Pathol., 1961, v39, 643.

2. Vernier, R. L., Worthen, H. G., Good, R. A., J. Pediat., 1961, v58, 620.

3. Heymann, W., Lund, H. Z., Pediatrics, 1951, v7, 691.

4. Kalant, N., Das Gupta, D., Despointes, R., Giroud, C. J. P., Am. J. Physiol., 1962, v202, 91.

5. Heymann, W., J. Pediat., 1961, v58, 609.

6. ———, in: Methods in Medical Research, A. C. Corcoran, Ed., Year Book Publishers Inc., Chicago, 1952, p264.

7. Lannigan, R., Brit. J. Exp. Path., 1963, v44, 326.

8. Kingsbury, F. B., Clark, C. P., Williams, G., Post, A. L., J. Lab. Clin. Med., 1926, v11, 981.

9. Varley, H., Practical Clinical Biochemistry, Interscience Publishers Inc., New York, 1954, p463.

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Assessment of Vitamin B_6 Nutriture in Pregnancy in the Rat.* (30627)

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Assessment of vitamin B_6 nutriture in pregnancy in the rat. Erythrocyte transaminase activities, both glutamic-oxalacetic (GOT) and glutamic-pyruvic (GPT), have been shown to be suitable parameters for assessment of vit B_6 nutriture in the male rat(1-3). Long-term studies(3) of the vit B_6 requirement of male rats suggested that the apparent requirement as judged by these criteria was slightly higher than that based on the criterion of maximal body weight gain, the most widely accepted criterion of nutriture in animal studies. Since body weight gain cannot be used as a criterion of vit B_6 adequacy in pregnancy and since the interpretation of xanthurenic acid (XA) excretion following a tryptophan load has been questioned(4), it seemed desirable to investigate the application of the erythrocyte transaminase activities to the assessment of vit B_6 nutriture during pregnancy. Accordingly, the response of erythrocyte transaminase activity to varying doses of vit B_6 was determined in pregnant rats and compared with the response of the tryptophan load test and the response of the hepatic transaminases. Hepatic GPT activity has been shown to be sensitive to vit B_6 nutriture(5) but to be decreased by the state of pregnancy(6).

Materials and methods. In Experiment 1, 23 pregnant and 21 nonpregnant female Wistar rats (average weight, 189 g) were divided into 3 groups. These received 16, 80, and 400 μg pyridoxine · HCl in daily oral doses from day 0 to day 18 of pregnancy. These doses were selected to approximate (i) the suggested National Research Council recommendation(7), (ii) the level considered adequate on the basis of earlier studies in the male(3), and (iii) a dose well in excess of both. A 20% casein, 10% corn oil diet lacking in vit B_6† was fed *ad libitum*. The animals were killed on day 19 of pregnancy by which time no deliveries had occurred.

In Experiment 2, 48 pregnant and 43 control rats (average weight 187 g) were divided into 5 groups and given daily oral doses of 15, 30, 60, 90, and 120 μg pyridoxine · HCl. Feeding began on day 2 of pregnancy and continued to day 20, a total of 19 days. The tryptophan load test was carried out on days 18 and 19 in the pregnant rats and days 17 and 20 in the non-pregnant animals. The rats were given, by stomach tube, a solution of L-tryptophan which provided 0.5 mg trypto-

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† Diet composition per 100 g: vitamin-free casein, 20 g; sucrose, 64.5 g; corn oil, 10 g; salts mixture,‡ 3.5 g; Alphacel, 2.0 g; thiamine • HCl, 0.5 mg; riboflavin, 1.0 mg; nicotinamide, 5.0 mg; calcium pantothenate, 2.0 mg; *p*-aminobenzoic acid, 2.0 mg; folic acid, 0.1 mg; biotin, 0.1 mg; vitamin B_{12}, 2 μg; choline, 200 mg; inositol, 200 mg; menadione, 0.1 mg; α-tocopherol, 6.0 mg; vit. A, 3000 IU; vit. D, 750 IU.

‡ Briggs, G. M., Williams, M. A., Fed. Proc., 1963, v22, 261; copper salt omitted.

TABLE I. Body Weight Gain and Obstetrical Performance of Exp 1 and 2.

Exp	Day of gestation	Dosage pyr. HCl, μg	Non-preg. group size	Non-preg. wt gain, g	Preg. group size	Preg. wt gain, g	Litter wt, g	Litter size No.	Avg fetal wt, g	Resorptions, No./litter
1	19	16	9	22.2 ± 2.75	8	84.1 ± 2.33	24.5 ± 1.85	11.4 ± .73	2.16 ± .07	.75 ± .41
		80	6	28.7 ± 4.11	8	95.6 ± 5.86	26.7 ± 1.19	12.2 ± .65	2.20 ± .08	.11 ± .12
		400	6	27.2 ± 3.06	7	96.0 ± 5.47	26.5 ± 1.11	11.4 ± .44	2.04 ± .05	.75 ± .34
2	21	15	8	33.9 ± 2.52	11	118.4 ± 4.74	48.3 ± 4.50	10.5 ± .79	4.61 ± .36	.64 ± .20
		30	10	44.1 ± 3.25	8	125.7 ± 5.02	44.1 ± 4.54	10.2 ± .44	4.29 ± .37	1.50 ± .92
		60	7	44.1 ± 5.03	11	137.1 ± 7.55	52.3 ± 3.13	11.6 ± .41	4.48 ± .21	.27 ± .14
		90	8	44.1 ± 3.85	10	138.7 ± 6.21	45.5 ± 4.85	10.3 ± 1.09	4.43 ± .21	2.20 ± 1.24
		120	10	41.6 ± 4.47	8	133.0 ± 4.08	53.3 ± 2.04	10.9 ± .77	5.01 ± .25	.12 ± .12

phan per gram initial body weight. The initial weight was chosen to overcome the disparity in weight gain between the pregnant and nonpregnant groups. Thus, the pregnant rats were given an average of 94 mg tryptophan, the controls, 96 mg. Xanthurenic acid was determined in 24-hour urine collections by the method of Wachstein and Gudaitis(8). The experiment was terminated on day 21 of pregnancy by which time 3 animals had delivered.

Transaminase activity of both liver and erythrocytes was determined as described by Heddle *et al*(9). Hemolysates for determination of erythrocyte enzyme activity were prepared by diluting one volume of saline-washed red cells with 2 volumes of deionized water. A 1% homogenate in phosphate buffer was prepared for measurement of liver enzyme activity.

Results and discussion. The body weight gain and obstetrical performance data in Exp. 1 and 2 are presented in Table I. Pregnant and nonpregnant groups receiving the lowest vit B_6 doses employed in either experiment (16 and 15 μg per day) gained less weight than comparable groups receiving higher doses. In Exp. 2, this decreased weight gain was significant in the pregnant group when compared with the groups receiving 60, 90, and 120 μg pyridoxine · HCl ($P<.05$, .01, and .001, respectively). Since there were no differences in the average litter weights, the decreased weight gain occurred at the expense of maternal tissues. The much larger weight gain of the pregnant animals in Exp. 2 is attributable to the longer gestation period—21 days as opposed to 19 days in Exp. 1. In neither experiment was the vit B_6 dose found to have a significant effect on total litter weight, litter size, fetal weight or number of resorptions.

The results of the transaminase determinations are presented in Table II and Fig. 1 and 2. In agreement with the earlier observations of Beaton *et al*(6), liver GPT activity was significantly decreased by pregnancy at all levels of vit B_6 ($P<.001$). Hepatic GOT activity of the pregnant groups was significantly higher than that of the control groups ($P<.01$) except at the 400 μg dose level. In the pregnant rats, neither GPT nor GOT activity responded to the 25-fold increase in vit B_6 dosage. In the nonpregnant rats, GPT activity did increase slightly.

Pregnancy appeared to cause an elevation in erythrocyte GOT activity at all levels of vit B_6 dosage except at the 16 μg level in Exp. 1. In Exp. 2, this increase was significant at all levels of vit B_6 ($P<.02$-$.001$).

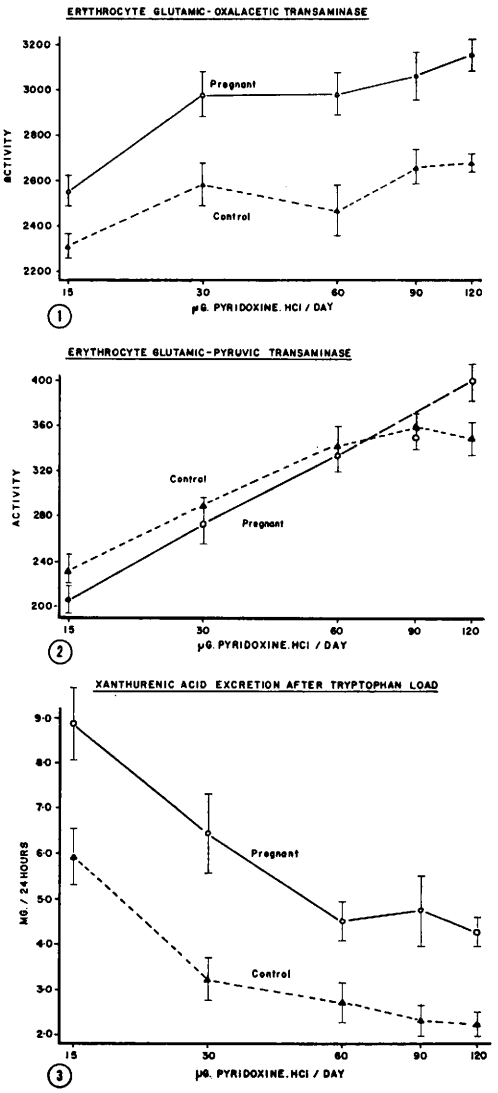

FIG. 1. Response of erythrocyte GOT activity to varying dosages of vit B_6. Vitamin dosage is plotted on a logarithmic scale. Enzyme activity is expressed as μg pyruvate produced per ml hemolysate per hour. Vertical lines represent the standard errors of the means.

FIG. 2. Response of erythrocyte GPT activity to varying dosages of vit B_6. Vitamin dosage is plotted on a logarithmic scale. Enzyme activity is expressed as μg pyruvate produced per ml hemolysate per hour. Vertical lines represent standard errors of the means. The solid line of the response curve of the pregnant rats is broken between 60 and 120 μg to indicate that the value at 90 μg is off the curve.

FIG. 3. Response of XA excretion following a tryptophan load (0.5 mg L-tryptophan per g body weight) to varying dosages of vit B_6. Vitamin dosage is plotted on a logarithmic scale. Excretion is expressed as mg XA excreted in 24 hours. Vertical lines represent standard errors of the means.

In Exp. 1, (Table II) the erythrocyte GOT activity of both pregnant and control groups at the 16 μg dose level was significantly lower than that at the 80 and 400 μg dose levels ($P<.05$-$.001$). In Exp. 2, (Fig. 1) the GOT activity in the pregnant group receiving the lowest vit B_6 dose (15 μg) was significantly

TABLE II. Hepatic and Erythrocyte Transaminase Activity in Exp 1.

Dosage pyr. HCl, μg	Hepatic GOT*		Hepatic GPT*		Erythrocyte GOT†		Erythrocyte GPT†	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant
16	528 ± 9.00	498 ± 5.15	35.5 ± 4.05	100 ± 11.2	2720 ± 128	2630 ± 39.3	175 ± 21.6	202 ± 11.8
80	545 ± 7.85	485 ± 3.38	41.1 ± 3.56	114 ± 8.11	3510 ± 73.4	3040 ± 114	300 ± 18.7	340 ± 18.7
400	550 ± 10.0	505 ± 13.9	36.9 ± 3.84	131 ± 16.6	3340 ± 104	2790 ± 180	384 ± 20.8	325 ± 29.2

* Activity expressed as mg pyruvate produced per g wet tissue per hr.
† Activity expressed as μg pyruvate produced per ml hemolysate per hr.

lower than all other dose levels ($P<.01$); in the nonpregnant group, the activity at the 15 μg dose level was significantly lower than that at the 90 and 120 μg dose levels only ($P<.01$). Erythrocyte GOT activity did not respond to increases in the vit B_6 dose above 30 μg pyridoxine · HCl per day. The experimental periods of 19 and 21 days employed in these experiments may have been too brief to induce the response seen in earlier more prolonged studies(2,3).

Erythrocyte GPT activity was more sensitive to the vit B_6 dose than GOT activity, as shown in Table II and Fig. 2. In each experiment, erythrocyte GPT activity in the pregnant rats increased significantly with each increment in vit B_6 dosage up to the highest dose employed (120 and 400 μg). Examination of the response (Fig. 2) shows it to be essentially linear. GPT activity of the control groups did not increase past 80 μg in Exp. 1 and 60 μg in Exp. 2. Thus, while GPT activity did not appear to level off in the pregnant rats at any of the doses used in this study, it reached a plateau between 60 and 80 μg pyridoxine · HCl per day in the nonpregnant animals. Pregnancy appeared to have a minimal effect on erythrocyte GPT activity; this was seen only at the higher dose levels of vit B_6. There were no significant differences between pregnant and control activity in either experiment except at the 120 μg dose level in Exp. 2 ($P<.05$).

The results of the tryptophan load test are shown in Fig. 3. Since the time of administration of the tryptophan did not affect the response, the results for the 2 days were combined for each group. Pregnancy resulted in a significantly higher XA excretion at all dose levels of vit B_6. The increased XA excretion may be due to changes in the enzymes involved in tryptophan metabolism because of shifts in hormonal balance in the course of pregnancy(10). However, the possibility must not be overlooked that other compounds present in the urine of the pregnant rats may be contributing to the apparent increase in XA excretion since the colorimetric assay employed in this study has been criticized as being nonspecific(11). XA excretion was responsive to vit B_6 at the lower doses only. Minimal excretion levels were achieved at about 30 μg in the nonpregnant control groups and 60 μg in the pregnant groups. Further increases in vit B_6 dose caused no further reduction in XA excretion. The decrease in excretion between the 15 and 30 μg doses was significant only in the controls ($P<.01$).

In choosing a parameter for the assessment of vit B_6 nutriture in pregnant rats, the most valuable would be one that is minimally affected by pregnancy *per se* and yet reflects the vit B_6 nutriture of the animal. The data obtained from these studies suggest that erythrocyte GPT activity is well suited to meet these qualifications. The state of pregnancy was found to have a marked effect on all of the parameters of vit B_6 nutriture measured except erythrocyte GPT. Furthermore, in the short experimental periods necessary for this type of study, erythrocyte GPT was found to be the most sensitive to the wide range of vit B_6 doses employed.

Studies of transaminase activity in the pregnant human have given rather unsatisfactory and inconclusive results. Borglin and Falk(12) determined plasma GOT and GPT in pregnant and nonpregnant women and could demonstrate no differences although the pregnant women showed abnormal tryptophan load tests. Cheney and Beaton(13) compared the response of the plasma and erythrocyte transaminase enzymes to vit B_6 depletion and repletion in nonpregnant rats. These studies indicated that the plasma enzymes were very variable and inconsistent in their response to vit B_6; the erythrocyte enzymes were consistent in their reflection of the vit B_6 nutriture of the animal. Glendening *et al* (14) measured GOT activity in whole blood of pregnant and nonpregnant women and could detect no differences between the pregnant and nonpregnant subjects. The present studies show that GOT activity actually increases in pregnancy in the rat.

The data obtained in this study provide evidence for an increased requirement of vit B_6 in pregnancy. A higher requirement is suggested by the continued response of erythrocyte GPT activity in the pregnant rat past the point where activity ceased to increase in the controls. An increased requirement is also suggested by the larger dose of vit B_6

required to lower XA excretion to minimal levels in the pregnant rat. The absence of any effect of vit B_6 dose, in the range employed, on the obstetrical performance of the various groups is not surprising since the leukocyte and plasma levels of pyridoxal phosphate in the cord blood of infants indicate that the fetus is supplied with vit B_6 at the expense of the maternal stores(10).

In order to define the vit B_6 requirement of the pregnant rat by means of erythrocyte GPT activity, it would be essential to determine the dose required to produce a plateau in the enzyme response(3). This would involve the administration of closely spaced doses of vit B_6 to establish, within a limited range, the point at which GPT ceased to respond. This approach is necessary since the absolute enzyme activities of the pregnant and nonpregnant rats may be different when their respective vit B_6 requirements have been met. Obviously, the present data are inadequate to define the vit B_6 requirement of the pregnant rat.

Summary. The response of several parameters of vit B_6 nutriture (erythrocyte GPT and GOT, hepatic GPT and GOT, and xanthurenic acid excretion following a tryptophan load) to dosages of vit B_6 ranging from 15 to 400 μg pyridoxine · HCl daily has been determined in pregnant and nonpregnant rats. All parameters except erythrocyte GPT appeared to be markedly affected by the state of pregnancy *per se*. Erythrocyte GPT was the most sensitive to the wide range of vit B_6 doses employed. Erythrocyte GPT appears to be well suited for use as a parameter for the study of vit B_6 requirements in pregnancy.

1. Cheney, M. C., Curry, D. M., Beaton, G. H., Can. J. Physiol. Pharmacol., 1965, v43, 579.
2. Cheney, M. C., Beaton, G. H., Can. J. Physiol. Pharmacol., 1965, v43, 591.
3. Beaton, G. H., Cheney, M. C., J. Nutrition, 1965, v87, 125.
4. Coursin, D. B., Am. J. Clin. Nutrition, 1964, v14, 56.
5. Caldwell, E. F., McHenry, E. W., Arch. Biochem. Biophys., 1953, v45, 97.
6. Beaton, G. H., Beare, J., Ryu, M. H., McHenry, E. W., J. Nutrition, 1954, v54, 291.
7. Committee on Animal Nutrition, Nutrient requirements of laboratory animals. Nat. Acad. of Sciences-Nat. Research Council Publication 990, Washington, D. C., 1962.
8. Wachstein, M., Gudaitis, A., Am. J. Clin. Path., 1952, v22, 652.
9. Heddle, J. G., McHenry, E. W., Beaton, G. H., Can. J. Biochem. Physiol., 1963, v41, 1215.
10. Wachstein, M., Vitamins & Hormones, 1964, v22, 705.
11. Satoh, K., Price, J. M., J. Biol. Chem., 1958, v230, 481.
12. Borglin, N. E., Falk, V., Acta Obst. Gynec. Scand., 1959, v38, 190.
13. Cheney, M. C., Beaton, G. H., Fed. Proc., 1964, v23, 875.
14. Glendening, M. B., Cohen, A. M., Page, E. W., Proc. Soc. Exp. Biol. and Med., 1955, v90, 25.

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Effect of Moderate Ingestion of Alcohol upon Serum Triglyceride Responses of Normo- and Hyperlipemic Subjects.* (30628)

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Several years ago we observed(1) that subjects having a behavior pattern (Type A) associated with increased incidence of clinical coronary heart disease(2,3) as a group clear exogenously-derived fat from their blood more slowly than a group of subjects having the converse, or Type B, behavior pattern. Having made this observation, we are pursuing studies concerning the mechanism responsible for this derangement. This cause could not reside in differences in dietary intake since the diets of the group were closely similar, both quantitatively and qualitatively, except

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