Vitamin B_{12} Absorption in Pregnancy and in the Newborn

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INCREASING interest is being shown in the role of vitamin B_{12} in pregnancy and in its transfer from mother to fetus. In a previous communication,[1] it was reported that a considerable quantity of radioactivity is transferred to the fetus when pregnant rats are injected with vitamin B_{12} labeled with Cobalt^{60}. Later it was demonstrated that serum vitamin B_{12} levels in the fetus at the time of delivery, measured from the umbilical cord blood, are invariably higher than those of the mother obtained at the same time.[2] This apparently "parasitic" action of the fetus in relation to its mother's vitamin B_{12} content has led us to study whether the pregnant woman, or rat, possesses a mechanism of compensation against excessive depletion of her vitamin B_{12} stores. The results of such a study, and some additional incidental findings regarding methods of studying this problem, are given in this report.

DETERMINATION OF THE ABSORPTION OF VITAMIN B_{12} BY PREGNANT AND NON-PREGNANT WOMEN

In view of a general, and understandable hesitancy on the part of obstetricians to ask mothers to allow their unborn or newborn infants to be subjected to the effects of radioactivity, however safe this may be, the following method of studying absorption was used:

Two groups of women, one pregnant and one non-pregnant group, were subdivided into three subgroups. The non-pregnant group consisted of women of childbearing age and of comparable background to the pregnant group. All women in subgroup A were given an oral dose of 1,000 μg of vitamin B_{12} while subgroup B and C received 500 μg and 250 μg respectively. Serum vitamin B_{12} levels were obtained on all subjects prior to vitamin B_{12} administration and one and one-half and three hours after the administration.

The resultant serum vitamin B_{12} levels are shown in Table I. An increase in serum vitamin B_{12} level of more than 160 μg is taken as a positive response, since this order of magnitude is beyond the error of our routine assay for this vitamin.

ABSORPTION OF VITAMIN B_{12} IN PREGNANT AND NON-PREGNANT RATS

Determination of Serum Level: In two separate studies, conducted three months apart, 26 adult female rats (three to four months old) were used. In each study the animals were bled by cardiac puncture, and 1.5 ml of blood was obtained for vitamin B_{12} assay according to the procedure described by Okuda[2,3]. The rats were randomly divided into two groups, A and B. Animals in group B were mated with adult male rats, while animals in group A served as controls. In both studies all mated animals became pregnant within four weeks. Shortly (one or two days) prior to delivery, rats in both groups A and B were again bled for B_{12} assay. The results of this study are given in Table II.

Determination of the Absorption of Vitamin B_{12} by Pregnant and Non-Pregnant Rats: The absorption of vitamin B_{12} by pregnant and non-pregnant rats was measured by feeding a measured amount (50 $\mu\mu$g) of radioactive vitamin B_{12} orally with a specific activity of 1,000 microcuries per mg. Two hours after feeding, the animals were injected with 50 $\mu\mu$g of unlabeled vitamin B_{12}. All animals, housed individually in separate cages, were given a basal casein diet.[4] Urine and feces were collected separately for eight days at

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two-day intervals. The details for the determination of radioactivity in the excreta are described in our previous communications.[5,6] The results of two studies performed at least three months apart are given in Table III. Half of the animals in each of the two study groups were mated and became pregnant. About ten days before delivery both pregnant and control rats were given 50 mmcg of vitamin B_{12} by mouth. The radioactivity in the urine and feces was determined by scintillation counting. The differences between the radioactivity of orally administered vitamin B_{12} and the fecal radioactivity is taken as a measure of the absorbed vitamin. The radioactivity in the fetus was also determined after digesting the tissues with 30 per cent KOH.

Determination of Radioactivity in Maternal Liver and Kidney and of the Fetus following Subcutaneous Administration of Radioactive Vitamin B_{12}: Twenty-four female adult rats (three to four months old) were used in two separate studies conducted four months apart. Half of the animals in each study were mated with adult males and the other half served as controls. Two or three days before delivery both pregnant and non-pregnant animals were injected subcutaneously with 10 $\mu\mu$g of

TABLE I

Response of Pregnant and Non-pregnant Women to Oral Tolerance Test for Vitamin B_{12}

Dosage in μg	Pregnant		Non-pregnant	
	Av. change in μg	Response, %	Av. change in μg	Response, %
250	15.5 (10)*	0	47 (10)	0
500	198.3 (13)	41.5	128 (12)	16.6
1,000	262.8 (9)	88.8	157.5 (7)	28.5

* Numbers in parenthesis denote the number of subjects used.

TABLE II

Plasma Vitamin B_{12} Levels of Pregnant and Control Rats

Study	Group	No. of rats	Serum B_{12} content in $\mu\mu g/ml \pm$ S.E.M.*		
			Before mating	Before delivery	6 weeks later
I	A	6 (control)	1.2 ± 0.06	—	1.1 ± 0.1
	B	8 (mated)	1.2 ± 0.12	0.66 ± 0.04	—
II	A	6 (control)	1.15 ± 0.09	—	1.1 ± 0.08
	B	6 (mated)	1.2 ± 0.14	0.6 ± 0.02	—

* S.E.M. Standard error of the mean.

TABLE III

Absorption of Orally Administered Vitamin B_{12} to Pregnant and Non-pregnant Rats

Study	No. of rats	Vitamin B_{12} activity in*			
		Urine	Feces	Absorbed B_{12}	B_{12} in fetus
I	8 (pregnant)	0.6 ± 0.05	10 ± 1.2	40	24 ± 3.6
	8 (control)	0.4 ± 0.12	29 ± 3.6	21	—
II	6 (pregnant)	0.5 ± 0.10	9.6 ± 0.8	40.4	26 ± 2.8
	6 (control)	0.35 ± 0.15	31 ± 2.9	19	—

* Expressed in $\mu\mu$g of vitamin B_{12}.

radioactive vitamin B_{12} and were sacrificed four days later. The livers and kidneys of the mothers and of the non-pregnant rats were assayed for their radioactivity contents, as were the offspring in their entirety. The results are shown in Table IV.

RESULTS

Pregnant women who were fed a dose of 1,000 μg of vitamin B_{12} showed a statistically significant increase in absorption of the vitamin as compared to non-pregnant women. An increase in serum vitamin B_{12} of 160 $\mu\mu g$ per ml or more was found in eight out of nine pregnant women, as compared with only two out of seven non-pregnant women. When the test dose was reduced to 500 μg the difference was not statistically significant, although it is suggestive of the greater absorption by the pregnant subjects. When the dosage was reduced further to 250 μg no significant increase in the serum level was observed in either group. When the results of the two higher dosages are taken together, the data again show that in pregnant women there is statistically significant greater absorption of vitamin B_{12}.*

This phenomenon was supported by results of experiments with rats. Thus, data tabulated in Table II clearly indicate that the vitamin B_{12} serum level decreased to almost one-half of its original value in the course of pregnancy. This result is clearly shown by comparing the serum vitamin B_{12} level of the rats with either the mean value of the same rats before mating or with the mean value of the non-pregnant controls. The serum vitamin B_{12} level of the control animals would suggest that the relatively lower levels during pregnancy are not the result of the process of bleeding.

Results in Tables III and IV indicate that the absorption of orally administered radioactive vitamin B_{12} is greater in the pregnant than in the non-pregnant rats. The increase in the absorption is approximately twofold (from approximately 20 $\mu\mu g$ to 40 $\mu\mu g$). It is interesting to note also that 60 per cent or more of the absorbed vitamin was concentrated in the fetus. The extraordinary ability of the fetus to draw vitamin B_{12} is again demonstrated in the experiment in which both pregnant and control rats were given 10 $\mu\mu g$ of Co^{60}-labeled B_{12} by the subcutaneous route. About 60 per cent of the injected vitamin B_{12} was concentrated in the fetus. More surprising, however, is the fact that the target organs of the pregnant animals contained much less radioactivity than those of the controls. These results, taken as a whole, indicate clearly that in pregnancy the absorbed vitamin B_{12} is concentrated in the fetus.

TABLE IV

Comparison of the Fate of Vitamin B_{12} Injected into Pregnant and Non-pregnant Rats

Study	No. of rats	Vitamin B_{12} content expressed as $\mu\mu g$ in			
		Liver	Kidneys	Fetus	$\mu\mu g$/fetus
A	6 (pregnant)	0.32 ± 0.06	0.17 ± 0.04	6.2 ± 1.0	0.6 ± 0.2
	6 (control)	1.3 ± 0.12	0.8 ± 0.10	—	—
B	6 (pregnant)	0.75 ± 0.17	0.28 ± 0.09	5.96 ± 0.8	0.46 ± 0.04
	6 (control)	1.50 ± 0.08	0.86 ± 0.10	—	—

Possible Interrelationship between Thyroid Activity and Vitamin B_{12} Metabolism: From what has been stated before, the fetal capacity for obtaining vitamin B_{12} from the available maternal supply is remarkable. It has been previously reported by the authors[2] that in spite of very low maternal serum vitamin B_{12} levels, the fetus could still show adequate or high serum levels. At that time allusion was made to the fact that the fetus of Case V,[2] in which neither the mother nor the fetus showed an appreciable serum level of vitamin B_{12}, had been admitted to the Pediatric Service of the Johns Hopkins Hospital with a classic picture of cretinism. An initial speculation as to a possible relationship between a depleted fetal vitamin B_{12} level and hypothryroid-

* $X^2 = 4.33$; significant $X^2 = 3.8$ (X^2 = chi sq)

ism was made at that time. Since then, a second case of athyroid cretinism has been admitted to the Johns Hopkins Hospital, in which the same fetal phenomenon was observed.

As a result of the findings in these cretins, studies were carried out in the Harriet Lane Home for Children which show that the administration of thyroid extract to cretins in infancy will rapidly return the vitamin B_{12} levels to normal.

At birth the second infant had no appreciable amount of vitamin B_{12} in the serum, while none was detected in the mother's blood at delivery. Six weeks postpartum, the mother's serum vitamin B_{12} level was noticed after initiation of thyroid therapy. Unfortunately there was no maternal vitamin B_{12} serum level available in this case.

Since in our previous report[2] the occurrence of very low maternal serum vitamin B_{12} levels is reported in the presence of normal or high fetal serum levels, and since the low fetal serum vitamin B_{12} levels were only obtained in those cases which clinically showed evidence of cretinism, we suggest that the fetal serum vitamin B_{12} level, rather than the maternal, is a significant finding in cretinism. The results of these studies are reported in Table V.

Because the incidence of cretinism is low, and because there is an even smaller likelihood of obtaining maternal and fetal serum vitamin B_{12} levels at the time of delivery of cretins except by the routine assay of these levels on thousands of cases, we have considered it worthwhile to report our findings on the aforementioned two cases in the hope of stimulating similar studies in other medical centers.

TABLE V

Thyroid Activity and Vitamin B_{12} Serum Level in Cretinism

	Date	Age	P.B.I.*	Clinical picture	Thyroid therapy	B_{12} serum level†
Patient D. W.	8/12/55	Birth	—	—	0	0
	10/7/55	7 weeks	0.6	Severe hypothyroid	Started on 1/4 g/day	0
	11/22/55	3 1/2 months	4.7	Euthyroid	3/4 g/day	157
	12/7/55	4 months	4.9	Euthyroid	3/4 g/day	222
	1/6/56	5 months	—	Euthyroid	3/4 g/day	325
Patient T. F.	3/28/56	7 weeks	2.0	Moderately hypothyroid	Started on 1/2 g daily	<50
	4/11/56	9 weeks	3.7	Slightly hypothyroid	3/4 g/day	303
	6/16/56	4 months	6.2	Euthyroid	1 g/day	—

* Protein-Bound Iodine.

† Expressed in $\mu\mu$g.

SUMMARY AND CONCLUSIONS

Absorption of orally administered vitamin B_{12} is significantly increased by pregnancy in the human and in the rat. As yet, pregnancy is the only known method of increasing vitamin B_{12} absorption in the adult.

In spite of increased vitamin B_{12} absorption,

the serum vitamin B_{12} level and the vitamin B_{12} content of the liver and kidney of pregnant rats actually show a decrease, presumably due to the demands of the fetus which seems to be the main beneficiary of the increased vitamin B_{12} absorption rate in pregnancy.

Oral tolerance tests can be used as a method of proving increased vitamin B_{12} absorption, provided the administered dose is large enough. The critical test dose in the experiment reported was 1,000 μg. This method of study obviates the use of radioactive materials in the pregnant state.

There is an, as yet, imperfectly understood relationship between thyroid function and vitamin B_{12} absorption in the earlier stages of life. It is suggested that abnormally low vitamin B_{12} serum levels may be found in the blood of newborns with cretinism. Administration of thyroid to newborn cretins rapidly raises the serum vitamin B_{12} to normal levels.

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