Vitamin B_{12} Serum Level and Pregnancy*

By KUNIO OKUDA, M.D., ANDRE E. HELLIGER, M.D., AND BACON F. CHOW, PH.D.

VITAMIN B_{12} is one of the essential factors in human nutrition[1] and in the maintenance of normal health.[2] It is, therefore, of interest to study the degree of tissue saturation with this vitamin in pregnant women and in their offspring, as measured by maternal and fetal serum vitamin B_{12} levels at the time of delivery. The results of such a study are presented in this communication.

EXPERIMENTAL

Vitamin B_{12} Assay: The procedure[3] for the determination of vitamin B_{12} activity in serum may be described briefly as follows: 2 ml of serum are added to a tube containing 8.0 ml of acetate buffer, pH 4.6. The tube is covered snugly with a piece of tinfoil and heated in a large boiling water bath for 30 minutes. After cooling with cold water, the tubes are centrifuged for 5 minutes and 6 ml of the clear supernatant fluid are removed and neutralized with 1 ml of Na_2HPO_4 (0.33 molar). 1.0, 2.0, and 3.0 ml of this mixture are then measured and assayed for the microbiological activity of vitamin B_{12} with the procedure of Skeggs and Wright.[4] The alkali-stable factor in the serum was determined by the procedure of Hoffman[5] *et al.*

RESULTS

Blood specimens for the vitamin B_{12} assay were obtained by venipuncture from the veins of a series of 25 pregnant women at the time of delivery and from the umbilical cords of their infants. The results of the assays, the ages of the mother and the weights of the infants at birth are shown in Table I. The ages of the women ranged from 15 to 41 years, with an average of 28 years. Parity of the women ranged from zero to eight. Approximately one-half of the subjects were white, and the other half were colored. The mean vitamin B_{12} serum level of the fetus was considerably higher than that of the mother, being 358 ± 36 and 120 ± 14 $\mu\mu$g/ml, respectively. The difference is statistically significant ($P<0.001$).

For comparison, blood specimens were drawn for vitamin B_{12} assay from non-pregnant women of comparable age; these subjects were employees of the Johns Hopkins Hospital and the School of Hygiene. The average vitamin B_{12} serum level of this group was 185 ± 12 $\mu\mu$g/ml. This was a selected group of women and may not be strictly comparable with our study group.

In a separate study, we recently measured the vitamin B_{12} serum level of another group of young, clinically healthy, non-pregnant women, selected at random from the Baltimore population.[4] The mean serum vitamin B_{12} value of this group was 220 ± 21 $\mu\mu$g/ml. Thus the serum levels of both groups of non-pregnant subjects were significantly higher than those of the pregnant women at the time of delivery.

In Figure 1 the vitamin B_{12} serum values of mothers are plotted as abscissa against those of the cord serum as ordinate. At a 45° angle a theoretical line is drawn on which points would fall if the maternal and cord values were equal. The points would be below or above this line, if the maternal values were larger or smaller than the fetal values. In every one of the 25 cases studied the cord level was at least equal to, or in excess of, that of the mother. There was no detectable amount of vitamin B_{12} activity in cord serum extract after alkali treatment; therefore, the higher vitamin B_{12} value in fetal serum is not due to desoxyriboside. The probability that all dif-

From the Department of Biochemistry, School of Hygiene and Public Health, and from the Department of Obstetrics, Johns Hopkins Hospital, Baltimore, Maryland.

*Not presented at the Symposium.

The authors acknowledge with thanks the grant-in-aid and materials from Merck and Company, and from the National Vitamin Foundation, and from Lederle Laboratory Division of the American Cyanamid Company, and the service of Miss Ann Knepshield for the vitamin B_{12} assay.

TABLE I

Serum B_{12} Levels of Mother and Fetus

Patient	Age	Race	Para	Mother serum B_{12}	Fetus, cord serum	Wt. of newborn
				μμg/ml	*μμg/ml*	*g*
1	20	C	III	114	333	2560
2	15	C	0	247	925	2805
3	37	C	I	87	281	2880
4	21	W	IV	119	128	3620
5*	19	W	II	<40	65	3960
6	22	W	I	230	<A270	3400
					<B330	3200
7	21			163	350	
8	26	C	I	117	280	2970
9	28	W	I	170	385	3840
10	41	C	III	~64	~262	2980
11	24	C	II	93	216	3950
12	32	W	III	105	420	4600
13	23	C	0	160	400	2878
14	27	W	IV	106	~410	
15	25	C	I	~40	347	3055
16	18	C	I	72	310	2615
17	28	W	0	319	435	3125
18	27	C	0	160	595	3750
19	21	C	0	115	275	2270
20	25	W	I	~53	178	3425
21	29	C	I	150	444	3900
22	29	C	I	<15	667	3050
23	35	W	IV	~90	178	3300
24	28	C	II	101	295	3075
25	36	C	VIII	84	461	3170
				119.8 ± 14.1	357.6 ± 35.8	t = 4.17

* Cretinism.

ferences would be of the same numerical sign in a population of 25 is extremely small.

DISCUSSION

Although vitamin B_{12} is considered to be essential for the growth of children, its stimulatory effect cannot be demonstrated easily in clinically healthy full-term[6] or premature infants, but can manifest itself among undernourished children.[6,7] In the light of our results, this difference in response may be due to the variation in the reserve of vitamin B_{12} in the young. The unique property of the fetus to concentrate vitamin B_{12} was reported in our previous communication,[8] where radioactive vitamin B_{12} was injected into pregnant rats about two weeks before parturition. In relation to the body weight, a large portion of the injected radioactivity, taken as a measure of vitamin B_{12}, was found in the fetus. Besides vitamin B_{12}, ascorbic acid[9] is the only other vitamin known to be concentrated in the offspring in a similar manner.

The serum level of vitamins may be considered an index of tissue saturation. Diseases due to vitamin B_{12} deficiency (such as pernicious anemia), or situations in which the dietary intake is inadequate[10] or absorptive capacity poor, will be accompanied by low vitamin B_{12} serum levels. On the other hand, elevated levels of the vitamin may be totally irrelevant to tissue saturation, since in certain diseases, such as leukemia[11] and diabetes with retinopathy,[12] the serum level may be elevated, presumably because of the destruction of tissues to which vitamin B_{12} is bound. It is our belief that a low vitamin B_{12} level reflects poor tissue reserves of this vitamin, although the reverse may not be necessarily true.

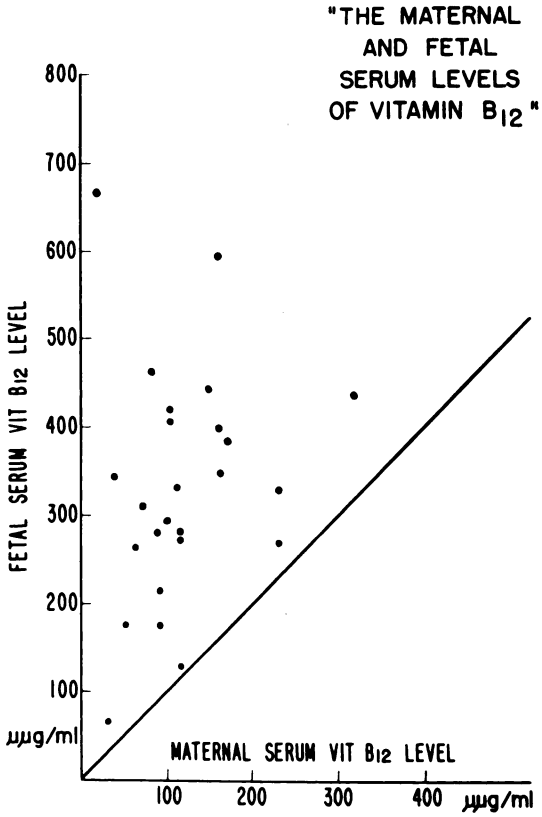

Figure 1

While our results definitely show abnormally low vitamin B_{12} serum levels in the mothers at the time of delivery, any justification for administering this vitamin to pregnant women must be based on the advisability of repleting the vitamin B_{12} level of the mother and insuring an adequate supply to the fetus. No clinical data are yet available to justify the general use of this vitamin in pregnancy.

With the limited number of cases studied, it is not possible to correlate the level of vitamin B_{12} in the cord serum either with the parity of the mothers or with the weight of the newborn infants. It is of interest to record that the vitamin B_{12} level in the cord serum of Case 5 was abnormally low for this series. Seven weeks after delivery this infant was admitted to the pediatrics department of the Johns Hopkins Hospital with marked and classical signs of hypothyroidism and bone growth retardation, as seen in cretinism.

It might be of interest to speculate whether a certain fetal level of vitamin B_{12} is necessary, in certain as yet unspecified stages of intrauterine life, for the proper development of unspecified fetal organs (e.g. the thyroid gland or bone).

SUMMARY

Vitamin B_{12} serum levels of mother and fetus were determined in 25 women ranging in age from 15 to 41 years, and in their first to ninth pregnancy. It was found that the vitamin B_{12} serum level of the maternal blood was considerably lower than that of the nonpregnant women of comparable age, and also lower than that of the fetal cord level. These findings may indicate that vitamin B_{12} is drawn from the mother to the fetus.

ACKNOWLEDGMENT

The authors express their appreciation for the cooperation and assistance of Dr. N. J. Eastman.

REFERENCES

1. Wokes, F., Badenoch, J., and Sinclair, H. M.: Human dietary deficiency of vitamin B_{12}. Am. J. Clin. Nutrition 3: 375, 1955.
2. Jolliffe, N., Funaro, R., Frontali, G., Maggioni, G., Corbo, S., and Lanciano, G.: Vitamin B_{12} as a growth factor in Italian children on diets low in animal protein. *Current Research on Vitamins in Trophology*, Nutrition Symposium Series No. 7, The Natl. Vitamin Foundation, New York, 1953, p. 119.
3. Okuda, K., Wood, R. D., Lang, C. A., and Chow, B. F.: Serum levels of vitamin B_{12} in man. *Fed. Proc.* 13: 1546, 1954.
4. Skeggs, H. R., Nepple, H. M., Valentik, K. A., Huff, J. W., and Wright, L. D.: Observations on the use of Lactobacillus leichmannii 4797 in the microbiological assay of vitamin B_{12}. *J. Biol. Chem.* 184: 211, 1950.
5. Hoffman, C. E., Stokstad, E. L. R., Franklin, A. L., and Jukes, T. H.: Response of Lactobacillus leichmannii 313 to the anti-pernicious anemia factor. *J. Biol. Chem.* 176: 1465, 1948.
6. Chow, B. F.: Sequelae to the administration of vitamin B_{12} to humans. *J. Nutrition* 43: 323, 1951.
7. Wetzel, N. C., Hopwood, H. H., Kuechle, M. E., and Grueninger, R. M.: Growth failure in school children: Further studies of vitamin B_{12} dietary supplements. J. Clin. Nutrition 1: 17, 1952.
8. Chow, B. F., Barrow, L., and Ling, C. T.: The distribution of radioactivity in the organs of the fetus or of young rats borne by mothers injected

with vitamin B_{12} containing Co^{60}. *Arch. Biochem. & Biophysics* 34: 151, 1951.

9. JAVERT, C. T., and STANDES, J.: Vitamin C plasma and prothrombin concentration in threatened, spontaneous, and habitual abortion. *Surg., Gynec. and Obst.* 76: 115, 1943.
10. EDDY, W. H.: *Vitaminology.* The Williams and Wilkins Co., Baltimore, 1949.
11. BEARD, M. F., PITNEY, W. R., and SANNEMAN, E. H.: Serum, concentrations of vitamin B_{12} in patients suffering from leukemia. *Blood* 9: 789, 1954.
12. CHOW, B. F., ROSEN, D. A., and LANG, C. A.: Vitamin B_{12} serum levels and diabetic retinopathy. *Proc. Soc. Exper. Biol. & Med.* 87: 38, 1954.