sensitive to x-rays than in rats, as reflected by induced oligospermia with the same low dose (9,10). Since TEM is a radiomimetic drug (1,2), it is surprising that, unlike the rat(2), mice treated with the same dose did not show structural defects in spermatogenesis. However, our interval betwen TEM treatment and fixation of testes may not have been long enough for manifestation of changes.

Summary. Triethylenemelamine (TEM) has been shown to affect adversely the reproductive capacity of male mice by acting on and through spermatozoa without altering their number, motility, or appearance. Females artificially inseminated with spermatozoa from the ductus deferentes and cauda epididymides of treated males showed a marked reduction in per cent fertility as well as litter size. Spermatozoa appeared more sensitive to TEM than late spermatids. No obvious structural changes were induced in testicular elements during treatment. The motility of spermatozoa was unchanged by exposure to a high concentration of TEM *in vitro*.

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Pyridoxal Phosphate in Plasma and Leukocytes of Normal and Pregnant Subjects Following B_6 Load Tests.* (25515)

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The concentration of pyridoxal phosphate, the coenzymatically available active form of Vit. B_6, has been estimated in leukocytes of normal subjects(1,2), and in those of pregnant women and cord blood(2), using a modification of the manometric method of Umbreit *et al.*(3). Boxer(1) also tried to utilize the method for determination of pyridoxal phosphate in total blood, but did not find measurable quantities in most human bloods unless the subjects were given oral doses of Vit. B_6. Comparisons of plasma and leukocyte values are herein described. Normal subjects, pregnant women and cord blood of newborn babies were studied. In addition, load tests were performed in normal controls, in a group in last trimester of pregnancy and in women who delivered their babies one day prior to test. We investigated whether load tests would give information not readily obtainable by single plasma or leukocyte determinations.

Method. Whole blood contains interfering substances necessitating use of diluted blood (1). Preliminary experiments were, therefore, undertaken to establish optimal dilution for performance of test. Plasma diluted 1:6, 82% of added Vit. B_6 could be recovered, whether or not its initial content was normal or relatively large, *e.g.*, obtained from individuals who had ingested a large amount of Vit. B_6. In contrast, in whole blood diluted 1:6, only 68%, and in packed red cells, only 61% of added pyridoxal phosphate could be recovered (Table I). For simultaneous estimation of the vitamin in leukocytes and plasma, 5 ml

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TABLE I. % Recovery of Added Pyridoxal Phosphate Standard (4 mγ). (Each figure is average of at least 5 determinations.)

Analysate	Undiluted	1:3	1:6
Plasma	13.7	61	82
Whole blood	7.2	59	68
Red cells	0	50	61

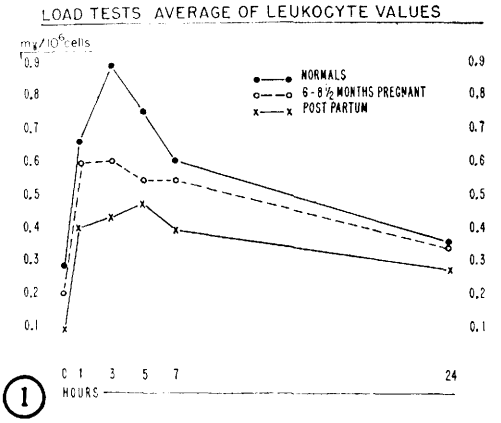
of venous blood was collected in a tube containing about 0.02 g of sodium versenate. Leukocytes were isolated using freeze-dried phytohemagglutinin prepared from navy beans. Separated leukocytes were treated as previously described. The plasma obtained was diluted 1:6 with distilled water. The assay method was based on coenzyme function of pyridoxal phosphate in a tyrosine decarboxylase system. Tyrosine decarboxylase prepared from Vit. B_6-free preparation of *Streptococcus fecalis,* was obtained from Dept. of Bacteriology, Rutgers Univ., New Brunswick. Minor changes were made in technic as described by Boxer *et al.*(1). Larger flasks (volume 15 ml) were used so that larger samples could be utilized. One cc of the diluted sample was put into flasks together with 1 cc of acetate buffer pH 5.5, ½ cc of L-tyrosine suspension of 25 g/100 ml buffer and ½ cc of a suspension of 4 mg tyrosine decarboxylase/ml water. This amount was considered optimal since increase in concentration of the decarboxylase suspension did not result in increase in amount of CO_2 given off. Instead of n-heptane, Brodie's solution was used as manometer fluid. All determinations were done in duplicate within 12 hours after sample had been obtained. Even at 4°C, some loss of Vit. B_6 occurred after 24 hours in plasma and after 48 hours in leukocytes. When duplicate manometer movements were used for calculation of standard deviation of all plasma results, this value came to ± 0.5 mγ/ml.

Results. 1. *Normal values*: concentration of B_6 in plasma and leukocytes was determined in 27 normal men and 20 normal women. The samples were usually obtained in the morning. No vitamin preparations had been taken by these individuals for at least 3 months prior to the test. In men, range of pyridoxal phosphate in leukocytes was 0.15 to 0.36 mγ/million cells with average of 0.23 (± 0.05). In women, the range was 0.14 to 0.30 mγ average 0.22 mγ (± 0.05/million cells. Plasma values were 5.2 to 16.2 mγ/ml, average 10.5 (± 2.5) in men and 5.2 to 12.0 mγ/ml, average 8.4 (± 2.5) in women. In these figures are not included 2 plasma determinations of 33 mγ/ml in a man and 18 mγ/ml in a woman. No obvious reason for these exceedingly high values could be found.

In 4 men and 2 women B_6 levels were measured 2-4 times at weekly intervals. Considerable variations in apparently healthy individuals were observed (Table II). Leukocytes and plasma levels showed, within a relatively short time, variations extending through the whole range in normal individuals.

2. *Mothers at term and cord blood of their babies*: Venous blood was obtained from 19 mothers at time of delivery, and from cord blood of their babies. Leukocyte values in maternal blood varied from 0.02 to 0.19 mγ, average 0.09 mγ, and in cord blood from 0.11 to 1.22 mγ, average 0.47 mγ. Respective plasma values varied from less than 2 to 8.6 mγ/ml, average 4.3 mγ/ml, and in cord blood from 10.8 to 49.2 mγ/ml, average 23.2 mγ. The difference between cord blood and blood of mothers is highly significant ($p<0.01$). Also significantly lower leukocytic and plasma values were noted in women at time of delivery ($p=0.075$) compared to normal women.

3. *Load Tests*: Three groups of individuals were used consisting of 9 normal controls, 5 men and 4 women, 7 women in third trimester of pregnancy, and 9 women 24 hours after delivery. Following withdrawal of a venous blood specimen in the morning, the experimental subjects were given an oral dose of 100 mg of pyridoxine hydrochloride (Merck). Blood samples were withdrawn after 1, 3, 5, and 7 hours, and final sample 24 hours after administration of the vitamin (Fig. 1, 2). In all 3 groups, peak values were reached on the average somewhat earlier in leukocytes than in plasma. The greatest rise in both leukocytes and plasma occurred in normal controls, and the lowest in women at term. Women in last trimester of pregnancy showed intermediate values.



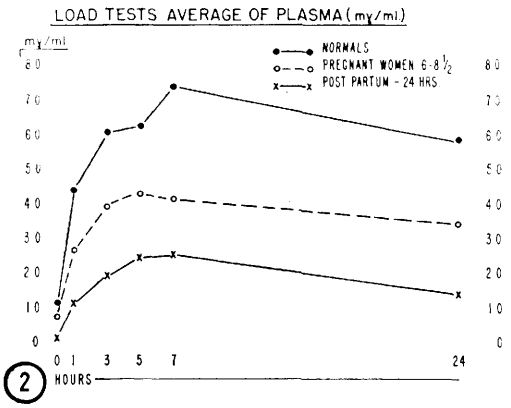


FIG. 1. Avg of pyridoxal phosphate values in leukocytes before and after oral administration of 100 mg pyridoxine hydrochloride in a group of normal controls, women 6 to 8½ mo pregnant and 24 hr post partum.

FIG. 2. Avg of pyridoxal phosphate values in plasma before and after oral administration of 100 mg pyridoxine hydrochloride in a group of normal controls, women 6 to 8½ mo pregnant and 24 hr post partum.

Twenty-four hours after administration of the vitamin, level of leukocytic B_6 in all 3 groups was slightly higher than normal average values. There was no statistically significant difference in amount of Vit. B_6 present in leukocytes of controls and the 2 groups of pregnant women. In contrast, plasma values after 24 hours were very high in control subjects averaging 60 mγ/ml while in women at term they averaged only 15 mγ/ml. In women in third trimester of pregnancy, 24 hour plasma averaged 35.5 mγ/ml. The difference of these values between pregnant women and normal controls, ($p = 0.05$) and between women after delivery and normal controls ($p < 0.01$) is statistically significant. It is interesting to note that preload determinations in women in third trimester of pregnancy revealed normal values for both plasma and leukocytes in 6 out of 7 cases.

Comment. Although average plasma values were somewhat lower in normal women than men, this difference is of questionable significance. Repeated weekly determinations of pyridoxal phosphate in plasma and leukocytes in the same individuals revealed considerable variations in both estimations (Table II). A close parallelism of leukocyte and plasma values was, on the other hand, consistently observed in blood of women in labor and in cord blood of their babies. Both plasma and leukocytes of the cord contained on the average 5 times as much Vit. B_6 as did maternal blood.

Vitamin load tests revealed considerably lower peak values in both plasma and leukocytes, in women at term and to a lesser degree in later pregnancy in comparison to normal controls (Fig. 1, 2). Twenty-four hours after administration of test dose of Vit. B_6, plasma values remained high in normal controls, but were very low in delivering women, indicating that pyridoxine is taken up avidly by the maternal tissues. That the amount of leukocytic vitamin was not significantly different in the 3 groups 24 hours after pyridoxine had

TABLE II. Result of Weekly Determinations of Plasma and Leucocyte Pyridoxal Phosphate Levels in 6 Control Subjects.

		Plasma, mγ/ml					Leukocytes, mγ/million cells				
Subject	Sex	1	2	3	4	Range	1	2	3	4	Range
E.L.	♂	12.8	10.7	10.0	9.4	9.4-12.8	.15	.26	.27	.18	.15-.27
H.M.	♂	15.2	11.4			11.4-15.2	.21	.23			.21-.23
T.V.	♂	11.0	5.2	7.2	8.1	5.2-11.0	.34	.16	.22	.15	.15-.34
A.N.	♂	10.5	8.8	10.7	5.3	5.3-10.7	.17	.33	.18	.24	.17-.33
C.F.	♀	6.5	6.7	5.9		5.9- 6.7	.23	.21	.14		.14-.23
P.T.	♀	7.4	7.6	5.3		5.3- 7.6	.23	.19	.16		.16-.23

been administered suggests that normal tissue level of vitamin had been restored for the time being, even in the most deficient group.

At termination of normal pregnancy, B_6 values are significantly depressed in maternal leukocytes and plasma. In blood of women in last trimester of pregnancy, however, normal average amounts of the vitamin are found although these individuals have a distinct abnormality in tryptophane metabolism which can be readily corrected by administration of pyridoxine(4). This relative pyridoxine deficiency can be demonstrated by the Vit. B_6 load test as described here. From a practical point of view, determination of plasma values 24 hours following administration of Vit. B_6 may be useful in evaluating the B_6 saturation of tissues in clinical subjects.

Summary. A method for determination of pyridoxal phosphate in human plasma is described. The amount of coenzymatically active form of Vit. B_6 was determined simultaneously in blood of normal subjects, delivering women, and in cord blood of their babies. In general, amounts of pyridoxal phosphate in plasma and leukocytes showed parallel changes. They were low in maternal blood, and high in cord blood. Following oral administration of 100 mg pyridoxine hydrochloride, increases were observed in both plasma and leukocytic B_6 values within a few hours. Considerably higher peaks were seen in normal controls as compared to women in last trimester of pregnancy and at time of delivery. The most significant difference between pregnant and nonpregnant subjects was noted in plasma values 24 hours following administration of vitamin. The possible practical application of these findings for evaluation of a latent B_6 deficiency in clinical subjects is suggested.

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Effects of Epinephrine on Toxicities of Several Local Anesthetic Agents. (25516)

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Apparently the opinion is held generally that inclusion of a vasoconstrictor, such as epinephrine, in solution with local anesthetic, such as procaine, affords considerable protection against danger of systemic intoxication by the anesthetic when such solution is injected into soft tissues. This protective action is said to result from slower, more gradual absorption of the anesthetic because of localized vasoconstriction, so that mechanisms for detoxication and elimination of the anesthetic are more nearly able to keep pace with absorption. This, then, prevents development of excessively high blood stream concentration of the toxic agent. Goodman and Gilman(1) state that this is true, although no reference is made to experimental verification. Campbell and Adriani(2) state that systemic toxicity of procaine after infiltration is reduced 30% or more by addition of 1:100,000 epinephrine to the solution, although, no source is given for the information. Sollmann(3) holds a similar view supported by reference to observations by Hatcher and Eggleston(4), Eggleston and Hatcher(5), Zaus and Moody(6), and Beutner, Prusmack, and Miller(7). The conclusions of Hatcher and Eggleston and by Eggleston and Hatcher were based on too few observations, without adequate controls. Since only 2 animals were used, both surviv-