AN EVALUATION OF THE FOLATE STATUS OF PREGNANT WOMEN FROM THREE ETHNIC GROUPS

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INTRODUCTION

"Folate deficiency is one of the commonest dietary deficiencies found in clinical practice, ranking with iron deficiency in frequency" (Chanarin, 1979). Worthington-Roberts (1981) states that folate deficiency is the most common hypovitaminosis in humans and is by no means confined to the indigent population of the world. Among Americans, nutritional deficiency of folate appears to be prevalent among those of poor economic status (Herbert et al., 1975); however, if dietary habits are compromised, middle to upper-class women are not exempt from becoming folate deficient (Kitay, 1979). Furthermore, when compared to a deficiency of Vitamin B_{12}, a nutritional deficiency of folate is a far more common cause of nutritional megaloblastic anemia (Herbert, 1970).

Pregnancy remains the most common cause of clinical folate deficiency anemia (Pitkin, 1980). Since pregnancy imposes increased demands for almost all nutrients, the Recommended Daily Allowance for pregnant women has been adjusted to meet these demands. With respect to folate, the RDA is doubled during gestation. Folate demands increase specifically as a result of maternal erythropoiesis and fetal-placental growth (Kitay et al., 1975), but the

synthesis of new tissue represents the most important factor in the increased folate requirement (Hurley, 1980).

The consequences of folate deficiency during and after pregnancy are still controversial. Previous studies have suggested folate deficiency in the mother is associated with a number of obstetric complications, low birth weight and/or fetal malformations (Daniel et al., 1971). More recently, a study by Gross et al. (1974) reported abnormal or delayed post-natal development may occur in children born to mothers with severe folate deficiency during pregnancy. In addition, other evidence suggests that inadequate levels of folate during pregnancy may result in a depresssion of fetal growth persisting into the first year of life (Hurley, 1980).

Research concerning folate deficiency in pregnant women has been extensive outside the United States. Cooper et al. (1970) cite a long list of these studies conducted abroad. In the United States, Herbert et al. (1975) point out that numerous cases of folate-deficient megaloblastic anemia have been reported, but the incidence has been evaluated in only a few studies. Sauberlich (1978) states that megaloblastic anemia resulting from folate deficiency does occur in a small, though significant, number of pregnant women in developed nations. The incidence of this condition may vary from three to twenty-two percent in this country (Hurley, 1980).

Many of the studies conducted in the United States have focused on lower socioeconomic groups. However, the majority of them have involved pregnant women of the Black or Caucasian races and rarely included women of Hispanic descent. Although the Ten-State Nutrition Survey did include Hispanic women, the data were not analyzed in such a way as to separate the population of pregnant women into ethnic groups (Rosenburg et al., 1979). Additionally, there is little research to ascertain whether different ethnic groups are more or less at risk.

In reference to the findings by the Ten-State Nutrition Survey regarding folate status, Herbert et al. (1975) state that "mean values may obscure the existence of a substantial number of actual values which are sufficiently below the mean as to suggest widespread deficiency". In fact, 26.5% of all the women surveyed in the state of Massachusetts, who were included in the Ten-State Nutrition Survey, "had red cell folate levels below the acceptable range and this group included an undetermined number of pregnant women, many already receiving prenatal care" (Rosenburg et al., 1979).

In view of this information, folate deficiency of varying degrees may exist in a significant percentage of women attending prenatal clinics. Therefore, research into the folate status of these three ethnic groups was conducted

to provide information regarding ethnic differences, while adding to the current body of knowledge regarding folate status during pregnancy. Results of this study also include the analysis of several hematological parameters and demographic data. Information regarding trimester and gravidity was also collected to evaluate the influence of these factors on folate status during pregnancy.

STATEMENT OF THE PROBLEM

Pregnant women tend to have lower blood folate levels than nonpregnant women. It has been speculated that a large portion of pregnant women may have a compromised folate status. The purpose of this study was to investigate the following questions: Is there a significant difference in the blood folate status of Caucasian, Black and Hispanic women attending a prenatal clinic? Is there a significant difference between the blood folate values for pregnant women at this clinic and the standard literature values for blood folate during pregnancy?

HISTORICAL PERSPECTIVE

Folic acid ($C_{19}H_{19}N_7O_6$) is one of a group of related compounds that function as coenzymes for several metabolic processes required by all vertebrates. Some of the major functions in humans include nucleic acid synthesis and metabolism, porphyrin synthesis, methylation, and growth (Kutsky, 1981). As a result, all new cell formation is dependent upon a satisfactory supply of folate; requirements are related to the amount of tissue growth occurring at any time (Hibbard, 1975). Hence, there is an increased need for folate in both pathological conditions and during the physiological demands of infancy, adolescence, and pregnancy (Rothman, 1970). Of and by itself, the increase in maternal erythropoiesis of pregnancy requires additional amounts of folate (Pitkin, 1976).

Although folate deficiency in pregnancy can exist alone, it is not uncommon to observe a coexisting iron deficiency (Kitay, 1979). In addition, if iron deficiency is severe enough, it may mask the morphological changes of an underlying megaloblastic anemia (Chanarin et al., 1965 and Rothman, 1970). Megaloblastic changes were found in 26% of pregnant subjects on initial examination by Lowenstein et al. (1966), but additional cases were detected only after iron

therapy. Chanarin et al. (1965) also stated that iron deficiency may not only mask morphological evidence of a megaloblastic anemia, but may also participate in the production of folate deficiency during pregnancy. It has been suggested that this occurs because iron deficiency produces an additional stress on folate metabolism during pregnancy, which may convert a sub-clinical folate deficiency into overt megaloblastic anemia (Chanarin et al., 1965).

In 1842, the first clinical picture of folate deficiency was presented in Boston (Channing, 1842). Occasional reports of a severe, and rapidly progressing, "Addisonian Type" anemia associated with pregnancy and the puerperium continued to appear in the medical literature. Osler, in England, while noting its similarity to Addison's Anemia, commented that permanent recovery could occur (Osler, 1919). Investigations in India by Wills in the early 1930's, where "pernicious anemia of pregnancy" was quite prevalent, led to successful treatment with Marmite, a yeast extract (Roe, 1978). Until this time, it had been believed that the causative agent was of an infectious etiology; however, by 1945 the curative factor was isolated and synthesized. This anemic condition was regarded as somewhat of a rarity until the beginning of the 1950's, when reports appeared giving the incidence of this anemia in a particular pregnant population (Rothman, 1970).

At first, most of the studies were retrospective; however, when an understanding of folate metabolism was established, prospective reports began to appear (Kitay, 1969). Intensive studies of both folate metabolism and its role in reproduction were begun initially in Great Britian and later in the United States (Hibbard et al., 1965). As diagnostic methods evolved and became more widely employed, the reported incidence of folate deficiency anemia in pregnancy rose from 1 in 250 to 1 in 70 confinements (Kitay, 1979). The reported incidence of folate deficiency during pregnancy continues to vary throughout the world, partly because of differences in dietary intake and partly because of the different diagnostic criteria employed (Rothman, 1970; Herbert, 1970; Fletcher et al., 1971; Pitkin, 1976; Bell, 1977). The reported incidence of folate deficiency in pregnancy will vary when even the same investigators use different tests (Rothman, 1970).

The most obvious clinical sign of folate deficiency is macrocytic anemia (Worthington-Roberts, 1981). The term "macrocytic anemia of pregnancy" developed as a result of observing macrocytes in peripheral blood. Yet as Kitay (1979) points out, this is a relatively late finding in folate deficiency. Another abnormal hematological development of folate deficiency in pregnancy is the formation of megaloblasts in the bone marrow (Rothman, 1970), but these

changes are also a late manifestation of folate deficiency (Hibbard et al., 1965). In fact, actual megaloblastic anemia is the final manifestation of total exhaustion of folate stores (Herbert, 1962). Therefore, when detection of megaloblastic anemia occurs in mid or late pregnancy, the existence of defective folate metabolism or inadequate intake earlier in pregnancy is strongly implied (Hibbard, 1975).

Before macrocytic or megaloblastic anemia is evident, several preliminary biological and morphological signs of folate deficiency during pregnancy may occur. One biological parameter that has been used to aid in the diagnosis of folate deficiency in pregnancy is the increased urinary excretion of formimino-glutamic acid (FIGLU). In the non-pregnant state, urinary FIGLU excretion has been shown to increase after twelve weeks of folate depletion (Herbert, 1962). This increase in urinary FIGLU excretion also can occur after twelve weeks gestation (Hibbard, 1964 and Hibbard et al., 1965). In addition, Hibbard et al. (1965) state that some women show a positive FIGLU test even before the trophoblast and fetus are sufficiently developed to utilize large amounts of folic acid. Edelstein et al. (1966) noted similar increases in urinary FIGLU excretion and additionally observed substantial increases during the sixth through twelfth weeks postpartum. It was suggested as a result of these studies that although urinary FIGLU

excretion increased during pregnancy, it could not be used to assess folate status, but rather could only be used to evaluate folate metabolism (Hibbard, 1964; Hibbard et al., 1965 and Edelstein et al., 1966). In addition, Hibbard (1964) found abnormal marrow morphology correlated with increased urinary FIGLU excretion.

However, other investigators did not consistently find increased FIGLU excretion associated with hematological findings during pregnancy and questioned its value as a diagnostic test of folate status. Chanarin et al. (1963) found normal excretion of urinary FIGLU in pregnant patients with megaloblastic anemia. In another group of anemic pregnant patients with megaloblastic marrow change, Chisholm and Sharp (1964) found more than 50% had normal FIGLU excretion. From the sixteenth week of gestation through the puerperium, a decline of urinary FIGLU excretion was observed by Chanarin et al (1965) in subjects who developed megaloblastic anemia. Therefore, Chanarin (1964) stated the estimation of urinary FIGLU excretion is unreliable in the diagnosis of megaloblastic anemia in pregnant or nonpregnant subjects. Kitay (1969) and Rothman (1970) cite several more conflicting studies concerning the reliability of increased urinary FIGLU excretion as a test to define a state of folate deficiency in pregnancy. As a result of these conflicting reports and the lack of consistent correlation with

hematological findings, increased urinary FIGLU excretion appears to be an unreliable indicator of folate deficiency during pregnancy.

The earliest morphological change that occurred in experimental folate deficiency was increased neutrophil nuclear hypersegmentation (Herbert, 1962). This change was observed several weeks before increased urinary FIGLU excretion occurred. According to Rothman (1970), one-half to two-thirds of patients with elevated neutrophil hypersegmentation have megaloblastic marrow changes. Limited studies suggest that a positive correlation exists between neutrophil nuclear hypersegmentation and folate deficiency in pregnancy (Kitay et al., 1969). The presence of an increased proportion of hypersegmented neutrophils in peripheral blood films "was the most valuable single aid in the diagnosis of megaloblastic anemia in pregnancy" (Chanarin et al., 1965). Lowenstein and associates (1966) also found a correlation between increased neutrophil hypersegmentation and megaloblastic marrow change. In 238 pregnant patients studied by Varadi et al. (1966), a 75% correlation between megaloblastic erythropoiesis and neutrophil hypersegmentation was reported. Hibbard and Hibbard (1971) found that 95% of their pregnant patients with megaloblastic erythropoiesis had high neutrophil lobe indices. However, the majority of studies

have failed to show a positive correlation between elevated hypersegmentation and the diagnosis of folate deficiency in pregnancy. Neither Chanarin et al (1965), Lowenstein et al. (1966), Varadi et al. (1966), Hibbard et al. (1971), or Herbert et al. (1975), could confirm the value of increased neutrophil hypersegmentation as a measure of folate deficiency during pregnancy. In fact, Herbert et al. (1975) found a decrease in the number of lobes of granulocyte nuclei. Therefore, the use of neutrophil nuclear hypersegmentation may be of value only in providing some evidence of folate deficiency as the cause of an anemia in pregnancy but not reliable as a screening test to detect patients "at risk".

The first sign of folate deficiency in a nonpregnant individual is a subnormal level of serum folate (Kitay, 1979). This can occur within three weeks on a folate deficient diet (Herbert, 1962). In the pregnant patient, several studies have confirmed that serum folate values fall consistently with advancing gestation, reaching their lowest concentrations at delivery (Chanarin et al., 1965; Edelstein et al., 1966; Temperley et al., 1968; Willoughby et al., 1968; Landon et al., 1971; Hall et al., 1976 and Tso et al., 1980). In addition, other investigations (Chisholm et al., 1964; Giles et al., 1966; Lowenstein et al., 1966 and

Temperley et al., 1968) have reported that there are good correlations between megaloblastic anemia or marrow changes and low serum folate. Chanarin et al. (1965) have suggested that the decline in serum folate during pregnancy may indicate a subclinical folate deficiency. However, Hall et al. (1976) suggest that this fall in serum folate concentration occurs as a result of plasma volume expansion and should not be regarded as pathological. Therefore, the use of serum folate concentration alone to assess the folate status of a pregnant population would be subject to criticism. However, in conjunction with another biochemical test such as erythrocyte folate concentration, its diagnostic reliability would increase.

The best biochemical indicator of folate deficiency during pregnancy is the erythrocyte folate level, which is a reflection of tissue stores (Kitay, 1979). Although red cell folate concentration does not begin to fall until the eighteenth week of folate deprivation for a nonpregnant subject (Herbert, 1962), this decline may become evident earlier in pregnancy as a result of accelerating demands. Reports from Chanarin et al. (1968) and Herbert et al. (1975), indicate that a fall in red blood cell folate can occur as early as twelve weeks gestation. Investigations by Varadi and associates (1966) found red cell folate low in nearly

all pregnant subjects with megaloblastic marrow changes. In a study by Chanarin et al. (1968), almost 40% of pregnant women who developed megaloblastic anemia had very low red blood cell folate values. Rothman (1970) cites several other studies which found correlations between reduced levels of erythrocyte folate and other hematological findings during pregnancy. Hershko and colleagues (1975), also found that red cell folate values are better indicators of folate status than serum folate, based on response-to-therapy studies. According to Chanarin (1976 and 1979), a low red cell folate is "unequivocal evidence of folate deficiency". However, Sauberlich (1978) and Hoffbrand (1978) suggest that a more positive diagnosis of folate deficiency can be made if both serum and red blood cell folate values are low. In support of this statement, Herbert et al. (1975) showed a significant correlation between serum and red cell folate levels in determining folate deficiency in a pregnant population.

Concomitant with the search for the most definitive method for diagnosing folate deficiency during pregnancy, is the experimentation for confirmation that folate deficiency is responsible for a number of obstetric complications and/or fetal malformations. Extensive studies with rats using either folate antagonists or folate depletion diets have

produced spontaneous abortions with fetal deaths early in gestation and/or a variety of malformations, including cleft lip and palate, hydrocephaly, eye defects, failure of closure of thoracic and abdominal walls, and numerous abnormalities affecting the nervous, skeletal and cardiovascular systems (Hurley, 1980).

The association between folate deficiency and maternal or fetal morbidity and mortality is still inconclusive. Reports indicating a high correlation between abruptio placentae and folate deficiency have not yet been confirmed. Hibbard (1964), Hibbard et al. (1965), Streiff and Little (1967), and Stone et al. (1967) report that the incidence of folate deficiency was somewhere between sixty to ninety percent in cases of abruptio placentae. In contrast, other investigations have been unable to confirm any direct relationships between abruptio placentae and folate deficiency (Varadi et al., 1966; Whalley et al., 1969 and Pritchard et al., 1971). According to Kitay (1969), part of the problem in finding a significant correlation with this obstetric complication resides in the retrospective nature of a majority of the investigations.

In addition to abruptio placentae, an increased incidence in spontaneous abortions associated with folate deficiency has been reported by some of the previous

investigators (Hibbard, 1964; Hibbard et al., 1965 and Hibbard, 1975). However, Streiff and Little (1965), Chanarin et al. (1968) and Pritchard et al. (1971) have failed to confirm a correlation between folate deficiency and spontaneous abortion.

During the 1960's, several conflicting studies regarding the effect of folate deficiency on birth weight and prematurity were reported (Rothman, 1970). In 1970, Baumslag and associates showed not only a reduction of incidence in prematurity, but also an increase in birth weight as a direct result of folate supplementation in a group of pregnant women whose dietary intake of folate was known to be very low. Iyengar et al. (1975) and Rolschau et al. (1979) confirmed the work of Baumslag and associates (1970) reporting an increase in birth weight in their studies and, in addition, showed evidence of an increase in placental weight.

Although it is easy to show relationships between fetal malformations and severe folate deficiency, it is difficult to show definite correlations with marginal or subclinical folate deficiency during pregnancy. Early studies found a higher incidence of fetal malformations with folate deficency during pregnancy when increased FIGLU excretion was used to establish the deficiency (Hibbard et al., 1965 and Hibbard and Smithells, 1965). Observations reported by

Scott and associates (1970) using other diagnostic tests, including marrow aspiration, did not support the previous studies, even in subjects whose folate deficiency was severe enough to cause overt megaloblastic anemia.

As a result of the conflicting evidence generated by these and other retropsective investigations, Hall (1972) conducted a very large prospective study, which confirmed the work of Scott and colleagues (1970) and found no significant correlation between fetal malformations and folate deficiency during pregnancy. However, Hibbard (1975) found the incidence of folate depletion in relation to fetal malformations highly significant when erythrocyte folate levels were used as diagnostic criteria and the women were selected before sixteen weeks gestation.

In another prospective study, which was restricted to women in their first trimester, Smithells et al. (1976) found more infants with neural tube defects born to women with significantly lower levels of serum and red cell folate than in the controls. To confirm the possible specific relationship between central nervous system defects and folate deficiency during pregnancy, the same investigators conducted another study and confirmed their previous work reporting significant differences between supplemented and unsupplemented women giving birth to fetuses with neural

tube defects (Smithells et al., 1980).

Recently an investigation was undertaken by Laurence and associates (1981) to prevent recurrence of neural tube defects by administering folate preconceptually and during gestation. They reported that they were extremely successful (100%) among the compliant subjects. In another study, involving recurrence of neural tube defects, Laurence and colleagues (1980) conducted retrospective and prospective studies on the same subjects and investigated the effect of maternal diet on the fetus using dietary counseling in place of folate supplements. The results showed a 50% reduction in relative risk in the counselled group, indicating the possible benefits of thorough and supportive dietary counseling. They suggest that improving the quality of the diet early in pregnancy may improve environmental factors acting on the fetus.

In addition to obstetric complications and fetal malformations, evidence suggests that postnatal problems such as depressed growth during the first year of life and possibly longer, may be associated with maternal folate deficiency (Hurley, 1980). Abnormal or delayed behavorial development was found in eight out of fourteen children whose mothers had had severe folate deficiency during pregnancy (Gross et al., 1974). Therefore, the elucidation of the potential for

deleterious effects from compromised folate status during gestation that may interfere with optimal fetal development and subsequent development of the infant are of obvious importance. The results of studies by Newberne and Wilson (1972) have also suggested that the effects of maternal nutrition are carried over into the later life of the offspring.

Apart from malabsorption problems, defects in folate metabolism, certain antagonistic drugs, and excessive alcohol consumption, it is generally accepted that the major contributing factor to folate deficiency during pregnancy is poor dietary intake. Rothman (1970) cites several studies that showed a high correlation between megaloblastic anemia or low erythrocyte folate levels and inadequate diets. The effect of low dietary intake of folate on whole blood folate levels was compared in both nonpregnant and pregnant women by Van de Mark and Wright (1972). They found a highly significant correlation. While investigating the possible residual effects of oral contraceptive agents in pregnant women, Martinez and Roe (1977) also found a strong correlation between diet and folate status. In addition, a seasonal variation either in the incidence of megaloblastic anemia (Chanarin et al., 1968 and Fleming, 1970), whole blood levels of folate (Daniel et al., 1970), or erythrocyte levels of folate (Martinez and Roe, 1977) has been

found in folate-deficient women during pregnancy.

Although the National Academy of Science recommends 800 micrograms per day of folate for pregnant women, the average intake in the United States varies considerably and is usually much less. In one survey, conducted by the Joint FAO/WHO Expert Group in 1970, it was estimated that the average intake of free folate was 37-279 micrograms/day. Intakes of less than 10% of the Recommended Daily Allowance were found in 50% of a group of pregnant teenagers, while the other half ingested less than 50% of the RDA (Daniel et al., 1971). Van de Mark and Wright (1972) reported the average intake of another group of pregnant adolescents was 184 micrograms per day, which is less than one-fourth of the RDA. Folate intakes were between 39-697 micrograms per day in a group of pregnant women, according to Moscovitch and Cooper (1973). Recent information, calculated from average per capita disappearance of principal foods in the U.S., indicates the folate availability may only be 227 micrograms per capita per day (Tamura and Stokstad, 1981). Therefore, these studies and others indicate the NAS recommendation for folate for pregnant women is not being met. Similar evidence of very low folate intakes from other developed countries suggest that the average folate intake for pregnant women is less than one-third of the RDA. Elsborg and Rosenquist

(1978) report the average intake in one group of pregnant women in Denmark to be less than one-third of the 400 micrograms per day which is the FAO/WHO Expert Group recommended daily allowance. Canadian intakes average 100-200 micrograms per day of folate for pregnant women (Hoppner et al., 1973).

Unfortunately, whether the investigators choose to analyze or calculate the content of folate in their subject's diets, the amount of available information involving the use of either method is both limited and conflicting. To complicate the problems of analysis, opinions differ regarding not only the methodology for analysis, but also which form of folate is the most biologically available. Baker and DeMaeyer (1979) reference several conflicting reports concerning the bioavailability of different forms of folate. As a result, food tables compiled from this data which are used to calculate folate from dietary recalls and food diaries are considered provisional, since many values are based on limited information and are far from adequate (Perloff and Butrum, 1977).

The question of dosage and benefits of folate supplements during pregnancy for either the mother and/or fetus has also been the subject of much research, and again, the evidence has been conflicting (Kitay, 1969; Rothman, 1970

and Hemminki et al., 1978). Despite differences of opinions and in view of the limitations of current methods for assessing folate status, many investigators advocate supplementation, since they believe the increased demands may not be met through diet alone. Although supplementation through food fortification has been proven to be an effective means to increase serum and red blood cell folate levels in a population consuming a limited diet (Colman et al., 1975), the feasibility or practicality of a similar program in the U.S. is questionable. Direct supplementation of target groups who appear to be at risk, such as pregnant women, seems to be the most effective method at this time.

Ethnic differences in both serum and red cell folate levels were found in the Ten-State Nutrition Survey, indicating that twice as many Hispanics and Blacks fall into the medium-high risk range than Whites (Sauberlich, 1977). It appeared that this difference might be related to income, yet it may also be a reflection of cultural dietary habits. In addition to these reports, studies by Garn and associates (1976 and 1977) have shown lower hemoglobin and hematocrit values in Blacks than in Whites, both in nonpregnant and pregnant subjects. Since very few studies have indicated that Hispanics were included in research populations, Hispanics also may have race-specific differences in hematological indies.

From the literature reviewed, it is possible that the prevalence of folate deficiency globally and in the U.S. may be underestimated. Many groups-such as pregnant women, infants, adolescents, and certain minorities-may be at higher risk for developing a compromised folate status. Most studies have not included pregnant minorities from the same geographical population; therefore, it was decided to investigate the folate status of pregnant women from three ethnic groups that are predominant in this area. Since earlier research has indicated that a relationship between age or trimester and folate status may exist, these parameters were also included.

HYPOTHESIS

The following null hypothesis was tested in this study: There is no significant difference in the folate status of pregnant Caucasian, Black and Hispanic women attending a prenatal clinic when compared to the normal folate status values for pregnant women.

The following null sub-hypotheses were also tested:

1) There is no significant difference in folate status among the Caucasian, Black and Hispanic pregnant women.

2) There is no significant correlation between folate status and age among the pregnant women.

3) There is no significant correlation between folate status and trimester among the pregnant women.

The minimum level for rejection of the null hypothesis or sub-hypotheses was $p \leq 0.05$. The independent variables were race, age and trimester. The dependent variables were serum and red blood cell folate values.

METHODS AND MATERIALS

The subjects for this study were selected from women at the time of their first prenatal visit to an outpatient clinic in Houston, Texas. Blood samples were collected over a period of three months from July through September, 1980. Subjects were not required to fast before this initial visit. Information regarding age, race, trimester and gravidity was recorded during this initial visit by outpatient clinic personnel, before the subjects reported to the hematology department. Subjects found to be using any type of vitamin or mineral supplement were excluded from this study. None of the subjects in this study had received prenatal supplements and all were of middle-income socioeconomic status. Socioeconomic status was estimated, based in the cost for the prenatal services provided by the clinic which was prepaid by the patient at the time of the initial visit. There were thirty-seven White, twenty-two Blacks, fifty-four Hispanics, and one Indian woman for a total of 114 subjects.

Phlebotomists from the hospital laboratory collected the venous blood samples as ordered by the clinic physicians for routine determination of the following blood indices: Hemoglobin (Hgb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular

Hemoglobin Concentration (MCHC), Red Blood Cell Count (RBC), and White Blood Cell Count (WBC). All of these parameters were analyzed by the laboratory technicians using a Coulter counter and other instruments. After these analyses were performed, the remaining blood was used for the folate analyses.

Approximately 2-3 ml of each blood sample was centrifuged to separate the cells from the serum within two hours from the time of collection. The serum was then transferred into another test tube which was previously prepared with ascorbic acid to preserve the folate for testing. The blood remaining in the centrifuged tube was used for red blood cell analysis. All the tubes were capped with parafilm, immediately frozen, and kept frozen until just prior to analysis.

Serum folate levels were determined by radioassay using radioactively labeled I^{125} pterolglutamic acid (PGA). Two Quanta-Count Folate kits (#19-1001) were purchased from Bio-Rad Laboratories for the biochemical analyses. Each sample of the subject's serum or diluted whole blood was mixed with a folate-protecting buffer and labeled with I^{125}(PGA) derivative. During a 15 minute incubation period at $100^{o}C$, the normal serum folate-binding proteins were inactivated while the folate was stabilized by the buffer. A measured amount of folate-binding protein was added to each tube and the

mixture was incubated again for 30 minutes at room temperature. During this second incubation period, competitive binding occurred between the labeled I^{125}(PGA) and the unlabeled folate for sites on the folate-binding protein. The folate concentration is a measure of the degree to which the binding of labeled PGA is inhibited by the unlabeled folate. This value of folate concentration was derived by comparison to a standard curve.

The radioactivity of the subject samples and folate standards were counted using a Tracor Gamma Trac 2200 counter in the Radiation-Safety Laboratory at the University of Texas Health Science Center at Houston. Before each batch of samples and standards were counted, background readings were taken and the instrument was calibrated with I^{131}. Each sample tube and each standard tube were counted for five minutes and the average counts per minute were used for the calculations. The serum folate concentration for each sample was derived from a standard curve which was prepared for each batch counted.

The percent trace binding by the folate protein was calculated to see if the level of sensitivity and precision fell within the acceptable range of 45-75%. The average percent trace binding for the six batches was 53.6%.

STATISTICAL ANALYSIS

All data were analyzed using the Statistical Package for the Social Sciences on the Dec-20 computer at Texas Woman's University. Analysis of vaiance was used to test the effects of race, age, trimester and gravidity on the hematological parameters provided from the hospital; analysis of variance was also used to test the effects of the same variables on serum and red blood cell folate concentrations. Regression analysis was used to determine correlations between dependent and independent variables.

RESULTS AND DISCUSSIONS

A total of 114 subjects were involved in this study. They were predominately primagravidas in their second trimester and in their early twenties. Table 1 contains average values for Hgb, Hct, MCV, MCHC, WBC, and RBC for these subjects. These parameters were analyzed by the Hematology laboratory in the hospital and were provided with each blood sample collected for this study. The average values for each parameter for the subjects fall within the normal range for these indices.

Race and age distributions for subjects in this study are presented in Table 2. The youngest age in this study was fourteen and the oldest was thirty-nine. The predominate age group was twenty to twenty-four with the majority of subjects age twenty-four.

It is not uncommon for women seeking prenatal care in many clinics to wait until their second trimester. In this study, more than two-thirds (69.3%) of the subjects were in their second trimester at the time of their first visit, as shown in Table 3. Many investigators believe that this is a serious problem because folate supplementation will not begin until after the period of maximum susceptibility to a folate deficiency, which occurs in the first trimester (Herbert et al., 1975; Hibbard, 1975 and Weiner, 1980).

TABLE 1

Average Values for Selected Hematological Parameters

Parameter	Number of Subjects	Average Values *	Acceptable Values
Hgb(g/dl)	114	11.9 ± 1.0 (8.8-14.5)	11.2 ± 0.7
Hct(vol%)	114	34.3 ± 3.1 (26.0-43.0)	32.7 ± 2.3
MCV(m^3)	114	87.9 ± 4.9 (68.2-98.2)	88.0 ± 4.8
MCH(pg)	114	30.6 ± 1.9 (22.9-34.1)	30.3 ± 1.6
MCHC (g/dl)	114	34.7 ± 0.6 (33.0-36.1)	35.0 ± 1.5
WBC_3 ($10^3/mm^3$)	112	9.1 ± 2.4 (4.7-15.5)	10.0 ± 4.9
RBC_6 ($10^6/mm^3$)	112	3.9 ± 0.4 (3.0-5.3)	4.1 ± 0.3

*mean ±SD

TABLE 2

Race and Age Distribution of Subjects

Age Range	White	Black	Hispanic	Percent
14			1	0.9
15-19	9	2	17	24.6
20-24	18	8	20	40.3
25-29	7	7	7	18.4
30-34	3	3	7	11.4
35-39	1	2	2	4.4
Total	38	22	54	100.0

TABLE 3

Distribution of Subjects by Trimester at Time of Entry into Study

Trimester	Number	Percent of Total
First	31	27.2
Second	79	69.3
Third	4	3.5

The majority of these subjects (82%) were primagravidas. The highest gravidity recorded was eight, but this occurred in only one subject (Table 4). The multiparous subjects comprised 17.5% of the study, as also shown in Table 4.

TABLE 4

Distribution of Subjects by Gravidity

Gravidity	Number	Percent of Total
1	93	81.6
2	8	7.0
3	7	6.1
4	5	4.4
8	1	0.9

The analysis of patient serum samples gave results that were within the normal values reported for serum folate levels. However, analysis of red blood cell folate resulted in conflicting data. It was determined that the amount of serum in all the tubes with the red blood cells was insufficient to allow for accurate aliquoting. Therefore, biochemical analysis of red blood cell folate was discontinued at this point. In addition, because of the need to prepare standard curves each time an analysis was performed, there

was only enough of some of the reagents to analyze eighty serum samples. Further analyses of serum folate concentrated on the samples from the Hispanic and White subjects.

Serum folate concentrations were analyzed by one way analysis of variance (ANOVA). Average serum folate concentrations were not significantly different between ethnic groups. The average serum folate values for eighty subjects are shown in Table 5. After removing the small number of serum folate values representing the Black subjects included in the one way ANOVA, chi-square analysis was performed on the remaining White and Hispanic serum folate values to see if there were any significant differences with these two groups. Again, average serum folate concentrations were not significantly different between Whites and Hispanics.

TABLE 5

Mean and Range Values of Serum Folate Levels (ng/ml) for Each Ethnic Group

Group	Number of Subjects	Mean Serum Values ± Standard Deviation	Ranges
White	27	9.1 ± 5.2	(2.6-25.1)
Black	7	9.8 ± 4.1	(4.7-15.7)
Hispanic	46	9.3 ± 4.2	(2.0-20.6)

The serum folate values for the White and Hispanic subjects were classified according to the indices reported by Sauberlich et al. (1977) as either deficient (less than 3.0 ng/ml), low (3.0 - 5.0 ng/ml) or average (greater than 5.0 ng/ml)(Table 6). This analysis shows that out of 73 subjects, 19.2% had low serum folate values and 2.7% had values falling within the deficient category also shown in Table 6. Almost 30% (8) of the White subjects and 17.4% (8) of the Hispanic subjects had serum folate levels that fell into the low-to-deficient categories (Table 6). There were, however, no significant differences between Hispanic and White subjects.

TABLE 6

Percent of Subjects with Deficient, Low, and Average Serum Folate Values (ng/ml)

	3.0 (deficient)	3.0-5.0 (low)	5.0 (average)
White	1	7	19
Hispanic	1	7	38
Total	2	14	57
Percent	2.7%	19.2%	78.1%

Table 7 shows the average values for selected hematological parameters that were significantly different ($p \leq 0.05$) between ethnic groups when analyzed by one way ANOVA. Hgb, Hct, and MCHC were not significantly different; however, the data indicated that values for the Black subjects were lower than for either the Hispanics or Whites. The predominating pattern that was observed for these values was: Black < Hispanic < White.

TABLE 7

Average Values for Selected Hematological Parameters by Ethnic Group

Parameters	White	Black	Hispanic
MCV(m^3)	89.3 ± 3.7^a	85.3 ± 7.4^b	88.1 ± 4.0^a
MCH(pg)	31.1 ± 1.5^a	29.5 ± 2.9^b	30.6 ± 1.6^a
WBC($10^3/mm^3$)	9.8 ± 2.9^a	7.6 ± 1.5^b	9.3 ± 2.5^a
	(n=37)	(n=22)	(n=54)

*note: values represent means $\pm$SD and those values within the same row followed by different superscripts are significantly different ($p \leq 0.05$) by one way ANOVA and Student-Newman-Keuls analysis

Garn et al. (1976) reported a difference of 0.64 g/100ml in Hgb values between Black and White women during pregnancy. This value was significantly different ($p \leq 0.0001$). In this

study, a difference of 0.63 g/100ml in Hgb values was observed between Black and White subjects (Table 8). The same investigators also report lower Hct values between Black and White pregnant women (Garn et al., 1977). Differences in Hct values between Black and White subjects also were observed in this study but they were not significant. Mean values for Black and White subjects were 33.5% and 35.0% respectively. The predominating pattern observed again for these values was: Black < Hispanic < White.

TABLE 8

Average Values of Hemoglobin by Ethnic Group

	Black	Hispanic	White
Hgb(g/dl)	11.54 ± .72 (10.2-13.3)	11.86 ± 1.1 (8.8-14.3)	12.17 ± .99 (9.5-14.5)
	(n=22)	(n=54)	(n=37)
Ten-State*	(11.73 ± 1.3)		(12.37 ± 1.1)

*Garn et al., 1976

The blood parameters shown in Table 1 were not significantly different between trimesters, except for MCHC as shown in Table 9. These blood parameters were analyzed to determine if the length of gestation had a significant effect on the average values. RBC values declined during gestation,

although the change was not statistically significant. Serum folate values fell with increasing length of gestation, which has been reported by other investigators (Hall et al., 1976; Chanarin, 1979 and Tso et al., 1980).

TABLE 9

Average Values of Mean Corpuscular Hemoglobin Concentration by Trimester

Parameter	Trimester (1)	Trimester (2)	Trimester (3)
MCHC(g/dl)	34.8 ± 0.6^{a}	34.7 ± 0.6^{a}	35.0 ± 0.6^{b}

*note: values represent means $\pm$SD and those values followed by different superscripts are significantly different ($p \leq 0.05$) by oneway ANOVA and Student-Newman-Keuls analysis

There were significant differences between several blood parameters and certain gravidities as shown in Table 10 by one way ANOVA. Hgb and Hct were significantly different between gravid one and gravids two and three. The cause for these differences is unknown at this time. There were no differences in the average values of MCV, MCH, and WBC parameters when analyzed using one way ANOVA between different gravidities. There was, however, a trend observed for the serum folate levels to decrease with each subsequent pregnancy, but this decrease with increasing gravidity was not statistically significant.

TABLE 10

Average Values of Selected Hematological Parameters by Gravidity

Parameter	Gravid (1)	Gravid (2)	Gravid (3)
Hgb(g/dl)	11.8 ± 1.0^{a}	12.5 ± 1.4^{b}	12.5 ± 0.8^{b}
Hct(vol%)	34.0 ± 2.9^{a}	36.6 ± 4.3^{b}	35.7 ± 2.6^{b}
MCHC(g/dl)	34.8 ± 0.6^{a}	34.2 ± 0.8^{b}	35.1 ± 0.4^{a}
RBC(10^6/mm^3)	3.9 ± 0.3^{a}	4.3 ± 0.6^{bc}	4.0 ± 0.3^{ac}

*note: values represent means $\pm$SD and those values within the same row followed by different superscripts are significantly different ($p \leq 0.05$)

No significant relationship was found between the length of gestation and the average serum folate levels by chi-square analysis. Chi-square analysis was also used to investigate the relationships between trimester and serum folate values in all three ethnic groups, and also between White and Hispanic subjects. No significant relationships were found; however, 30% (16) of the subjects in their second trimester had serum folate levels less than 5 ng/ml as previously reported.

Chi-square analysis showed that there was no effect on serum folate levels of gravidity for the White or Hispanic subjects. Twenty-two percent of the primagravida White and

Hispanic subjects in their second trimester had serum folate values less than 5 ng/ml.

When the blood parameters listed in Table 1 for all three ethnic groups were analyzed using Spearman's correlation statistical test, no correlation was found between any of the parameters and serum folate values. Again, because of the small number of serum folate values representing the Black subjects, these values were removed and the same statistical analysis was applied to see if any correlation existed between the White and Hispanic serum folate values and the blood parameters listed in Table 1. No correlation was found between the blood parameters and the serum folate values for the White and Hispanic subjects.

Because of the disparity in the number of subjects in each group the analysis of variance was repeated using a Kruskal-Wallis nonparametric ANOVA. This analysis was performed only on those parameters that were significantly different by parametric analysis. This nonparametric statistical test was used to determine if the ethnic groups that were studied were significantly different for certain hematological parameters. The effects of trimester and gravidity on Hgb, MCHC and RBC were analyzed.

These analyses showed there were significant differences ($p \leq 0.05$) between ethnic groups for MCV, MCH, and WBC

as shown in Table 11. These same parameters were previously shown to be significantly different ($p \leq 0.05$) between ethnic groups using one way ANOVA as shown in Table 7. The same pattern was also observed between the ethnic groups. Average Black values were the lowest and average White values were the highest for these parameters. MCHC was the only blood parameter shown to be effected by trimester (Table 12), using Kruskal-Wallis ANOVA. Using one way ANOVA, MCHC was also shown to be significantly different between trimesters (Table 9). In Table 13, the effect of gravidity on Hgb, MCHC, and RBC is shown. The differences between gravid one, two and three for these blood parameters is significantly different at the p 0.05 level.

TABLE 11

The Effect of Race on Selected Hematological Parameters

Parameter	Race and Rank	Corrected Chi-square	Level of Significance
MCV(m^3)	B$\prec$H$\prec$W	7.349	0.025
MCH(pg)	B$\prec$H$\prec$W	8.173	0.017
WBC($10^3/mm^3$)	B$\prec$H$\prec$W	12.508	0.002

*note: Kruskal-Wallis ANOVA at ($p \leq 0.05$) level
B=Black (n=22); H=Hispanic (n=54); W=White (n=37)

TABLE 12

The Effect of Trimester on Mean Corpuscular Hemoglobin Concentration

Parameter	Trimester and Rank	Corrected Chi-square	Level of Significance
MCHC(g/dl)	$2 < 1 < 3$	8.161	0.017

*note: Kurskal-Wallis ANOVA at ($p \leq 0.05$) level
First trimester (n=31); Second trimester (n=79);
Third trimester (n=4)

TABLE 13

The Effect of Gravidity on Selected Hematological Parameters

Parameters	Gravidity and Rank	Corrected Chi-square	Level of Significance
Hgb(g/dl)	$3 < 2 < 1$	7.089	0.029
MCHC(g/dl)	$2 < 1 < 3$	7.421	0.024
RBC($10^6/mm^3$)	$1 < 3 < 2$	6.170	0.046

*note: Kruskal-Wallis ANOVA at ($p \leq 0.05$) level
Gravidity-1 (n=93); Gravidity-2 (n=8); Gravidity-3 (n=7)

This study was predominately comprised of young primagravidas in their second trimester. The result of the biochemical data suggest that the majority of the subjects did

not have a compromised folate status. Serum folate levels indicative of a deficiency occurred in only two women. Most of the women also had blood indicies that fell within the acceptable ranges for the several hematological parameters that were analyzed. Iron deficiency anemia, which is common during pregnancy, was not apparent in this particular group of pregnant women. In general, this group was not a "high risk" group based on the average values observed. However, the average Hgb (11.9 g/100ml) and Hct (34.3%) values for the Hispanic women in this study were lower than those reported by Jacob et al. (1976). Also, the lower Hgb and Hct values found for the Black women in this population, support the results found in the Ten-State Nutrition Survey and those reported by Garn et al. (1976 and 1977), that suggest race-specific differences exist in normal pregnant subjects. Both parametric and nonparametric ANOVA statistics showed significant differences involving the same variables, except for the effect of gravidity on Hct; nonparametric ANOVA did not show a significant difference.

CONCLUSIONS

In conclusion, the null hypothesis which stated there was no significant difference in the folate status of pregnant Caucasian, Black, and Hispanic women attending a prenatal clinic when compared to the normal folate status values for pregnant women was not rejected. Results from this study show that the majority of these pregnant women had folate values well within the acceptable range. However, some of the subjects did have serum folate levels that were low and this may possibly indicate a compromised folate status.

There was no significant difference in folate status among the three ethnic groups; therefore, the null sub-hypothesis was not rejected. This may imply that there are no race-specific differences in folate values during pregnancy in contrast to what has been observed for Hgb and Hct values between Black and Caucasian women.

Since no correlation was found between folate status and age, this null sub-hypothesis was also not rejected. A possible explanation of this result might be the fact that the majority of the pregnant women in this study were in their twenties. This age group is not considered to be a high risk group when compared to those women who are over

thirty-five or are young adolescents.

Because there was no significant difference found between folate status and trimester this null sub-hypothesis was not rejected. Folate levels did decline slightly with increased length of gestation. This result suggests the possibility of a subclinical folate deficiency for subjects who are in a marginal nutritional status prior to pregnancy.

IMLICATIONS FOR FURTHER RESEARCH

This study has shown that the majority of pregnant women attending this clinic did not have a compromised folate status. However, the women were primarily from a middle income level and in their twenties. These are, therefore, not considered high risk groups. A study with subjects receiving prenatal care at a free clinic would include pregnant women from lower income levels and possibly at greater risk for developing a compromised folate status. Also, a larger number of young adolescents and women over thirty years of age would help to determine if these women are at higher risk during pregnancy. In addition, to rule out the possibilities of iron deficiency anemia and/or pernicious anemia, blood tests assessing iron status and vitamin B_{12} status should also be included with the folate analysis.

Food frequency questionnaires, including a special section listing folate-rich foods and twenty-four hour recalls for each subject should be conducted at the initial visit. This information could be used to compare dietary folate intake with serum and red blood cell folate levels. The percent of dietary folate consumed per day on the basis of caloric intake could also be analyzed from this data.

More information regarding the length of time between

each pregnancy would be necessary to more accurately analyze the effect of gravidity on folate status and other blood indices. The use of weeks, in addition to trimester, would be more helpful in evaluating the effect of gestation time on folate status and other parameters.

LITERATURE CITED

Baker, S.J., and DeMaeyer, E.M.: Nutritional anemia: its understanding and control with special reference to the works of the WHO. Am. J. Clin. Nutr. 2:368, 1979.

Baumslag, N., Edelestein, T., and Metz, J.: Reduction of incidence of prematurity by folic acid supplementation in pregnancy. Br. Med. 1:16, 1970.

Bell, W.R.: Hematologic abnormalities in pregnancy. Med. Clin. North Am. 61:165, 1977.

Chanarin, I.: Studies on urinary formimino-glutamic acid excretion. Proc. R. Soc. Med. 57:384, 1964.

Chanarin, I.: Investigation and management of megaloblstic anemia. Heamatol. 5:747, 1976.

Chanarin, I.: Distribution of folate deficiency. In Botez, M.I., and Reynolds, E.H., eds.: Folic Acid in Neurology, Psychiatry, and Internal Medicine. 1st ed. New York: Raven Press Books, Ltd., 1979.

Chanarin, I., Rothman, D., and Berry, V.: Iron deficiency and its relation to folic-acid status in pregnancy: results of a clinical trial. Br. Med. J. 1:480, 1965.

Chanarin, I., Rothman, D., Ward, A., and Perry, J.: Folate status and requirement in pregnancy. Br. Med. J. 2: 390, 1968.

Chanarin, I., Rothman, D., and Watson-Williams, E.J.: Normal formiminoglutamic acid excretion in megaloblastic anemia in pregnancy. Studies on histidine metabolism in pregnancy. Lancet 1:1068, 1963.

Channing, W.: Notes on anaemia, principally in its connections with the puerperal state, and with functional disease of the uterus; with cases. New Engl. Q. J. Med Surg. 1:157, 1842.

Chisholm, M., and Sharp, A.A.: Formimino-glutamic acid excretion in anemia of pregnancy. Br. Med. J. 2:1366, 1964.

Colman, N., Larsen. J.V., Barker, M., Barker, E.A., Green, R., and Metz, J.: Prevention of folate deficiency by food fortification. III. Effect in pregnant subjects of varying amounts of added folic acid. Am. J. Clin. Nutr. 28:465, 1975.

Cooper, B.A., Cantlie, G.S.D., and Brunton, L.: The case for folic acid supplements during pregnancy. Am. J. Clin. Nutr. 23:848, 1970.

Daniel, W.A., Mounger, J.R., and Perkins, J.C.: Obstetric and fetal complications in folate-deficient adolescent girls. Am. J. Obstet. Gynecol. 111:233, 1971.

Edelstein, T., Stevens, K., Brandt, V., Baumbslag, N., and Metz, J.: Tests of folate and vitamin B_{12} nutrition during pregnancy and the puerperium in a population subsisting on a suboptimal diet. J. Obstet. Gynaecol. Br. Commonw. 73:197, 1966.

Elsborg, L., and Rosenquist, A.: Folate intake by teenage girls and by pregnant women. Int. J. Vitam. Nutr. Res. 49:70, 1978.

Fleming, A.F.: Seasonal incidence of anemia in pregnancy in Ibadan. Am. J. Clin. Nutr. 23:224, 1970

Fletcher, J., Gurr, A., Fellingham, F.R., Prankerd, T.A., Brant, H.A., and Menzies, D.N.: The value of folic acid supplements in pregnancy. J. Obstet. Gynaecol. Br. Commonw. 78:781, 1971.

Food and Nutrition Board: Recommended Dietary Allowances. 9th rev. ed., 1980. Washington, DC: National Academy of Sciences, 1977.

Garn, S.M., Shaw, H.A., and McCabe, K.: Black-white hemoglobin differences during pregnancy. Ecol. Food N. 5:99, 1976.

Garn, S.M., Shaw, H.A., Gurie, K.E., and McCabe, K.: Apportioning black-white, hemoglobin and hematocrit differences during pregnancy. Am. J. Clin. Nutr. 30:461, 1977.

Giles, C.: An account of 335 cases of megaloblastic anemia of pregnancy and the puerperium. J. Clin. Pathol. 19:1, 1966.

Gross, R.L., Newberne, P.M., and Reid, J.V.O.: Adverse effects of infant development associated with maternal folic acid deficiency. Nutr. Rep. In. 10:241, 1974.

Hall, M.H.: Folic acid deficiency and congenital malformations. J. Obstet. Gynaecol. Br. Commonw. 79:159, 1974.

Hall, M.H., Pirani, B.B.K., and Campbell, D.: The cause of the fall in serum folate in normal pregnancy. Br. J. Obstet. Gynaecol. 83:132, 1976.

Hemminki, E., and Starfield, B.: Routine administration of iron and vitamins during pregnancy: review of controlled clinical trials. Br. J. Obstet. Gynaecol. 85:404, 1978.

Herbert, V.: Experimental nutritional folate deficiency in man. Trans. Assoc. Am. Physicians 75:307, 1962.

Herbert, V.: Introduction: Symposium on Folic Acid Deficiency. Am. J. Clin. Nutr. 23:841, 1970.

Herbert, V., Colman, N., Spivack, M., Ocasio, E., Ghanta, V., Kimmel, K., Brenner, L., Freundlich, J., and Scott, J.: Folic acid deficiency in the United States: Folate assays in a prenatal clinic. Am. J. Obstet. Gynecol. 123:175, 1975.

Hershko, C., Grossowicz, N., Rachmilewitz, M., Kesten, S., and Izak, G.: Serum and erythrocyte folates in combined iron and folate deficiency. Am. J. Clin. Nutr. 28:1217, 1975.

Hibbard, B.M.: Role of folic acid in pregnancy. J. Obstet. Gynaecol. Br. Commonw. 71:529, 1964.

Hibbard, B.M.: Folates and the fetus. S. Afr. Med. J. 49:1223, 1975.

Hibbard, B.M., and Hibbard, E.D.: Neutrophil hypersegmentation and defective folate metabolism in pregnancy. J. Obstet. Gynaecol. Br. Commonw. 78:776, 1971.

Hibbard, B.M., Hibbard, E.D., and Jeffcoate, T.N.A.: Folic acid and reproduction. Acta. Obstet. Gynecol. Scand. 44:375, 1965.

Hibbard, E.D., and Smithells, R.W.: Folic acid metabolism and human embryopathy. Lancet 1:1254, 1965.

Hoffbrand, A.V.: Effects of folate deficiency in man. In Rechigl, M. Jr., ed.: CRC Handbook Series in Nutrition and Food. Vol. III. Florida: CRC Press, Inc., 1978.

Hoopner, K., Lampi, B., and Perrin, D.E.: Folacin activity of frozen convenience foods. J. Am. Dietet. Assoc. 63:536, 1973

Hurley, L.S.: Developmental Nutrition. 1st ed. New Jersey: Prentice-Hall, Inc., 1980.

Iyengar, L., and Rajalakshmi, K.: Effect of folic acid supplement on birth weights of infants. Am. J. Obstet. gynecol. 122:332, 1975.

Jacob, M., Hunt, I.F., Dirige, O., and Swendseid, M.E.: Biochemical assessment of the nutritional status of low-income pregnant women of Mexican descent. Am. J. Clin. Nutr. 29:650, 1976.

Kitay, D.Z.: Folic acid deficiency in pregnancy. Am. J. Obstet. Gynecol. 104:1067, 1969.

Kitay, D.Z.: Folic acid and reproduction. Clin. Obstet. Gynecol. 22:809, 1979.

Kitay, D.Z., and Harbort, R.: Iron deficiency and folic acid deficiency in pregnancy. Clin. Perinatol. 2:255, 1975.

Kitay, D.Z., Hogan, W.J., Eberle, B., and Mynt, T.: Neutrophil hypersegmentation and folic acid deficiency in pregnancy. Am. J. Obstet. Gynecol. 104:1163, 1969.

Kutsky, R.J.: Handbook of Vitamins, Minerals and Hormones. New York: Litton Educational Publishing Inc., 1981.

Landon, M.J., and Hytten, F.E.: The excretion of folate in pregnancy. J. Obstet. Gynaecol. Br. Commonw. 78:769, 1971.

Laurence, K.M., James, N., Miller, M., and Campbell, H.: Increased risk of recurrence of pregnancies complicated by fetal neural tube defects in mothers receiving poor diets, and possible benefit of dietary counselling. Br. Med. J. 281:1592, 1980.

Laurence, K.M., James, N., Miller, M.H., Tennant, G.B., and Campbell, H.: Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural tube defects. Br. Med. J. 282:1509, 1981.

Lowenstein, L., Cantlie, G., Ramos, O., and Brunton, L.: Incidence and prevention of folate deficiency in a pregnant clinic population. Can. Med. Assoc. J. 95:797, 1966.

Martinez, O., and Roe, D.A.: Effects of oral contraceptives on blood folate levels in pregnancy. Am. J. Obstet. Gynecol. 128:255, 1977.

Moscovitch, L.F., and Cooper, B.A.: Folate content of diets in pregnancy: comparison of diets collected at home and diets prepared from dietary records. Am. J. Clin. Nutr. 26:707, 1973.

Newberne, P.M., and Wilson, R.B.: Prenatal malnutrition and postnatal responses to infection. Nutr. Rep. In. 5:151, 1972.

Osler, W.: The severe anemias of pregnancy and the postpartum state. Br. Med. J. 1:1, 1919.

Perloff, B.P., and Butrum R.R.: Folacin in selected foods. J. Am. Dietet. Assoc. 70:161, 1977.

Pitkin, R.M.: Nutritional support in obstetrics and gynecology. Clin. Obstet. Gynecol. 19:499, 1976.

Pitkin, R.M.: Nutritional requirements in normal pregnancy. Diabetes Care 3:472, 1980.

Pritchard, J.A., Scott, D.E., and Whalley, P.J.: Maternal folate deficiency and pregnancy wastage. IV. Effects of folic acid supplements, anticonvulsants, and oral contraceptives. Am. J. Obstet. Gynecol. 109:341, 1971.

Roe, D.A.: Lucy Wills (1888-1964) A biographical sketch. J. of Nutr. 108:1379, 1978.

Rolschau, J., Date, J., and Kristoffersen, K.: Folic acid supplement and intrauterine growth. Acta. Obstet. Gynecol. Scand. 58:343, 1979.

Rosenberg, I.H., and Dyer, J.: The prevalence and causes of folic acid deficiency in the United States. In Botez, M.I., and Reynolds, E.H., eds.: Folic Acid in Neurology, Psychiatry and Internal Medicine. 1st ed. New York: Raven Press Books Ltd., 1979.

Rothman, D.: Folic acid in pregnancy. Am. J. Obstet. Gynecol. 108:149, 1970.

Sauberlich, H.E.: Detection of folic acid deficiency in populations. In Food and Nutrition Board: Folic Acid, Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Washington, DC: National Academy of Sciences, 1977.

Sauberlich, H.E.: Vitamin indicies. In Committee on Nutrition of the Mother and Preschool Child. Food and Nutrition Board Laboratory Indicies of Nutrtional Status in Pregnancy. Washington, DC: National Academy of Sciences, 1978.

Scott, D.E., Whalley, P.J., and Pritchard, J.A.: Maternal folate deficiency and pregnancy wastage. II. Fetal Malformations. Obstet. Gynecol. 36:26, 1970.

Smithells, R.W., Sheppard, S., and Schorah, C.J.: Vitamin deficiences and neural tube defects. Arch. Dis. Child. 51:944, 1976.

Smithells, R.W., Sheppard, S., Schorah, C.J., Seller, M.J., Nevin, N.C., Harris, R., Read, A.P., and Fielding, D.W.: Possible prevention of neural-tube defects by periconceptional vitamin supplementation. Lancet 1:339, 1980.

Stone, M.L., Luhby, A., Feldman, R., Grodon. M., and Cooperman, J.M.: Folic acid metabolism in pregnancy. Am. J. Obstet. Gynecol. 99:638, 1967.

Streiff, R.R., and Little, A.B.: Folic acid deficiency as a cause of uterine hemorrhage in pregnancy. J. Clin. Invest. 44:1102, 1965.

Streiff, R.R., and Little, A.B.: Folic acid deficiency in pregnancy. New Eng. J. Med. 276:776, 1967.

Tamura, T., and Stokstad, E.L.R.: Folate intake provided by average U.S. diet, Unpublished data presented at ad hoc Review Group Meeting, Federation of American Societies for Experimental Biology 3/23-24/81. In U.S. Department of Health and Human Services. Bureau of Foods. Food and Drug Administration. A Review of Folate Intake, Methodology, and Status. FDA 223-79-2275, November 1981.

Temperley, I.J., Meehan, M.J.M., and Gatenby, P.B.B.: Serum folic acid levels in pregnancy and their relationship to megaloblastic marrow change. Br. J. Haematol. 14:13, 1968.

Tso, S.C., and Wong, R.L.C.: Folate status in pregnant Chinese women in Hong Kong. Int. J. Gynaecol. Obstet. 18:290, 1980.

U.S. Department of Health, Education and Welfare. Ten-State Nutrition Survey 1968-1970. Vols. I-V DHEW Pubn. Nos. (HSM) 72-8130 through 72-8134, 1972.

Van de Mark, M.S., and Clever Wright, A.: Hemoglobin and folate levels of pregnant teenagers. J. Am. Dietet. Assoc. 61:511, 1972.

Varadi, S., Abott, D., and Elwis, A.: Correlation of peripheral white cell and bone marrow changes with folate levels in pregnancy. J. Clin. Pathol. 19:33, 1966.

Weiner, M.: The significance of marginal nutritional deficiency during pregnancy. Nutr. Rep. In. 21:653, 1980.

Whalley, P.J., Scott, D.E., and Pritchard, J.A.: Maternal folate deficiency and pregnancy wastage. I. Placental abruption. Am. J. Obstet. Gynecol. 105:670, 1969.

Willoughby, M.L.N., and Jewell, F.G.: Folate status throughout pregnancy and in the postpartum period. Br. Med. J. 4:356, 1968.

Worthington-Roberts, B.S.: The water soluble vitamins. In Worthington-Roberts, B.S., ed.: Contemporary Developments in Nutrition. St. Louis: C.V. Mosby Co., 1981.