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We should like to acknowledge the assistance of Barbara Lenes Mohandes and Jayne Rosenthal of Barnard College.

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Received July 10, 1957. P.S.E.B.M., 1957, v96.

Pyridoxal Phosphate (B_6-al-PO_4) Levels of Circulating Leukocytes in Maternal and Cord Blood.* (23468)

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In the later stages of pregnancy there exists a disturbance of Vit. B_6 metabolism. Following a tryptophan load test large amounts of xanthurenic acid appear in the urine(1) and the excretion pattern of tryptophan metabolites on chromatographic analysis deviates from the normal(2). This is immediately corrected by administration of relatively small amounts of pyridoxine(1,2). Furthermore pregnant women retain administered Vit. B_6 to a larger extent than do normal controls(3). The present report deals with the estimation of the coenzymatically available active form of Vit. B_6, namely pyridoxal phosphate, in the only readily accessible prototype tissue, *i.e.* leukocytes, in the blood of non-pregnant controls, mothers at term and in the cord blood of their babies.

Material and methods. Blood samples were taken from non-pregnant healthy women, women during delivery and from the cord blood of their babies. The pregnant women were divided into 3 groups. One group received no vitamin supplementation containing Vit. B_6. A second group had taken, during the course of their pregnancy, vitamin preparations which contained some Vit. B_6 in an uncontrolled and irregular manner. A third group had received, for a period of at least 14 days prior to their confinement, 2 tablets of 5 mg each pyridoxine hydrochloride daily. 5-10 ml venous blood was collected into siliconed test tubes containing a small amount of disodium versenate. Pyridoxal phosphate was estimated in isolated leukocytes by a recently developed method(4) which is based on the coenzyme function of B_6-PO_4 in a tyrosine decarboxylase system from *Streptococcus faecalis*(5). The leukocytes were isolated within a few hours after the blood had been obtained(5a). To the packed leukocytes 0.25 ml of 0.5 *N* NaOH was added and the tubes immersed in a boiling water bath for 5 minutes. The alkaline hydrolisates were kept in the refrigerator at 4°C and worked up within 24 hours. All analyses were made in triplicate and the samples were assayed at random.

Results. The results are summarized in Table I. In the first experimental group consisting of 60 non-pregnant, healthy women of child-bearing age, the amount of pyridoxal phosphate varied from 0.11 to 0.79 mγ/million leukocytes, averaging 0.32 ± 0.02 mγ/million leukocytes. The second group consisted of 51 women at term who had received no Vit. B_6 supplementation during their pregnancy. The B_6-al-PO_4 levels varied between

* This work was aided by grants-in-aid and material from Merck and Co. and National Vit. Fn. We are indebted for valuable advice to Dr. G. E. Boxer, Merck Inst. for Therapeutic Research.

TABLE I. Pyridoxal Phosphate (B_6-al-PO_4) Levels of Circulating Leucocytes.

Group	No. of cases	Age range	Avg age	Range of B_6-al-PO_4 content (mγ/million cells)	Avg B_6-al-PO_4 content (mγ/million cells)	Probability relation	
I. Non-pregnant controls (N)	60	19–45	28	.11– .79	.32 ± .02	M., N.V. *vs* N.	<.01
II. A. Pregnant women at term without vit. B_6 medication (M., N.V.)	51	19–40	27	.01– .36	.16 ± .01	M., N.V. *vs* C., N.V.	<.01
B. Cord blood (C., N.V.)	36			.13– .93	.44 ± .04		
III. A. Pregnant women at term with some vit. B_6 medication (M., V_1)	69	19–45	27	.06– .44	.20 ± .01	M., V_1 *vs* C., V_1	<.01
B. Cord blood (C., V_1)	61			.12–1.02	.47 ± .02	M., N.V. *vs* M., V_1	<.05
IV. A. Pregnant women at term with controlled vit. B_6 medication (M., V_2)	26	16–41	27	.04– .61	.28 ± .03	M., V_2 *vs* C., V_2 M., N.V. *vs* M., V_2	<.01 <.01
B. Cord blood (C., V_2)	22			.18–1.24	.55 ± .05	C., N.V. *vs* C., V_2	<.04

0.01 and 0.36, averaging 0.16 ± 0.01 mγ per million cells. The third experimental group was composed of 69 women at term who had occasionally taken Vit. B_6 containing preparations. Their pyridoxal phosphate level ranged between 0.06 and 0.44, averaging 0.20 ± 0.01 mγ/million cells. The 26 subjects in the fourth group had taken Vit. B_6 regularly for at least 14 days prior to delivery. Their blood level varied between 0.04 and 0.61 and averaged 0.28 ± 0.03 mγ B_6-al-PO_4/million leukocytes. The difference in the vitamin level between the control group and the pregnant group without vitamin supplementation is highly significant. Occasional intake of Vit. B_6 led to a moderate increase of leukocytic B_6 of doubtful significance. However, the level of the coenzyme form of Vit. B_6 is significantly increased in those women who ingested pyridoxine regularly. The vitamin content of their leukocytes approached that of the normal control group.

The amount of leukocytic pyridoxal phosphate was significantly greater in the cord blood as compared to the maternal blood in all groups examined. The cord blood of 26 infants borne by the women without vitamin supplementation contained between 0.13 and 0.93 (average 0.44 ± 0.04) mγ B_6-al-PO_4/million leukocytes, and that of 61 infants of women who had occasionally taken the vitamin between 0.12 and 1.12 (average 0.47 ± 0.02) mγ B_6-PO_4/million leukocytes. The highest level was found in the last group in 22 infants of mothers supplemented by vit. B_6. The levels of pyridoxal phosphate in these infants ranged between 0.18 and 1.24 mγ with an average of 0.55 ± 0.05 mγ/million leukocytes. The difference between the cord blood values of babies whose mothers were not Vit. B_6 supplemented and those who received the vitamin is statistically significant.

Comment. A decrease of circulating Vit. B_{12} similar to that of Vit. B_6 was recently described in the serum of pregnant women by Okuda *et al.*(6) and by Baker *et al.*(7). Okuda and his coworkers also found much higher Vit. B_{12} levels in the cord blood as compared to maternal blood. The concentration of glutamic-aspartic transaminase, a Vit. B_6 dependent enzyme, is much greater in the fetal than in the maternal blood(8). On the other hand, Friedman *et al.*(9) found no difference in amount of Vit. B_6 in blood, urine or skin of non-pregnant controls and women at term. This might, however, be explained by

difficulties with the bioassay used by these investigators and by the fact that all forms of the vitamin, metabolically active as well as inactive, were measured.

Present evidence indicates that Vit. B_6 is a very important nutrient that is essential for the human being(10). The central nervous system of the newborn infant appears to be particularly sensitive to a conditioned Vit. B_6 deficiency(11). The importance of Vit. B_6 for reproduction(12) and for normal fetal and infant development(13) has also been proven in the experimental animal. Obviously pregnancy exacts large demands on the maternal store of Vit. B_6. The results here reported suggest that the growing fetus may attract maternal Vit. B_6 across the placental barrier. It is possible to increase the amounts of circulating pyridoxal phosphate to almost normal control levels by oral administration of pyridoxine to pregnant women, and further to increase fetal levels, suggesting the probability of a placental transfer of the vitamin. It is interesting to note that a daily dose of 10 mg pyridoxine was found previously to rectify the abnormal tryptophan load tests in pregnant women. Supplementation of the normal diet by a daily dose of 10 mg pyridoxine hydrochloride has been suggested, and preliminary results have been reported indicating that such a regime may be of benefit to pregnant women, since there was a significant decrease of preeclampsia in a vitamin-supplemented as compared to a similar untreated control group(14).

Summary. Comparison of the pyridoxal phosphate content of circulating leukocytes in non-pregnant controls, women at term and umbilical cord blood shows significantly higher values in normal controls as compared to women at term. The cord blood shows the highest levels. Pyridoxine administration elevates the B_6-al-PO_4 levels in pregnant women to a very marked, and that of cord blood to a moderate, degree. It is concluded that the growing fetus can successfully compete with the mother for this important vitamin.

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