Vitamin B-6 nutriture and plasma diamine oxidase activity in pregnant Hispanic teenagers[1-3]

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ABSTRACT Vitamin B-6 status was assessed by measuring erythrocyte glutamic-pyruvic transaminase (EGPT) indices in 122 pregnant Hispanic teenagers. Seventeen percent were vitamin B-6 deficient (EGPT indices > 1.25) at the initial interview (first or second trimester). A daily supplement of 5 mg vitamin B-6, beginning at initial interview, did not reduce prevalence of vitamin B-6 deficiency at final interview (third trimester). No association was found between EGPT indices > 1.25 and the outcome of pregnancy. The activity of diamine oxidase (DAO), a vitamin B-6–dependent enzyme produced by the placental decidua, was measured in maternal plasma. At initial and final interviews, plasma-DAO activity was increased by in vitro addition of pyridoxal-5'-phosphate. The activity in early pregnancy was positively associated with dietary vitamin B-6 intake and was lower in teenagers with EGPT indices > 1.25 at the final interview. Findings suggest that plasma-DAO activity is influenced by vitamin B-6 status. *Am J Clin Nutr* 1986;44:907–13.

KEY WORDS Vitamin B-6 status in pregnancy, vitamin B-6 supplementation in pregnancy, vitamin B-6 and pregnancy outcome, plasma diamine oxidase activity and vitamin B-6 status

Introduction

Biochemical vitamin B-6 deficiency occurs frequently during pregnancy as measured by abnormal tryptophan-load tests (1, 2), low pyridoxal-5'-phosphate (PLP) levels in maternal plasma (3–5), and elevated indices of glutamic-oxalacetic transaminase (EGOT) (6) and glutamic-pyruvic transaminase (EGPT) (7) in erythrocytes. Vitamin B-6 supplementation in amounts two to four times the Recommended Dietary Allowance (RDA) (8) of 2.6 mg/day for pregnant women has been reported as necessary to achieve normal nonpregnant values for the tryptophan-load test and PLP titers (9).

Maternal biochemical vitamin B-6 deficiency may result in a reduced activity or decreased saturation of vitamin B-6–dependent enzymes in the placenta. Vitamin B-6 is a cofactor for diamine oxidase (DAO), an enzyme that is produced by placental decidua (10, 11). During pregnancy, DAO is found in maternal plasma, where its activity increases with gestational age during the first half of pregnancy and then reaches a plateau (12). Low DAO activity in plasma has been associated with pregnancy wastage, such as spontaneous abortion (13, 14) and stillbirth (12). Ramsay et al (15) observed a trend toward lower DAO activity in placental tissue of vitamin B-6–deficient Kenyan women.

We investigated vitamin B-6 status (as indicated by the EGPT index) and plasma-DAO activity in 122 pregnant teenagers of Hispanic descent in Los Angeles, CA. Because vitamin B-6 is a cofactor for DAO, the effect of the in vitro addition of PLP on plasma-DAO activity was also determined.

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Methods

Subjects

Determinations of vitamin B-6 nutriture were conducted in a subsample of 122 pregnant Hispanic teenagers from a population of 138 subjects (mean age, 15.9 ± 0.8 yr) in Los Angeles who were studied to determine the effect of zinc supplementation on zinc status and outcome of pregnancy. The teenagers received care from a Maternity and Infant Care (MIC) Project that provided services not ordinarily available in public health prenatal clinics. For example, the staff of the MIC Project includes specialists, such as nutritionists and health educators; appointments are scheduled within 10 days; no charge is made for medical care; and access to the Supplemental Food Program for Women, Infants, and Children (WIC) is facilitated. These procedures may contribute to the high attendance rate at MIC Projects.

Details of the zinc-supplementation study are reported elsewhere (16). In summary, the teenagers were randomly divided into control and zinc-supplemented groups. At the initial interview, both groups were issued a daily vitamin and mineral supplement that contained 5 mg of vitamin B-6 as pyridoxine HCl. Because there was no difference in vitamin B-6 status between the two groups, the total number of teenagers were grouped together in the present study.

Teenagers who were in the study long enough to take the supplements for more than 60 days and who obtained one or more refills of 60 capsules from the clinic pharmacy were classified as compliers. Of the teenagers who returned to the clinic during the third trimester, 93% were compliers (n = 82 of 89). The study protocol was approved by the Human Subjects Protection Committee, University of California, Los Angeles, and by the Research Committee of the University of Southern California School of Medicine.

Outcome of pregnancy—follow-up data

Follow-up data—including placental and infant weights, Apgar scores, and outcomes of pregnancy—were obtained from the hospital records of the 89 women who had returned for the final interview. Criteria for diagnosing suboptimal outcomes including premature rupture of membranes, pregnancy-induced hypertension, and prematurity were described in a previous paper (16).

Laboratory assays

All measurements were made twice during pregnancy, once at the initial interview (at a mean of the 17th wk of pregnancy) and again at the final interview (at a mean of the 36th wk of pregnancy). A 10-mL blood sample from the antecubital vein was collected into a heparinized tube and centrifuged at 1500 × g for 10 min. The plasma was removed and frozen at −20°C in foil-wrapped tubes. The packed erythrocytes were diluted with an equal volume of double-distilled water and frozen at −20°C in foil-wrapped polyethylene bottles. All samples were processed and frozen within 2 h after collection. Sample preparation and enzyme analyses were done under subdued light.

The diluted erythrocytes were hemolyzed by freezing and thawing three times. Enzyme-saturation tests for EGPT were conducted using the procedure of Tonhazy et al (17) as modified by Cinnamon and Beaton (18). An enzyme-saturation index was determined by calculating the ratio of PLP-stimulated enzyme activity to unstimulated activity. An EGPT index > 1.25 indicated a vitamin B-6 deficiency (19).

Plasma-DAO activity was determined by the method of McEwen (20). The effect of in vitro addition of PLP on DAO activity was measured by comparing values obtained from assays with and without the addition of 0.05 mL of 0.5 mg PLP/mL solution to the reaction mixture prior to incubation. We calculated an enzyme-saturation index for DAO, using the same procedure as described for EGPT.

Statistical analyses

We used t tests to compare group means and analysis of covariance to control for the effect of gestational age (21). The Pearson chi-square test was used to analyze categorical data and, in cases of small sample size, Fisher's exact test was employed (21). McNemar's test (22) was used to test for the significance of changes in the number of teenagers with biochemical vitamin B-6 deficiencies at the final as compared to the initial interview. In all statistical tests, values of $p < 0.05$ were reported. All levels of significance were reported without adjusting for multiple tests.

Results

Vitamin B-6 status

The mean EGPT indices for all teenagers in the study and for those who returned for a final interview during the third trimester are given in **Table 1.** There was no significant difference in the mean EGPT index at the initial as compared to the final interview (paired t test). At the initial interview, 17% of the subjects were classified as vitamin B-6 deficient (EGPT index > 1.25); at the final interview, 27% were deficient. This increase was only of borderline significance ($p < 0.08$).

Plasma-DAO activity

Mean plasma-DAO activities at initial and final interviews are shown for the compliers, grouped according to their vitamin B-6 status at the final interview (**Table 2**). When PLP was added in vitro, the mean plasma-DAO activity was increased ($p < 0.02$). The increases occurred in both the B-6–adequate and B-6–deficient teenagers at the initial and also at the final interview.

Three factors were considered in comparing plasma-DAO activity in the teenagers: *1*) vitamin B-6 status as determined by the EGPT index, *2*) the in vitro addition of the cofactor, PLP, and *3*) the stage of pregnancy (initial or

TABLE 1
Biochemical vitamin B-6 status in pregnant Hispanic teenagers at initial and final interviews

	EGPT index		Vitamin B-6 deficient
	n	$\bar{x} \pm SD$	
			%*
Initial interview			
All teenagers	122	1.14 ± 0.17	17
Teenagers who returned for a final interview	89	1.13 ± 0.17	17
Final interview	89	1.17 ± 0.18	27

* EGPT index > 1.25.

final interview) (Table 2). Mean plasma-DAO activity without added PLP was significantly lower ($p < 0.04$) at the initial interview in the B-6–deficient as compared to the B-6–adequate teenagers when the linear effect of gestational age was controlled. In addition, when PLP was added, plasma-DAO activity tended to be lower ($p < 0.06$) in the vitamin B-6–deficient than in the vitamin B-6–adequate teenagers. During pregnancy (final minus initial values), mean DAO activity increased significantly ($p < 0.05$) only in the vitamin B-6–deficient teenagers. Thus, at the final interview, mean plasma-DAO activities were not significantly different in the vitamin B-6–deficient as compared to the vitamin B-6–adequate teenagers.

The DAO index was calculated from the ratio of DAO activity stimulated with added PLP to DAO activity without added PLP. The DAO index was not significantly different in B-6–deficient as compared to the B-6–adequate teenagers at either the initial or the final interviews (Table 2).

Vitamin B-6 status and the outcome of pregnancy

Vitamin B-6 status of the teenagers at the final interview was not found to affect the mean placental weight, birth weight, or Apgar scores of their infants (**Table 3**).

Table 4 shows the mean plasma-DAO activity and vitamin B-6 status at the final interview in those teenagers with satisfactory or suboptimal outcomes of pregnancy. No significant differences in plasma-DAO activity or biochemical vitamin B-6 status were found in teenagers with satisfactory as compared to suboptimal outcomes of pregnancy. All 15 teenagers with suboptimal outcomes who gave a blood sample at the final interview were compliers, except one who experienced premature rupture of membranes.

Discussion

Vitamin B-6 status

The prevalence of vitamin B-6 deficiency as measured by the EGPT index (17) in the teenagers at the initial interview was lower (17%) than that reported for low-income pregnant

TABLE 2
Plasma-DAO activity (− and + PLP added in vitro) in compliers grouped by biochemical vitamin B-6 status at final interview

	Vitamin B-6 adequate		Vitamin B-6 deficient*	
	n	$\bar{x} \pm SD$	*n*	$\bar{x} \pm SD$
Plasma-DAO activity (−PLP)†				
Initial interview	55	84.8 ± 55.8	19	57.2 ± 36.4‡
Final interview	61	93.9 ± 51.2	21	101.9 ± 47.1§
Plasma-DAO activity (+PLP)†				
Initial interview	55	96.4 ± 61.2‖	18	69.6 ± 34.5‖
Final interview	61	116.5 ± 67.1‖	21	131.6 ± 59.4§‖
DAO index¶				
Initial interview	55	1.17 ± 0.25	18	1.22 ± 0.22
Final interview	61	1.29 ± 0.34	21	1.31 ± 0.31

* EGPT index > 1.25.
† nmoles 2,3-trimethylene--1,2 dihydroquinazolium hydroxide formed/0.3 mL/6 h.
‡ Significantly lower than B-6–adequate group when the linear effect of gestational age was controlled ($p < 0.04$, ANCOVA).
§ Significant increase at the final interview in B-6–deficient teenagers ($p < 0.05$, paired *t* test).
‖ Significantly higher than DAO activity − PLP ($p < 0.02$, paired *t* test).
¶ DAO index = (DAO activity + PLP)/(DAO activity − PLP).

TABLE 3
Selected measures of pregnancy outcome according to maternal vitamin B-6 status at the final interview

	Vitamin B-6 adequate		Vitamin B-6 deficient*	
	n	$\bar{x} \pm SD$	*n*	$\bar{x} \pm SD$
Placental weight (g)	57	641 ± 132	20	615 ± 134
Infant birth weight (g)	62	3435 ± 436	21	3357 ± 389
Apgar scores				
1-min after birth	62	8.1 ± 1.3	21	8.0 ± 1.8
5-min after birth	61	9.0 ± 0.4	21	8.7 ± 1.6

* EGPT index > 1.25.

women in Florida (68%) (7) and for pregnant women in Central Europe (50%) (6) as measured by the EGOT index. In the teenagers, the mean reported dietary vitamin B-6 intake was 1.5 ± 0.8 mg, or 58% of the RDA (16). This value is in the upper range of intakes (38–58% of RDA) reported by other investigators (7, 23, 24).

At the initial interview, no association was found between elevated EGPT indices and low dietary-vitamin B-6 intakes (data not presented). Supplementation with 5 mg of pyridoxine HCl/day beginning at about the 17th wk of pregnancy was not sufficient to reduce the prevalence of biochemical vitamin B-6 deficiencies at about the 36th wk as compared to the 17th wk in either the total group (Table 1) or in the compliers (data not presented).

Other investigators (4) assessed vitamin B-6 status by measuring PLP levels in maternal plasma and found that 4–10 mg of vitamin B-6/day was required to maintain plasma-PLP levels during pregnancy. Schuster et al (25) reported similar results. The relatively larger intakes of vitamin B-6 necessary to affect the biochemical indices of vitamin B-6 status in pregnancy have led some investigators (26) to question whether these indices represent a developing deficiency or a normal physiologic response to pregnancy.

TABLE 4
Plasma-DAO activity and biochemical vitamin B-6 status at the final interview in teenagers with satisfactory or suboptimal outcome of pregnancy

	Plasma DAO (−PLP)*		Vitamin B-6 status
	n	(*n* = 89) $\bar{x} \pm SD$	(*n* = 89) *n* deficient†/*n* cases (%)
Satisfactory outcome	74	95.1 ± 50.7	21/74 (28)
Suboptimal outcome			
Fetal death‡	1	73.9	0/1 (0)
PROM§	2	96.1 ± 30.4	1/2 (50)
PIH‖	9	99.7 ± 47.4	1/9 (11)
Prematurity¶	2	106.1 ± 34.1	1/2 (50)
Congenital anomaly**	1	168.9	0/1 (0)
Total suboptimal outcomes	15	103.0 ± 37.9	3/15 (20)

* nmoles 2,3-trimethylene--1,2 dihydroquinazolium hydroxide formed/0.3 mL/6 h.

† EGPT index > 1.25.

‡ At 38th wk of gestation.

§ Premature rupture of membranes.

‖ Pregnancy-induced hypertension.

¶ There were five cases of prematurity. Blood samples were not collected from three mothers, one whose infant died 3 h after birth.

** Infant born with congenital translocation of the great vessels of the heart.

Plasma-DAO activity and vitamin B-6 status

DAO found in maternal plasma arises from an uncharacterized spillage from cells of the placental decidua where DAO is synthesized (11). It has been reported (12) that plasma-DAO activity increases markedly during the first trimester of pregnancy and reaches a plateau at about the 20th wk. That report was confirmed in the present study by the observation that plasma-DAO activity was positively correlated with gestational age at the initial interview ($r = 0.31$, $p = 0.001$, $n = 85$) but not at the final interview (data not presented).

There are several findings in the present study that indicate a relationship of plasma-DAO activity to vitamin B-6 status. At the initial interview, mean plasma-DAO activity without added PLP (the coenzyme form of vitamin B-6) was significantly higher in teenagers who had adequate vitamin B-6 status as compared to those who were vitamin B-6 deficient (Table 2). This observation may indi-

cate that adequate vitamin B-6 status is necessary for plasma-DAO activity to rise maximally during early pregnancy.

From the initial to the final interview, however, mean plasma-DAO activity increased significantly only in the vitamin B-6–deficient group and at the final interview the plasma-DAO activity was no longer lower than in the vitamin B-6–adequate group. This increase in DAO values could have been due to the daily supplement of 5 mg pyridoxine HCl prescribed for all teenagers beginning at about the 17th wk of pregnancy. Without that supplement, DAO activity may not have increased during pregnancy in the vitamin B-6–deficient teenagers. The finding that plasma-DAO activity increased in teenagers who were vitamin B-6–deficient as measured by the EGPT index suggests that placental DAO may have a higher metabolic priority than maternal plasma EGPT for vitamin B-6.

Additional evidence of a relationship between plasma-DAO activity and vitamin B-6 status is the observation that plasma-DAO activity in early pregnancy was affected by the dietary intake of vitamin B-6. The initial mean DAO activity without added PLP was significantly lower ($p < 0.04$) in teenagers with dietary vitamin B-6 intakes $< \frac{2}{3}$ of the RDA as compared to those with intakes $\geq \frac{2}{3}$ of the RDA (69.0 ± 47.3 units and 90.3 ± 57.8 units, respectively). That relationship between initial DAO activity and vitamin B-6 intake was also observed when PLP was added in vitro (75.3 ± 42.0 units and 105.2 ± 54.1 units, respectively, $p < 0.04$).

Another finding linking plasma-DAO activity and vitamin B-6 is the consistent observation that plasma-DAO values were increased by the in vitro addition of PLP (Table 2). The DAO index, however, was not significantly different in the vitamin B-6–adequate as compared to vitamin B-6–deficient teenagers. A possible explanation for this observation is that the DAO apoenzyme may tend to be unstable if it is not saturated with PLP. The stability of some vitamin B-6–containing enzymes has been shown to be dependent on PLP availability (27).

Progress and outcome of pregnancy

An early investigation (28) of vitamin B-6 nutriture during pregnancy suggested that the nausea of pregnancy was associated with a vitamin B-6 deficiency. Because various factors (such as physiological changes and anxieties related to childbirth) may cause nausea during early pregnancy, it is difficult to ascertain the role of vitamin B-6 nutriture in the development of nausea (29). At the initial interview of this study, all teenagers were asked, "Do you often feel nauseated to the point where you eat less than usual?" The prevalence of biochemical vitamin B-6 deficiencies (17%) in teenagers who reported nausea did not differ from the prevalence (18%) in teenagers who did not report nausea. Thus, no evidence was obtained in the present study to relate vitamin B-6 deficiency and prevalence of nausea in pregnancy. These observations confirm those of Schuster et al (30) who reported no relationship between vitamin B-6 status and the incidence and degree of morning sickness during the first trimester of pregnancy.

Schuster et al (7) did not find an association between maternal vitamin B-6 status and infant birth weight. Similarly, we found no differences in mean infant birth weights or placental weights grouped by biochemical vitamin B-6 status as determined at the initial or final interview (Table 3).

Apgar scores have been associated with maternal biochemical vitamin B-6 status (7, 23). Schuster (7) found that mothers with adequate biochemical vitamin B-6 status had infants with higher Apgar scores than mothers with ficient biochemical vitamin B-6 status. In a study of infants with low Apgar scores (< 7), Roepke and Kirksey (23) found that low maternal dietary-vitamin B-6 intakes were associated with low levels of PLP in maternal plasma at delivery. Schuster et al (30) found that mothers who reported intakes of vitamin B-6 > 7.5 mg/day (or about three times the RDA for pregnancy) delivered infants with significantly higher Apgar scores. In the present study, neither the mean Apgar score nor the number of low scores (< 7) at 1- and 5-min postbirth was found to be related to biochemical vitamin B-6 status at either the initial or final interview. All infants had 5-min Apgar scores ranging from 8 to 10, except for one infant with a score of 2.

Mean plasma-DAO activity and the prevalence of vitamin B-6 deficiency were not significantly different in the teenagers with sub-

optimal outcomes of pregnancy compared to those with satisfactory outcomes (Table 4). However, there was an unusually low incidence of suboptimal outcomes in this population of teenagers, which may have been due, in part, to the prenatal care received at the MIC Project.

Studies report that low plasma-DAO levels are associated with certain disorders of placental function, such as spontaneous abortion (12, 13) and stillbirth (14). Therefore, it may be important to maintain high levels of DAO activity early in pregnancy. While the role of plasma DAO during pregnancy is not entirely understood, it has been suggested (31–33) that the interaction of polyamines and their products with diamine-degrading enzymes, such as DAO, may prevent the immune rejection of the fetoplacental unit.

A study by Baker et al (34) provided evidence that the vitamin B-6 that is taken up by the placenta is transferred to the fetus only after placental requirements are met. Plasma-DAO activity during the first half of pregnancy may thus indicate whether placental and therefore fetal supplies of vitamin B-6 are adequate. Plasma-DAO activity, however, has a high degree of interindividual variability and further study is needed to ascertain whether measurements of this enzyme would be a useful indicator of vitamin B-6 status during pregnancy.

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References

1. Wachstein M. Evidence for a relative vitamin B_6 deficiency in pregnancy and some disease states. Vitam Hormones 1964;22:705–19.
2. Sprince H, Lowry RS, Folsome CE, Behrman J. Studies on the urinary excretion of xanthurenic acid during normal and abnormal pregnancy: a study of the excretion of xanthurenic acid in normal nonpregnant, normal pregnant, pre-eclamptic, and eclamptic women. Am J Obstet Gynecol 1951;62:84–92.
3. Cleary RE, Lumeng L, Li T-K. Maternal and fetal plasma levels of pyridoxal phosphate at term: adequacy of vitamin B_6 supplementation during pregnancy. Am J Obstet Gynecol 1975;121:25–8.
4. Lumeng L, Cleary RE, Wagner R, Yu P-L, Li T-K. Adequacy of vitamin B-6 supplementation during pregnancy: a prospective study. Am J Clin Nutr 1976;29:1376–83.
5. Hamfelt A, Hahn L. Pyridoxal phosphate concentration in plasma and tryptophan load test during pregnancy. Clin Chim Acta 1969;25:91–6.
6. Heller S, Salkeld RM, Korner WF. Vitamin B-6 status in pregnancy. Am J Clin Nutr 1973;26:1339–48.
7. Schuster K, Bailey LB, Mahan CS. Vitamin B-6 status of low-income adolescent and adult pregnant women and the condition of their infants at birth. Am J Clin Nutr 1981;34:1731–5.
8. National Research Council, Food and Nutrition Board. Recommended dietary allowances. 9th ed. Washington, DC: National Academy of Sciences, 1980.
9. Dempsey WB. Vitamin B_6 and pregnancy. In: Committee on Dietary Allowances, Food and Nutrition Board. Human vitamin B_6 requirements. Washington, DC: National Research Council, National Academy of Sciences, 1978:202–9.
10. Mondavi B, Costa MT, Finazzi-Agro A, Rotilio G. Pyridoxal phosphate as a prosthetic group of pig kidney diamine oxidase. Arch Biochem Biophys 1967;119:373–81.
11. Southren AL, Kobayashi Y, Brenner P, Weingold AB. Diamine oxidase activity in human maternal and fetal plasma and tissues at parturition. J Appl Physiol 1965;20:1048–51.
12. Gahl WA, Raubertas F, Vale AM, Golubjatnikov R. Maternal serum diamine oxidase in fetal death and low birth-weight infants. Br J Obstet Gynecol 1982;89:202–7.
13. Weingold AB, Southren AL, Lee BO. A fetal salvage program: the use of plasma DAO as a fetal monitor. Int J Fertil 1971;16:24–35.
14. Southren AL, Kobayashi Y, Weingold AB, Carmody NC. Serial plasma diamine oxidase (DAO) assays in first- and second-trimester complications of pregnancy. Am J Obstet Gynecol 1968;96:502–10.
15. Ramsay VP, Neumann C, Clark V, Swendseid ME. Vitamin cofactor saturation indices for riboflavin, thiamine, and pyridoxine in placental tissue of Kenyan women. Am J Clin Nutr 1983;37:969–73.
16. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: effects on selected blood constituents and on the progress and outcome of pregnancy. Am J Clin Nutr 1985;42:815–28.
17. Tonhazy NE, White NG, Umbreit WW. A rapid method for the estimation of glutamic-aspartic transaminase in tissues and its application to radiation sickness. Arch Biochem Biophys 1950;28:36–42.
18. Cinnamon AD, Beaton JR. Biochemical assessment of vitamin B-6 status in man. Am J Clin Nutr 1970;23:696–702.
19. Sauberlich HE, Canham JE, Baker EM, Raica N, Herman YF. Biochemical assessment of the nutritional status of vitamin B-6 in the human. Am J Clin Nutr 1972;25:629–42.
20. McEwen CM. Serum amine oxidases in pregnancy. J Lab Clin Med 1964;64:540–7.
21. Dixon WJ, Massey FJ Jr. Introduction to statistical

analysis. New York: McGraw Hill, 1983:123–30, 256–64, 272–8.
22. McNemar Q. Psychological statistics. New York: John Wiley and Sons, 1962:224–7.
23. Roepke JLB, Kirksey A. Vitamin B-6 nutriture during pregnancy. I Vitamin B-6 intake, levels of the vitamin in biological fluids, and condition of the infant at birth. Am J Clin Nutr 1979;32:2249–56.
24. Shane B, Contractor SF. Assessment of vitamin B-6 status. Studies on pregnant women and oral contraceptive users. Am J Clin Nutr 1975;28:739–47.
25. Schuster K, Bailey LB, Mahan CS. Effect of maternal pyridoxine-HCl supplementation on the vitamin B-6 status of mother and infant and on pregnancy outcome. J Nutr 1984;114:977–88.
26. Reynolds RD, Leklem JE. Implications on the role of vitamin B_6 in health and disease—a summary. In: Reynolds RD, Leklem JE, eds. Vitamin B_6: its role in health and disease. New York: Alan R Liss, 1985: 481–9.
27. Kominami E, Kobayashi K, Kominami S, Katunuma N. Properties of a specific protease for pyridoxal enzymes and its biological role. J Biol Chem 1972;247: 6848–55.
28. Willis RS, Winn WW, Morris AT, Newsom AA, Massey WE. Clinical observations in treatment of nausea and vomiting in pregnancy with vitamin B_1 and vitamin B_6. Am J Obstet Gynecol 1942;44:265–71.
29. Williams SR. Nutritional guidance in prenatal care. In: Worthington-Roberts BS, ed. Nutrition in pregnancy and lactation. St Louis, MO: CV Mosby, 1981: 96.
30. Schuster K, Bailey LB, Dimperio D, Mahan CS. Morning sickness and vitamin B_6 status of pregnant women. Hum Nutr:Clin Nutr 1985;39C:75–9.
31. Byrd WJ, Jacobs DM, Amoss MS. Synthetic polyamines added to cultures containing bovine sera reversibly inhibit in vitro parameters of immunity. Nature (London) 1977;267:621–3.
32. Gaugas JM, Cursen P. Polyamine interaction with pregnancy serum in suppression of lymphocyte transformation. Lancet 1978;i:18–20.
33. Morgan DML, Illei G. Polyamine-polyamine oxidase interaction: part of maternal protective mechanism against fetal rejection. Br Med J 1980;280:1295–7.
34. Baker H, Frank O, Deangelis B, Feingold S, Kaminetzsky HA. Role of placenta in maternal-fetal vitamin transfer in humans. Am J Obstet Gynecol 1981;141: 792–6.