. P., Pratt, P. W., and Herbert, V.: Correlation of B_{12} binding proteins with disorders of B_{12} metabolism: relation to hypo- and hyperleucocytic states and leucocyte turnover, J. Clin. Invest. 45, 1016-1017 (1966).

6. Olesen, H., Andersen, M. P. and Amris, A.: Serum vitamin B_{12} binding capacity in patients with anemia, Scand. J. Haemat., 5, 235-240 (1968).

7. Lau, K-S., Gottlieb, C., Wasserman, L. R. and Herbert, V.: Measurement of serum B_{12} levels using radioisotope dilution and coated charcoal, Blood, 26, 202-214(1965).

8. Gottlieb, C., Lau, K-S., Wasserman, L. R. and Herbert, V.: Rapid charcoal assay for intrinsic factor, gastric juice unsaturated B_{12} binding capacity, antibody to IF, and serum unsaturated B_{12} binding capacity, Blood, 25, 875-884 (1965).

9. Clarke, D. D. and Nicklas, R.: A micromodification of the automatic determination of serum iron, Automation in Analytical Chemistry, Technicon Symposia 1967, vol. I, 181-184 (1967).

10. Okuda, K., Wood, R. D., Lang, C. A. and Chow, B. F.: Serum levels of vitamin B_{12} in man, Fed. Proc., 13, 471 (1954).

11. Ikemoto, N.: Clinical investigation on the metabolism of vitamin B_{12}. II. Vitamin B_{12} metabolism in leukemia, Naika-Hokan, 6, 350-359 (1959).

12. Chow, B. F., Wood, R., Hororick, A. and Okuda, K.: Agewise variation of vitamin B_{12} serum levels. J. Gerontol., 11, 142-146 (1956).

13. Jørgensen, F. S.: Vitamin B_{12} and its binding protein in cirrhosis and infectious hepatitis, Scand. J. Haemat., 7, 322-324 (1970).

14. Yamamoto, A. and Fukuda, M.: Clinical studies on serum transcobalamin. Changes of transcobalamin and transcobalamin subfractions following CN-B_{12} administration, in preparation.

15. Retief, E. P., Gottlieb, C. W., Kochwa, S., Pratt, P. W. and Herbert, V.: Separation of vitamin B_{12} binding protein of serum, gastric juice and saliva by rapid DEAE cellulose chromatography, Blood, 29, 501-516 (1967).

16. Mendelsohn, R. S., Watkin, D. M., Horbett, A. P. and Fahey, J. L.: Identification of the vitamin B_{12} binding protein in serum of normal and of patients with chronic myelocytic leukemia, Blood, 13, 740-747 (1958).

17. Meyer, L. M., Bertcher, R. W., Cronkite, E. P., Suarez, R. M., Miller, I. F., Mulzac, L. W. and Olivaretta, S. T.: Co^{60} vitamin B_{12} binding capacity of serum in persons with hematologic disorders, various medical diseases and neoplasma, Acta Med. Scand., 169, 557-575 (1961).

18. Hall, C. A. and Finkler, A. E.: Measurement of the amounts of the individual vitamin B_{12} binding proteins in plasma, II. Abnormalities in leukemia and pernicious anemia, Blood, 27, 618-622 (1966).

19. Fukuda, M.: Clinical significance of serum transcobalamin, Jap. J. Clin. Hematol., 11, 331-336 (1970).

20. Fukuda, M., Natori, H., Motoyama, K., Yamamoto, A. and Urushizaki, I.: Studies on fractionation of serum proteins by electrofocusing. II. On transcobalamin subfractions, The physico-chemical Biology, 14, 211-213 (1969).

21. Natori, H., Yamamoto, A., Motoyama, K. and Fukuda, M.: Analytical studies on transcobalamins and leucocyte binders from various animals, Jap. J. Nucl. Med., 7, 256 (1970).

22. Natori, H., Fukuda, M. and Urushizaki, I.: Clinical studies on vitamin B_{12} binding proteins. II. Vitamin B_{12} binding protein in leucocytes from normal human subjects and chronic myeloid leukemia, submitted for publication.