

Low Serum and Red Blood Cell Folate Are Moderately, but Nonsignificantly Associated with Increased Risk of Invasive Cervical Cancer in U.S. Women

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ABSTRACT Previous observational epidemiologic studies of folate and cervical cancer, as well as folate supplementation trials for cervical dysplasia, have produced mixed results. We examined the relationship between serum and RBC folate and incident invasive cervical cancer in a large, multicenter, community-based case-control study. Detailed in-person interviews were conducted, and blood was drawn at least 6 mo after completion of cancer treatment from 51% of cases and 68% of controls who were interviewed. Blood folate was measured with both microbiologic and radiobinding assays. Included in the final analyses were 183 cases and 540 controls. Logistic regression was used to control for all accepted risk factors, including age, sexual behavior, smoking, oral contraceptive use, Papanicolaou smear history and human papillomavirus (HPV)-16 serology. For all four folate measures, the geometric mean in cases was lower than in controls (e.g., 11.6 vs. 13.0 nmol/L, $P < 0.01$ for the serum radiobinding assay). Folate measures using microbiologic and radiobinding assays were correlated (serum: $r = 0.90$; RBC: $r = 0.77$). For serum folate, multivariate-adjusted odds ratios (OR) in the lowest vs. highest quartile were 1.3 [95% confidence interval (CI) = 0.8–2.9] and 1.6 (0.9–2.9), using the microbiologic and radiobinding assays, respectively. For RBC folate, comparable OR were 1.2 (0.6–2.2) and 1.5 (0.8–2.7). Similar risks were obtained when restricting analyses to subjects with a history of HPV infection. Thus, low serum and RBC folate were each moderately, but nonsignificantly, associated with increased invasive cervical cancer risk. These findings support a role for one-carbon metabolism in the etiology of cervical cancer. *J. Nutr.* 131: 2040–2048, 2001.

KEY WORDS: • *cervix neoplasms* • *serum folate* • *red blood cell folate* • *microbiologic folate assay* • *radiobinding folate assay* • *humans*

For the past 25 years, there has been credible speculation that folate inadequacy might be a risk factor for cervical neoplasia (1,2). The hypothesis that folate is involved in human carcinogenesis in general, and cervical carcinogenesis in particular, is biologically plausible. Low folate status may be important in cancer etiology because folate is required for DNA synthesis, repair and methylation (3–5). In cervical carcinogenesis, low folate may facilitate the incorporation of human papillomavirus (HPV),² a factor believed to be responsible for >90% of all invasive cervical cancers (6,7) into the

host genome. HPV integrates into the host genome of several cervical cancer cell lines at fragile sites made susceptible to breakage by inadequate folate (8–10).

The role of folate in the etiology of cervical cancer has been evaluated in many studies, but with mixed results (2,11–27). Of three clinical intervention trials with folate supplementation, one found improvement of cervical dysplasia (2), whereas two others did not (17,22). Case-control studies using dietary measures generally showed no association or only weak associations between folate intake and risk of cervical dysplasia or cancer (11–15,19–21,24,25). In many of these studies, crude associations were substantially attenuated when adjusted for accepted cervical cancer risk factors. Additionally, few early studies incorporated any measure of HPV infection. Finally, assessment of folate intake in these studies may have been imprecise because the usual adult diet is difficult to quantify,

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² Abbreviations used: CI, confidence interval; HPV, human papillomavirus; MTHFR, methylenetetrahydrofolate reductase; OD, optical density; OR, odds ratio(s); Pap, Papanicolaou.

and nutrient databases for folate are limited by the multiple forms, instability and variable bioavailability of folate in foods (28–33).

Serologic measures of folate allow better measurement of folate status than dietary intake measures (29,34), and RBC folate is a more reliable measure of folate status than serum folate because it integrates folate intake over several months, whereas serum folate fluctuates with daily intake (29,35–37). Case-control studies of cervical dysplasia and cancer that measured blood folate have generated mixed results (16,18,20,23,25–27). Of the five reports with invasive cervical cancer cases (12,13,15,16,26), only two (16,26) examined serum folate and none have yet examined RBC folate; therefore, additional studies of invasive cervical cancer that use serologic measures of folate status, especially RBC folate, and that also consider HPV status, may be informative.

In the 1980s, the National Cancer Institute conducted a large case-control study of incident invasive cervical cancer in five U.S. communities (38–40). Analyses of dietary data from this study found no clear association between folate intake and risk of invasive (13) or in situ (14) cervical cancer. The current paper examines the relationship between invasive cervical cancer risk and serologic measures of folate status. Because of the complexities of measuring folate, serum and RBC folate were measured with both radiobinding and microbiologic assays, which is rare in a large epidemiologic study. To facilitate adequate control for confounding, history of HPV infection was assessed with a serologic HPV-16 antibody assay, and all other known cervical cancer risk factors were assessed by a detailed in-person interview.

SUBJECTS AND METHODS

Study design. Eligible subjects were all women, aged 20–74 y, with histologically confirmed, primary incident invasive cervical cancer diagnosed from April 1982 through December 1983 in five areas reporting to the Comprehensive Cancer Patient Data System. Twenty-four hospitals in areas centered around Birmingham, AL; Chicago, IL; Denver, CO; Miami, FL; and Philadelphia, PA, participated. Up to two potential controls, matched by age (± 5 y), ethnicity (Caucasian, African American, Hispanic) and neighborhood (first six digits of a 10-digit telephone exchange), were identified by random digit dialing for each case. Approximately 25% of potential controls who had a previous hysterectomy were replaced.

Trained staff conducted interviews in the subjects' homes with structured questionnaires to obtain detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking and diet. Diet was assessed using a 75-item food-frequency questionnaire asking "usual adult frequency of consumption, ignoring any recent changes" (13). All study participants provided informed written consent. The study was approved by the Institutional Review Boards of the National Cancer Institute and of the five participating study centers. Additional details of the study design have been published (38–40).

For the biochemical component of the study, blood samples were drawn at least 6 mo after completion of treatment for cervical disease to minimize any effects of treatment or disease on blood nutrient status. Treatment included surgery (44%), localized radiation (18%) or both (28%). A small percentage of subjects (4%) received chemotherapy in addition to other treatments, and 6% of subjects were missing treatment information. Between March 1983 and October 1985, nonfasting blood samples were obtained; aliquots were stabilized with ascorbic acid (0.5% for serum and ~0.9% for whole blood) and frozen at -70°C until assayed (October 1988–January 1991). Hematocrits were determined in duplicate at the time of the blood collection.

Participation. A total of 480 eligible cases (73%) and 801 eligible controls (72%) were interviewed. Blood was obtained from

245 cases and 545 controls (51 and 68% of those interviewed, respectively). Reasons for nonparticipation in the blood draw included death (17% of cases, 0.4% of controls), contact and scheduling difficulties (15 and 17%), subject refusal (9 and 13%), hospital refusal (6 and 0%), cases who were not yet 6 mo post-treatment at the completion of the study (2 and 0%), and unsuccessful blood draws (2 and 1%), respectively.

Excluded from the epidemiologic analyses were all cases who received chemotherapy treatment ($n = 11$) and/or who had advanced (stage III or IV) disease ($n = 17$), cases with nonsquamous cell cervical cancer ($n = 28$), one control who reported possible cervical cancer and subjects whose ethnicity was other than Caucasian, African American or Hispanic ($n = 7$ cases, 2 controls). Other reasons for exclusion included insufficient blood for the folate assays, use of an antibiotic by the subject, which could have interfered with the microbiologic folate assay, and missing hematocrit data. Data from two serum microbiologic assay batches (8 cases, 22 controls) and 3 serum radiobinding assay batches (14 cases, 31 controls) were excluded due to quality control problems with these specific batches (see below). Because serum folate values are used to calculate RBC folate values, these samples were excluded for the RBC analyses as well. These exclusions were not mutually exclusive. The number of cases and controls included in each epidemiologic analysis were as follows: serum microbiologic assay, 170, 505; serum radiobinding assay, 169, 506; RBC microbiologic assay, 169, 504; and RBC radiobinding assay, 162, 496, respectively.

Laboratory methods. Serum and whole blood folate were measured in duplicate with a microbiologic assay (41) using *Lactobacillus rhamnosus* ATCC #7469 (formerly called *L. casei*) and with a radiobinding assay (42,43) using SimulTrac Slurry Kits (Becton Dickinson, Franklin Lakes, NJ). RBC folate was calculated from serum and whole blood folate measurements, corrected for hematocrit (42). Matched cases and controls were assayed consecutively within the same batch. Laboratory personnel were unaware of the case/control status of the samples.

In addition to each laboratory's own internal quality control procedures, laboratory reproducibility for the serum assays was monitored using blinded serum samples at low and normal folate concentrations. These samples were randomly inserted into each batch to comprise ~10% of the total number of samples. National Cancer Institute staff requested that four microbiologic and one radiobinding batches that failed to meet the Westgard multirule criteria (44) be repeated. At the time the assays were conducted, it was not possible to prepare whole-blood samples with predetermined folate concentrations to be used as blinded quality control samples. However, each laboratory's internal quality control samples included with the whole-blood study samples were evaluated using the Westgard rules, and eight microbiologic (but no radiobinding batches) had to be repeated. Additional laboratory problems suggested by review of the quality control samples after study completion resulted in the exclusion of two microbiologic serum assay and three radiobinding serum assay batches from the epidemiologic analysis. The CV for the remaining batches, based on the quality control material, was calculated using the variance component estimation procedure in SAS (45) and incorporated both within- and between-batch variability. Using blinded quality control material, the CV for the serum microbiologic assay was 11.6% and for the serum radiobinding assay was 5.2%. Using the laboratory's own quality control material, the CV for the whole-blood microbiologic assay was 11.4% and for the whole-blood radiobinding assay was 10.3%.

A test for HPV type-16 antibodies in serum has been developed only recently. In November–December 1998, we tested for HPV-16 seropositivity using a well-characterized virus-like particle ELISA (46). Samples were tested in duplicate; before they were averaged, the optical density (OD) readings of each duplicate were adjusted according to results of three control samples run in triplicate in each batch, to control for between-day and between-batch variability. An OD < 0.904 was classified as seronegative; an OD > 1.017 was classified as seropositive; and an OD between these values (3.6% of subjects tested) was considered indeterminate (47).

Statistical analyses. Statistical analyses were conducted using SAS version 6.12 for Windows (45). Correlations were measured

with Spearman's rank order correlation coefficient. χ^2 tests were used to determine whether significant differences for selected demographic and behavioral factors existed between cases and controls. Geometric means were calculated by transforming folate values with the natural logarithm, calculating the mean and then transforming back to standard units.

The odds ratio (OR) was the measure of association used to estimate the relative risk of cervical cancer. Folate quartiles were based on the frequency distribution among the controls. The highest quartile was used as the referent, or comparison, group. Logistic regression was used to obtain maximum likelihood estimates of the OR and 95% confidence intervals (CI), while adjusting for potential confounders (48). Comparable OR were found using unconditional regression models, adjusting for the study-matching factors, and conditional regression models. Therefore, unconditional regression models, which retained all of the cases and controls whose matched subjects did not participate in the blood draw portion of this study, were chosen for the detailed analyses and are presented throughout the results. Unless otherwise specified, all OR are adjusted for study matching factors (age, ethnicity, study site) and the following exposures related to risk in this study: HPV-16 seropositivity, number of sexual partners, age at first intercourse, years since last Papanicolaou (Pap) smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education and income. Potential confounding variables were entered into the models as categorical variables with missing data retained in a separate category. Control for confounding was considered adequate when the addition of a potential confounder or an increase in the number of strata of a confounder did not change the adjusted OR by ≥ 0.1 . Analyses with the RBC radiobinding assay data were adjusted for kit lot because two different kit lots were used for the whole-blood determinations. Tests for trend were obtained by assigning to each quartile the median folate concentration of the controls in that quartile and treating this as a continuous variable. Effect modification was assessed by examining stratum-specific OR and by using the likelihood ratio test to compare models with and without the interaction terms (49). All statistical tests were two-tailed. Differences with $P < 0.05$ or a CI that excluded 1.0 were considered significant.

RESULTS

Demographic and behavioral characteristics of study participants. In the original study design, potential controls had been individually matched to eligible cases on the bases of age, ethnicity and neighborhood. Among the subjects from whom blood was successfully drawn, the distribution of cases remained comparable to that of the controls on age, ethnicity and study site (Table 1). However, cases who donated blood appeared to be of a lower socioeconomic status than controls, based on their report of less education ($P = 0.001$) and lower income ($P = 0.001$).

Serum and RBC folate. For each of the four folate measures (serum and RBC folate measured by both the microbiologic and radiobinding assays), cases had lower geometric mean folate than controls (Table 2). The Third National Health and Nutrition Examination Survey reports somewhat higher blood folate using a radiobinding assay than we report here. For Caucasian women, mean age 43.2 y, unadjusted mean serum folate was 16.4 ± 0.5 nmol/L and RBC folate was 483.4 ± 9.7 nmol/L (50).

On the basis of a serum folate cut-off point of <6.8 nmol/L (<3 ng/mL) (51), 5–8% of subjects were classified as folate deficient by the two assays. On the basis of an RBC folate cut-off point of <317 nmol/L (<140 ng/mL) (51), only 1.5% of subjects were deficient using the microbiologic assay, whereas 27% were deficient using the radiobinding assay. However, RBC folate measured by the microbiologic assay used in this study tended to run high and probably underestimated the percentage of deficient subjects. In addition, it is important to

TABLE 1

Distribution of selected demographic variables for women from five U.S. communities who provided a blood sample, by case/control status

Demographic factor	Cases		Controls		P value ¹
	n	%	n	%	
Age, y					
<35	51	(21)	138	(25)	
35–44	70	(29)	161	(30)	
45–54	62	(25)	115	(21)	
55+	62	(25)	131	(24)	0.40
Ethnicity					
Caucasian	160	(65)	367	(67)	
African-American	55	(22)	137	(25)	
Hispanic	23	(9)	39	(7)	
Other	7	(3)	2	(0.4)	0.45
Study site					
Birmingham	56	(23)	105	(19)	
Chicago	42	(17)	121	(22)	
Denver	69	(28)	133	(24)	
Miami	33	(13)	75	(14)	
Philadelphia	45	(18)	111	(20)	0.35
Education					
<12 y	110	(45)	121	(22)	
12	74	(30)	180	(33)	
13–14 or trade school	32	(13)	110	(20)	
15–16	15	(6)	82	(15)	
≥ 17	14	(6)	52	(10)	0.001
Income					
$\leq \$10,000$	99	(40)	119	(22)	
$\$10,001–\$30,000$	95	(39)	267	(49)	
$> \$30,000$	44	(18)	145	(27)	
Unknown	7	(3)	14	(3)	0.001

¹ P-value for the chi-square test for a difference between cases and controls. Unknown categories are not included in the test.

recognize that elevated disease risks may occur at folate concentrations above the cut-off point for clinical deficiency (52).

Folate values using the microbiologic and radiobinding assays were correlated for both the serum ($r = 0.90$) and the RBC ($r = 0.77$) measures. This was reassuring because it implies that although absolute values might differ, both assays ranked individuals similarly, and thus reliably. The correlation between serum and RBC folate within each measure was less than between the two methodologies ($r = 0.72$ for serum and RBC folate using the microbiologic assay, and $r = 0.63$ for serum and RBC folate using the radiobinding assay).

Blood folate and invasive cervical cancer risk. The risk of invasive cervical cancer was moderately elevated (OR = 1.2–1.6 in the multivariate-adjusted models) in the lowest folate quartile compared with the highest folate quartile for all four blood folate measures (Table 3). Little confounding by HPV-16 status or other cervical cancer risk factors was observed.

The OR adjusted for age, race, site and HPV-16 seropositivity were recalculated by octile of blood folate to explore the risk gradient over a wider range of exposure. For both the serum and RBC models using the microbiologic assay, the OR between extreme octiles were similar to those between extreme quartiles. However, using the radiobinding assay, the

TABLE 2

Geometric mean serum and RBC folate levels for women from five U.S. communities, using microbiologic and radiobinding assays¹

Folate assay	n	nmol/L	P-value ²
Serum microbiologic assay			
Cases	170	19.1 (17.0–21.5)	
Controls	505	20.6 (19.4–22.0)	0.35
Serum radiobinding assay			
Cases	169	11.6 (10.8–12.5)	
Controls	506	13.0 (12.5–13.5)	<0.01
RBC microbiologic assay			
Cases	169	914.9 (846.2–989.1)	
Controls	504	967.5 (925.6–1011.2)	0.34
RBC radiobinding assay			
Cases	162	368.2 (349.4–388.0)	
Controls	496	384.0 (373.9–394.4)	0.13

¹ Values are geometric means (95% confidence interval).

² P value for t test of difference between cases and controls.

OR between extreme octiles were greater than between extreme quartiles (for serum < 7.9 nmol/L, OR = 2.58, 95% CI = 1.3–5.3; for RBC < 268 nmol/L, OR = 1.78, 95% CI = 0.9–3.7).

Inclusion of education and income in the models slightly attenuated the serum OR, but not the RBC OR. Adjustment for these should help control for inadequately measured life-

style factors and provide a conservative estimate of risk. Inclusion of intake of provitamin A carotenoids or vitamin C, other micronutrients postulated to reduce the risk of cervical cancer, modestly increased, rather than decreased the folate OR.

To integrate both serum folate measures and both RBC folate measures, risks were examined among subjects concurrently in the lowest quartile by both assay types compared with those concurrently in the highest quartile by both assay types. Similarly, elevated OR were noted in the low folate groups for both serum and RBC measures (OR = 1.6 for serum and 1.5 for RBC, Table 4). Risks were not noticeably strengthened by combining the microbiologic and radiobinding assays, probably because of the high correlation between the two. To simplify presentation of further epidemiologic analyses, these combined exposure models are presented.

HPV is believed to be responsible for >90% of all invasive cervical cancers (6,7). Therefore, we examined the association between folate and cervical cancer risk using only the controls seropositive for HPV-16. All cases were used in this analysis because we assumed that all cases had been exposed to oncogenic HPV at one time. We used the combined exposure model to examine subjects in the lowest quartile by both assay types compared with those in the highest quartile by both assay types. Low serum and RBC folate were nonsignificantly associated with increased cervical cancer risk, after controlling for exposure to oncogenic HPV in this manner (Table 5). Comparable results were observed when only HPV-16 seropositive cases were included; adjusted OR for low folate in the combined exposure models were 2.0 (0.5–8.9) for serum and 1.2 (0.2–5.9) for RBC.

TABLE 3

Invasive cervical cancer risk by serum and RBC folate levels, using microbiologic and radiobinding assays, for women in five U.S. communities

Folate quartile (nmol/L)	n cases/n controls	OR ¹ (95% CI)	OR ² (95% CI)	OR ³ (95% CI)
Serum microbiologic assay				
4 (≥34.4)	44/128	1.0	1.0	1.0
3 (19.0–34.3)	39/129	0.92 (0.6–1.5)	0.85 (0.5–1.5)	0.85 (0.5–1.5)
2 (12.5–18.9)	31/125	0.75 (0.4–1.3)	0.80 (0.5–1.4)	0.81 (0.4–1.5)
1 (<12.5)	56/123	1.39 (0.9–2.3)	1.46 (0.9–2.4)	1.27 (0.7–2.3)
P for trend		0.56	0.42	0.73
Serum radiobinding assay				
4 (≥17.1)	38/127	1.0	1.0	1.0
3 (13.0–17.0)	31/127	0.82 (0.5–1.4)	0.83 (0.5–1.5)	0.68 (0.4–1.3)
2 (9.7–12.9)	37/126	1.00 (0.6–1.7)	0.98 (0.6–1.7)	0.75 (0.4–1.4)
1 (<9.7)	63/126	1.76 (1.1–2.9)	1.92 (1.2–3.2)	1.63 (0.9–2.9)
P for trend		0.03	0.02	0.17
RBC microbiologic assay				
4 (≥1397)	39/126	1.0	1.0	1.0
3 (944–1396)	35/126	0.92 (0.5–1.6)	0.96 (0.6–1.7)	1.04 (0.6–1.9)
2 (699–943)	48/126	1.31 (0.8–2.2)	1.36 (0.8–2.3)	1.41 (0.8–2.6)
1 (<699)	47/126	1.29 (0.8–2.2)	1.30 (0.8–2.2)	1.18 (0.6–2.2)
P for trend		0.23	0.23	0.42
RBC radiobinding assay ⁴				
4 (≥471)	41/124	1.0	1.0	1.0
3 (391–470)	30/124	0.79 (0.5–1.4)	0.82 (0.5–1.4)	0.87 (0.5–1.6)
2 (319–390)	36/124	0.96 (0.6–1.6)	0.90 (0.5–1.6)	1.04 (0.6–1.9)
1 (<319)	55/124	1.60 (1.0–2.7)	1.64 (1.0–2.8)	1.49 (0.8–2.7)
P for trend		0.08	0.09	0.18

¹ OR, odds ratio; CI, confidence interval; adjusted for study matching factors (age, ethnicity, study site).

² Also adjusted for human papillomavirus-16 serologic status.

³ Adjusted for all of the above and number of sexual partners, age at first intercourse, years since last Pap smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education and income.

⁴ Adjusted for whole-blood assay kit.

TABLE 4

Invasive cervical cancer risk integrating two serum assays and two RBC assays for women from five U.S. communities

	<i>n</i> cases/ <i>n</i> controls	OR ¹ (95% CI)	OR ² (95% CI)
Serum microbiologic/serum radiobinding			
High/High ³	30/94	1.0	1.0
Low/Low	45/90	2.0 (1.1–3.7)	1.6 (0.7–3.6)
RBC microbiologic/RBC radiobinding ⁴			
High/High ³	28/86	1.0	1.0
Low/Low	35/80	1.7 (0.9–3.5)	1.5 (0.6–4.1)

¹ OR, odds ratio; CI, confidence interval; adjusted for age, ethnicity, study site, and human papillomavirus (HPV)-16 serologic status.

² Adjusted for age, ethnicity, study site, HPV-16 serologic status, number of sexual partners, age at first intercourse, years since last Pap smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education and income.

³ Referent group.

⁴ Adjusted for whole-blood assay kit.

The number of HPV-16 seropositive controls was small, generating potentially unstable results, and it is possible that many of the other control subjects had been previously exposed to HPV-16 or other oncogenic HPV types. We therefore considered number of sexual partners and age at first intercourse, which are accepted proxy variables for HPV exposure. We examined risks among all of the cases and only the controls with ≥ 2 sexual partners, and among all of the cases and only the controls with age at first intercourse ≤ 20 y. With the combined exposure model, risks were nonsignificantly but consistently elevated in the lowest folate quartiles for both serum and RBC measures (OR = 1.5–2.2) (Table 5).

We also assessed the relationship between folate and infection with oncogenic HPV. Among the controls, folate status was not predictive of detection of HPV-16 antibodies. OR with the combined exposure model, adjusted for age, ethnicity and study site, were 0.7 (0.3–1.9) for low serum folate and 1.2 (0.4–3.7) for low RBC folate.

Because of previous hypotheses linking oral contraceptive use to low folate status and thus increased risk of cervical abnormalities (1,2), we closely investigated these associations. Geometric mean serum and RBC folate, using either assay and adjusted for age, ethnicity and study site, was not significantly different between women who had used oral contraceptives and women who had not. We stratified women by never/ever oral contraceptive use and examined the association between blood folate and invasive cervical cancer risk within each stratum. We observed no elevation in risk by folate status among users of oral contraceptives, although it had been hypothesized that oral contraceptive use would have depleted cervical folate stores. Unexpectedly, however, we did find elevated risks for low folate among women who never used oral contraceptives. Among never-users, OR for low compared with high folate quartiles, using the combined exposure models adjusted for age, ethnicity, study site, years since last Pap smear and HPV serology, were 5.9 (1.9–21.4) for serum folate and 3.4 (0.9–12.5) for RBC folate. The test for effect modification was not significant for either combined exposure model ($P = 0.08$ for serum and 0.21 for RBC).

We further examined risks by duration of oral contraceptive use. Women with high folate had a pattern of increasing risks with increased years of oral contraceptive use, whereas women with low folate, hypothesized to be more susceptible to folate depletion by oral contraceptive use, had a pattern of constant risks with increased years of oral contraceptive use (Table 6). The test for effect modification was not significant for either combined exposure model ($P = 0.31$ for serum and 0.40 for RBC).

Subjects who participated in the blood draw component of the study, relative to all those who participated in the interview, were more often Caucasian, came preferentially from certain study sites and were of higher socioeconomic status, as measured by education and income. Thus, we explored whether there were differences in participation between the cases and controls that might lead to bias. The cases and controls who donated blood were comparable to each other in age, ethnicity and study site (Table 1); controls had been individually matched to cases on these factors in the original study design. To examine whether cases and controls differ-

TABLE 5

Invasive cervical cancer risk among women from five U.S. communities with a likely history of human papillomavirus (HPV) infection

	History of HPV infection based on		
	HPV-16 seropositivity OR ¹ (95% CI) <i>n</i> cases/controls	Number of sexual partners OR ² (95% CI) <i>n</i> cases/controls	Age at first intercourse OR ³ (95% CI) <i>n</i> cases/controls
Serum microbiologic/serum radiobinding			
High/High	1.0 30/14	1.0 30/52	1.0 30/56
Low/Low	2.4 (0.8–7.4) 45/13	2.0 (1.0–3.9) 45/50	1.6 (0.8–3.0) 45/62
RBC microbiologic/RBC radiobinding ⁴			
High/High	1.0 28/10	1.0 28/52	1.0 28/55
Low/Low	1.4 (0.5–4.8) 35/13	2.2 (1.0–4.9) 35/50	1.5 (0.8–3.1) 35/62

¹ OR, odds ratio; CI, confidence interval; all cases and HPV-16 seropositive controls only; adjusted for age, ethnicity, study site.

² All cases and controls with ≥ 2 lifetime sexual partners; adjusted for age, ethnicity, study site.

³ All cases and controls with age at first intercourse ≤ 20 y; adjusted for age, ethnicity, study site.

⁴ Adjusted for whole-blood assay kit.

TABLE 6

Invasive cervical cancer risk by duration of oral contraceptive use for women from five U.S. communities^{1,2}

	Duration of oral contraceptive use		
	Never OR (95% CI) <i>n</i> cases/ controls	<5 y OR (95% CI) <i>n</i> cases/ controls	≥5 y OR (95% CI) <i>n</i> cases/ controls
Serum microbiologic/ Serum radiobinding			
High/High	1.0 10/47	1.9 (0.6–6.5) 10/28	3.1 (0.9–10.8) 10/19
Low/Low	3.2 (1.3–8.7) 26/36	2.7 (0.8–8.7) 13/37	3.0 (0.7–11.9) 6/17
RBC microbiologic/ RBC radiobinding			
High/High	1.0 13/40	1.4 (0.4–5.4) 7/29	3.3 (0.8–13.7) 8/17
Low/Low	2.2 (0.8–6.5) 20/31	2.1 (0.5–8.6) 9/29	2.0 (0.5–8.6) 6/20

¹ Adjusted for age, ethnicity, study site, years since last Pap smear, and human papillomavirus-16 serologic status; RBC also adjusted for whole-blood assay kit.

² OR, odds ratio; CI, confidence interval.

entially participated in the blood draw component by socioeconomic status, number of sexual partners, age at first intercourse, time since last Pap smear, vitamin supplement use or other cervical cancer risk factors, we compared OR among all of the interviewed subjects with OR among only those participating in the blood draw. For each of these exposures, similar patterns of risk were seen, suggesting that participation bias was minimal.

We found some evidence of differential participation by folate intake. However, the correlation between folate intake and blood folate status was low (Spearman $r = 0.08-0.16$), indicating that the differential participation would have little influence on blood folate status. In addition, the OR between folate intake and cervical cancer risk were similar among blood donors and nondonors, again suggesting that participation bias was minimal.

To examine the possibility of low blood folate being the result of systemic effects of disease or treatment, we compared mean blood folate concentrations of the cases by stage of cancer and treatment received (surgery or radiation). None of the women included in our analyses had received chemotherapy. We found no evidence that either disease or treatment had reduced blood folate concentrations (Table 7) by the time blood was drawn, at least 6 mo after completion of treatment.

At the time of blood draw, subjects were questioned concerning whether they had changed their diet in the past 3 y (this time period encompassed diagnosis and treatment, if any, for the cases). For the subjects in this analysis, 33.9% of cases and 43.1% of controls reported they had made changes to their diet. Only 7.1% of cases and 8.5% of controls reported they had increased their fruit, vegetable and/or grain consumption; these percentages were not significantly different ($P = 0.55$). Of these, only two cases (1.1% of total) reported that they made these healthy improvements to their diet as a result of their cancer. Other subjects reported decreasing their food intake for reasons including weight loss, health (such as reducing cholesterol levels) and, for five cases (2.7%), a loss of appetite due to illness and/or treatment. The percentage of

cases (16.4%) and controls (18.9%) who reported decreasing their food intake was similar ($P = 0.45$). Only two cases (1.1%) and two controls (0.4%) specifically reported a decreased intake of fruits, vegetables, or grains.

DISCUSSION

Low blood folate was moderately and consistently associated with an increased risk of invasive cervical cancer (OR = 1.2–1.6), although the risks were not significant (Table 3). Risk was elevated for each of the four measures of folate status, i.e., serum folate measured with microbiologic and radiobinding assays, and RBC folate measured with both assays. The effect remained after adjustment for history of HPV infection and all other accepted cervical cancer risk factors. A threshold effect was evident, with risk clearly elevated among women in the lowest folate quartile. When subjects were concurrently classified by both microbiologic and radiobinding assays, OR were 1.6 for low relative to high serum folate and 1.5 for RBC folate.

Ziegler et al. (13,14), using the same study population as the current analysis, did not detect an association between folate intake and risk of invasive or in situ cervical cancer. In the current analysis, improved measurement of folate status using serologic measures could explain this discrepancy. Serologic measures assess folate status more accurately than dietary intake measures due to difficulty quantifying usual adult diet and limitations in databases for folate in foods (28–34). Some case-control studies relying on serum and RBC folate have provided evidence for a protective effect of folate (18,20,23), whereas others have not (16,25,27), and all studies but one (16) examined precancerous conditions, not invasive cancer. Like our retrospective study, the single prospective study reported nonsignificantly reduced risks with elevated serum folate (OR = 0.60, 95% CI 0.19–1.88) but was based on only 13 invasive and 26 in situ cervical cancer cases (26).

Among the women in our study, we found a strong and significant positive association between serum homocysteine and invasive cervical cancer risk (OR = 2.4–3.2, all 95% CI excluded 1.0, in the three highest homocysteine quartiles relative to the lowest quartile) (53). These results provide evidence that our moderate folate association is real. Elevated serum homocysteine is a sensitive indicator of folate inadequacy and an emerging biomarker of problems in one-carbon metabolism (54–58). Serum homocysteine was moderately and inversely correlated with blood measures of folate status in

TABLE 7

Serum and RBC folate levels by stage of cervical cancer and treatment for women from five U.S. communities^{1,2}

	Microbiologic assay		Radiobinding assay	
	<i>n</i>	nmol/L	<i>n</i>	nmol/L
Stage, Treatment		Serum		Serum
Stage I, Surgery only	61	7.7 (6.0–9.9)	63	5.2 (4.4–6.0)
Stage I, Any radiation	33	8.9 (6.5–12.1)	33	5.4 (4.4–6.5)
Stage II, Any radiation	25	9.1 (6.4–13.0)	24	4.9 (3.9–6.2)
		RBC		RBC
Stage I, Surgery only	61	409.3 (340.2–492.3)	61	163.9 (147.0–182.7)
Stage I, Any radiation	33	435.4 (347.2–545.8)	32	169.8 (147.6–195.2)
Stage II, Any radiation	24	412.3 (317.5–535.4)	22	159.4 (134.9–188.4)

¹ Values are geometric means (95% confidence interval) adjusted for age, ethnicity, study site.

² Within each group, no means are significantly different.

this population (Spearman $r = -0.3$ to -0.4). Homocysteine may be more predictive of cervical cancer risk than low folate because of problems in assessing dietary (29,30) and blood folate status (59) or, more likely, because it identifies additional abnormalities in one-carbon metabolism beyond low folate. Homocysteine can be elevated in response to low folate or low vitamin B-12 because both micronutrients are necessary for the conversion of homocysteine to methionine, or in response to low vitamin B-6, which is required for homocysteine degradation (58). Genetic polymorphisms that alter enzyme activity in the one-carbon metabolism pathway, such as C677T methylenetetrahydrofolate reductase (MTHFR), can also result in elevated homocysteine (57).

In most large epidemiologic studies, cost, feasibility and subject refusal limit the number of times blood can be drawn from subjects. A criticism of these studies is the relevance of data from a single blood draw to "usual" nutrient levels. A strength of the current study is that, although blood was drawn only once, blood folate status was measured with both long-term (RBC) and recent (serum) blood folate markers. However, RBC folate was not more predictive of reduced risk in this study; the OR for cervical cancer risk were similar with the serum and the RBC data. It is possible that folate intake was relatively stable for the women in our study. If similar results are found in other studies using both measures, this will simplify study design for epidemiologists because sample collection and assay is much simpler for serum folate.

We were also able to compare results using a microbiologic and a radiobinding assay. The assays were surprisingly well correlated ($r = 0.9$ for serum and 0.8 for RBC). The radiobinding assay was more reproducible (CV for serum folate = 5.2% for radiobinding and 11.6% for microbiologic), which may explain the modestly stronger associations seen with the radiobinding assay. Ultimately, however, it is not clear which assay is better for epidemiologic studies because the assays may not be measuring the same folate forms, and it is not known what folate forms are especially relevant to cancer. To complicate the picture further, the radiobinding and microbiologic assays give different results for RBC folate for subjects whose one-carbon metabolism is altered by the C677T MTHFR polymorphism (60).

A stronger association may exist at folate concentrations lower than those found in our study. The percentage of folate-deficient subjects in our study was relatively low (<10% for three of the assays) compared with another study that reported strong associations (with 14–24% of subjects deficient for serum folate and 41–52% for RBC folate) (20). However, folate values can vary greatly among laboratories (59) and complicate these comparisons. Cervical cancer is the third most common cancer in women worldwide (61); thus, associations at low folate concentrations may be magnified in developing countries.

Whitehead et al. (1) found megaloblastic cervical abnormalities in 19% of women using oral contraceptives, in the absence of low blood folate or vitamin B-12. No similar abnormalities were found in women not using oral contraceptives. Folic acid therapy was given to eight women using oral contraceptives and their abnormalities were reversed. The authors hypothesized that a localized folate deficiency existed in the cervical tissue of these women. Butterworth et al. (2) further postulated that this localized deficiency could provide an environment that could lead to cervical dysplasia; in a blind, randomized trial, women using oral contraceptives, with mild or moderate cervical dysplasia, showed significant improvement with folate supplementation of 10 mg daily for 3 mo ($P < 0.05$). In an additional sample of 40 healthy hospital

workers, RBC folate was 30% lower in oral contraceptive users compared with nonusers ($P < 0.01$), and among oral contraceptive users, RBC folate was 15% lower in women with dysplasia compared with healthy volunteers (P reported as not significant) (2). However, in a follow-up study among women with mild or moderate cervical dysplasia (80% of whom were oral contraceptive users), no significant improvement was found with folate supplementation of 10 mg/d for 6 mo (17). In the current study, there was no difference in geometric mean serum or RBC folate between women who had used oral contraceptives and those who had not. We did not find an increased risk with low serum or RBC folate among women who used oral contraceptives, even when we focused on women who used oral contraceptives the longest. In fact, we found the strongest inverse association with folate among women who never used oral contraceptives, although the interaction was not significant ($P = 0.08$ for serum and 0.21 for RBC). We did not have a measure of localized folate status in the cervix.

HPV infection is believed to be etiologically associated with most cases of cervical cancer, although only a small minority of women who are HPV-positive progress to cervical cancer (62). If folate helps prevent the incorporation of the HPV virus into the genome, this may explain why only some women infected with HPV progress to cervical cancer. When we restricted our analyses to women believed to have a history of HPV infection, using only controls seropositive to HPV-16, with multiple sexual partners or first intercourse at an early age, the association between folate and cervical cancer risk remained. Thus, low folate could be involved in the progression of cervical cancer after HPV infection. However, among the controls in our study, folate was not predictive of detection of HPV-16 antibodies in serum, suggesting that low folate is unrelated to risk of being infected with HPV.

Our serologic characterization of a history of HPV infection had several important limitations. The HPV-16 virus-like particle ELISA test, which uses serum, may be insensitive relative to DNA hybridization assays, which require cervical tissue scrapings (62), and HPV antibody titers may decrease after surgical treatment for cervical cancer (63). Furthermore, we tested only for antibodies to HPV-16, the most prevalent oncogenic HPV type, which accounts for >50% of invasive cervical cancer in the United States (6), but other oncogenic HPV types exist. Given these limitations, although only 36% of the cases tested seropositive for HPV-16, for the purpose of the HPV stratified analysis, we assumed that all cases, irrespective of their current status by this assay, had once been infected with an oncogenic HPV. Among controls, 15% tested seropositive for HPV-16, similar to a 12% prevalence recently reported among U.S. blood donors, using the same ELISA serologic HPV-16 assay that we used (46). We therefore did not assume false negatives among the controls.

The elevated risk noted in this study is unlikely to be the result of confounding by inadequately measured exposures. Adjustment for potential confounding by accepted cervical cancer risk factors had little effect on the OR. Addition of HPV-16 serologic status to the models actually increased the OR; thus, it is unlikely that better measurement of history of HPV infection would substantially attenuate the effect. Inclusion in the multivariate models of education and income, indicators of poor diet and/or unhealthy lifestyle, only slightly attenuated the OR, suggesting that other lifestyle factors would have little influence on risks. Folate sources such as orange juice and green leafy vegetables are also sources of vitamin C and carotenoids. However, adjustment for intake of

these two micronutrients did not attenuate the folate associations.

Participation bias is also unlikely to explain our findings. Cases and controls who participated in the blood phase of the study did not differ from each other in the study matching factors of age, ethnicity and study site. In addition, the same patterns of risk were seen for education, income and other cervical cancer risk factors in all subjects interviewed and in the subgroup who participated in the blood draw. Furthermore, although 17% of cases had died before the blood draw, any bias would have attenuated the OR if low folate was associated with more advanced disease.

To minimize the possibility that advanced disease or deteriorating health influenced the results, we excluded all stage III and IV cases ($n = 17$) from our analyses. To minimize any treatment effect, blood was collected at least 6 mo after completion of treatment, and those who received chemotherapy ($n = 11$) were excluded from the analyses. In addition, for the women in the analyses, we found no evidence that either disease stage or the treatment received reduced blood folate. Finally, in a small prospective cohort study, serum folate measured at baseline was inversely related to subsequent cervical cancer incidence (26), suggesting that low folate preceded disease.

It is unlikely that the folate would have degraded during storage. Folate was found to be stable in plasma samples frozen for 4 y at -20°C (64), and our samples were stored at -70°C . In addition, both our serum and RBC samples were stabilized with ascorbic acid to keep the folate in a reduced state (65). If any degradation did occur, the resulting misclassification of folate status would have attenuated the OR.

It is unlikely that deliberate improvements in diet after diagnosis of cervical cancer could have biased our results. Because diet-disease relationships with cervical cancer were not well established or publicized at the time our study was conducted, it is not likely that subjects made long-term, healthy diet changes as a result of their disease. Furthermore, at the time of blood draw, subjects were questioned concerning whether they had changed their diet in the past 3 y. An analysis of these data indicated that only two cases reported that, as a result of their cancer, they increased fruit, vegetable and/or grain intake, which are potential sources of folate. A much larger number of cases and controls reported these improvements to their diet for a variety of health reasons. Similarly, only five cases reported a loss of appetite due to illness or treatment, whereas many more cases and controls decreased their intake for reasons such as weight loss.

The increase in invasive cervical cancer risk that we observed with low serum and RBC folate was moderately strong and consistently seen across our four measures of folate status. Our aim was to include new measures of folate status, as well as consider HPV status and other cervical cancer risk factors, to test a hypothesis that has not been consistently supported or refuted in the epidemiologic literature. Because of its size and design, our study provides a robust test of whether folate may be critical at any stage of cervical carcinogenesis. The relationship is biologically plausible, due to folate's role in DNA synthesis, repair and methylation, and is also supported by our strong homocysteine results. The U.S. Food and Drug Administration now requires that enriched grain products be fortified with folic acid at $140\ \mu\text{g}/100\ \text{g}$ of grain product (66). This requirement was established to help women consume at least $400\ \mu\text{g}$ of folic acid daily to reduce the incidence of neural tube defects. Future studies should explore the effects of fortification on the folate status of women at high risk for cervical cancer and monitor its effect on cervical cancer incidence.

ACKNOWLEDGMENTS

The authors are grateful to Barbara Strupp, Alan Mathios and Patrick Stover for invaluable advice and to Katrina Wahl for technical assistance.

LITERATURE CITED

- Whitehead, N., Reyner, F. & Lindenbaum, J. (1973) Megaloblastic changes in the cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. *J. Am. Med. Assoc.* 226: 1421-1424.
- Butterworth, C. E., Hatch, K. D., Gore, H., Mueller, H. & Krumdieck, C. L. (1982) Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am. J. Clin. Nutr.* 35: 73-82.
- Eto, I. & Krumdieck, C. L. (1986) Role of vitamin B12 and folate deficiencies in carcinogenesis. *Adv. Exp. Med. Biol.* 206: 313-330.
- Mason, J. B. & Levesque, T. (1996) Folate: effects on carcinogenesis and the potential for cancer chemoprevention. *Oncology* 10: 1727-1744.
- Butterworth, C. E. (1992) Effect of folate on cervical cancer. Synergism among risk factors. *Ann N.Y. Acad. Sci.* 669: 293-299.
- Bosch, F. X., Manos, M. M., Munoz, N., Sherman, M., Jansen, A. M., Peto, J., Schiffman, M. H., Moreno, V., Kurman, R., Shah, K. V. & International Biological Study on Cervical Cancer (IBSCC) Study Group (1995) Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J. Natl. Cancer Inst.* 87: 796-802.
- Lowy, D. R., Kimbauer, R. & Schiller, J. T. (1994) Genital human papillomavirus infection. *Proc. Natl. Acad. Sci. U.S.A.* 91: 2436-2440.
- Popescu, N. C., DiPaolo, J. A. & Amsbaugh, S. C. (1987) Integration sites of human papillomavirus 18 DNA sequences on HeLa cell chromosomes. *Cytogenet. Cell Genet.* 44: 58-62.
- Popescu, N. C., Amsbaugh, S. C. & DiPaolo, J. A. (1987) Human papillomavirus type 18 DNA is integrated at a single chromosome site in cervical carcinoma cell line SW756. *J. Virol.* 51: 1682-1685.
- Gallego, M. I., Zimonjic, D. B., Popescu, N. C., DiPaolo, J. A. & Lazo, P. A. (1994) Integration site of human papillomavirus type-18 DNA in chromosome band 8q22.1 of C4-1 cervical carcinoma: DNase I hypersensitivity and methylation of cellular flanking sequences. *Genes Chromosomes Cancer* 9: 28-32.
- Brock, K. E., Berry, G., Mock, P. A., MacLennan, R., Truswell, A. S. & Brinton, L. A. (1988) Nutrients in diet and plasma and risk of in situ cervical cancer. *J. Natl. Cancer Inst.* 80: 580-585.
- Verreault, R., Chu, J., Mandelson, M. & Shy, K. (1989) A case-control study of diet and invasive cervical cancer. *Int. J. Cancer* 43: 1050-1054.
- Ziegler, R. G., Brinton, L. A., Hamman, R. F., Lehman, H. F., Levine, R. S., Mallin, K., Norman, S. A., Rosenthal, J. F., Trumble, A. C. & Hoover, R. N. (1990) Diet and the risk of invasive cervical cancer among white women in the United States. *Am. J. Epidemiol.* 132: 432-445.
- Ziegler, R. G., Jones, C. J., Brinton, L. A., Norman, S. A., Mallin, K., Levine, R. S., Lehman, H. F., Hamman, R. F., Trumble, A. C., Rosenthal, J. F. & Hoover, R. N. (1991) Diet and the risk of in situ cervical cancer among white women in the United States. *Cancer Causes Control* 2: 17-29.
- Herrero, R., Potischman, N., Brinton, L. A., Reeves, W. C., Brenes, M. M., Tenorio, F., de Britton, R. C. & Gaitan, E. (1991) A case-control study of nutrient status and invasive cervical cancer. I. Dietary indicators. *Am. J. Epidemiol.* 134: 1335-1346.
- Potischman, N., Brinton, L. A., Laiming, V. A., Reeves, W. C., Brenes, M. M., Herrero, R., Tenorio, F., de Britton, R. C. & Gaitan, E. (1991) A case-control study of serum folate levels and invasive cervical cancer. *Cancer Res.* 51: 4785-4789.
- Butterworth, C. E., Hatch, K. D., Soong, S., Cole, P., Tamura, T., Sauberlich, H. E., Borst, M., Macaluso, M. & Baker, V. (1992) Oral folic acid supplementation for cervical dysplasia: a clinical intervention trial. *Am. J. Obstet. Gynecol.* 166: 803-809.
- Butterworth, C. E., Hatch, K. D., Macaluso, M., Cole, P., Sauberlich, H. E., Soong, S., Borst, M. & Baker, V. V. (1992) Folate deficiency and cervical dysplasia. *J. Am. Med. Assoc.* 267: 528-533.
- Buckley, D. I., McPherson, R. S., North, C. Q. & Becker, T. M. (1992) Dietary micronutrients and cervical dysplasia in southwestern American Indian women. *Nutr. Cancer* 17: 179-185.
- VanEenwyk, J., Davis, F. G. & Colman, N. (1992) Folate, vitamin C, and cervical intraepithelial neoplasia. *Cancer Epidemiol. Biomark. Prev.* 1: 119-124.
- Liu, T., Soong, S., Wilson, N. P., Craig, C. B., Cole, P., Macaluso, M. & Butterworth, C. E., Jr. (1993) A case control study of nutritional factors and cervical dysplasia. *Cancer Epidemiol. Biomark. Prev.* 2: 525-530.
- Childers, J. M., Chu, J., Voigt, L. F., Feigt, P., Tamimi, H. K., Franklin, E. W., Alberts, D. S. & Meyskens, F. L. (1995) Chemoprevention of cervical cancer with folic acid: a phase III Southwest Oncology Group intergroup study. *Cancer Epidemiol. Biomark. Prev.* 4: 155-159.
- Kwasniewska, A., Tukendorf, A. & Senczuk, M. (1997) Folate deficiency and cervical intraepithelial neoplasia. *Eur. J. Gynaecol. Oncol.* 18: 526-530.
- Wideroff, L., Potischman, N., Glass, A. G., Greer, C. E., Manos, M. M., Scott, D. R., Burk, R. D., Sherman, M. E., Wacholder, S. & Schiffman, M. (1998) A nested case-control study of dietary factors and the risk of incident cytological abnormalities of the cervix. *Nutr. Cancer* 30: 130-136.
- Kanetsky, P. A., Gammon, M. D., Mandelblatt, J., Zhang, Z., Ramsey, E.,

- Dnistrian, A., Norkus, E. P. & Wright, T. C. (1998) Dietary intake and blood levels of lycopene: association with cervical dysplasia among non-Hispanic, black women. *Nutr. Cancer* 31: 31-40.
26. Alberg, A. J., Selhub, J., Shah, K. V., Viscidi, R. P., Comstock, G. W. & Helzlsouer, K. J. (2000) The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. *Cancer Epidemiol. Biomark. Prev.* 9: 761-764.
27. Goodman, M. T., McDuffie, K., Hernandez, B., Wilkens, L. R. & Selhub, J. (2000) Case-control study of plasma folate, homocysteine, vitamin B12, and cysteine as markers of cervical dysplasia. *Cancer* 89: 376-382.
28. Life Sciences Research Office FASEB (1995) Third report on nutrition monitoring in the United States. U.S. Government Printing Office, Washington, DC.
29. Anderson, S. A. & Talbot, J. M. (1981) A review of folate intake, methodology, and status. Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, MD.
30. Subar, A. F., Block, G. & James, L. D. (1989) Folate intake and food sources in the US population. *Am. J. Clin. Nutr.* 50: 508-516.
31. Herbert, V. (1987) Recommended dietary intakes (RDI) of folate in humans. *Am. J. Clin. Nutr.* 45: 661-670.
32. Sauberlich, H. E., Kretsch, M. J., Skala, J. H., Johnson, H. L. & Taylor, P. C. (1987) Folate requirement and metabolism in nonpregnant women. *Am. J. Clin. Nutr.* 46: 1016-1028.
33. Gregory, J. F. (1989) Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. *Adv. Food Nutr. Res.* 33: 1-101.
34. Glynn, S. A. & Albanes, D. (1994) Folate and cancer: a review of the literature. *Nutr. Cancer* 22: 101-119.
35. Herbert, V. (1967) Biochemical and hematologic lesions in folic acid deficiency. *Am. J. Clin. Nutr.* 20: 562-569.
36. Gibson, R. S. (1990) Principles of Nutritional Assessment. Oxford University Press, New York, NY.
37. Colman, N. (1981) Laboratory assessment of folate status. *Clin. Lab. Med.* 1: 775-796.
38. Brinton, L. A., Schairer, C., Haenszel, W., Stolley, P., Lehman, H. F., Levine, R. & Savitz, D. A. (1986) Cigarette smoking and invasive cervical cancer. *J. Am. Med. Assoc.* 255: 3265-3269.
39. Brinton, L. A., Huggins, G. R., Lehman, H. F., Mallin, K., Savitz, D. A., Trapido, E., Rosenthal, J. & Hoover, R. (1986) Long-term use of oral contraceptives and risk of invasive cervical cancer. *Int. J. Cancer* 38: 339-344.
40. Brinton, L. A., Hamman, R. F., Huggins, G. R., Lehman, H. F., Levine, R. S., Mallin, K. & Fraumeni, J. F., Jr. (1987) Sexual and reproductive risk factors for invasive squamous cell cervical cancer. *J. Natl. Cancer Inst.* 79: 23-30.
41. Scott, J. M., Ghanta, V. & Herbert, V. (1974) Trouble-free microbiologic serum and red cell folate assays. *Am. J. Med. Technol.* 40: 125-134.
42. Longo, D. L. & Herbert, V. (1976) Radioassay for serum and red cell folate. *J. Lab. Clin. Med.* 87: 138-151.
43. Waxman, S., Schreiber, C. & Herbert, V. (1971) Radioisotopic assays for measurement of serum folate levels. *Blood* 38: 219-228.
44. Westgard, J. O., Barry, P. L., Hunt, M. R. & Groth, T. (1981) A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin. Chem.* 27: 493-501.
45. SAS Institute Inc. (1996) The SAS System for Windows, Release 6.12 TS Level 0020. SAS Institute, Cary, NC.
46. Strickler, H. D., Kirk, G. D., Figueroa, P., Ward, E., Braithwaite, A. R., Escoffery, C., Drummond, J., Goebel, B., Waters, D., McClimens, R. & Manns, A. (1999) HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. *Int. J. Cancer* 80: 339-344.
47. Strickler, H. D., Hildesheim, A., Viscidi, R. P., Shah, K. V., Goebel, B., Drummond, J., Waters, D., Sun, Y., Hubbert, N. L., Wacholder, S., Brinton, L. A., Han, C. L., Nasca, P. C., McClimens, R., Turk, K., Devairakkam, V., Leitman, S., Martin, C. & Schiller, J. T. (1997) Interlaboratory agreement among results of human papillomavirus type 16 enzyme-linked immunosorbent assays. *J. Clin. Microbiol.* 35: 1751-1756.
48. Breslow, N. E. & Day, N. E. (1980) Statistical Methods in Cancer Research. Vol 1, The Analysis of Case-Control Studies. Int. Agency Res. Cancer, Lyon, France.
49. Hosmer, D. W. & Lemeshow, S. (1989) Applied Logistic Regression. John Wiley & Sons, New York, NY.
50. Ford, E. S. & Bowman, B. A. (1999) Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. *Am. J. Clin. Nutr.* 69: 476-481.
51. Senti, F. R. & Pilch, S. M. (1984) Assessment of the folate nutritional status of the U.S. population based on data collected in the second National Health and Nutrition Examination Survey, 1976-1980. Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, MD.
52. Scott, J. M. (1999) Folate and vitamin B12. *Proc. Nutr. Soc.* 58: 441-448.
53. Weinstein, S. J., Ziegler, R. G., Selhub, J., Fears, T. R., Strickler, H. D., Brinton, L. A., Hamman, R. F., Levine, R. S., Mallin, K. & Stolley, P. D. (2001) Elevated serum homocysteine levels and increased risk of invasive cervical cancer in US Women. *Cancer Causes Control* (in press).
54. Savage, D. G., Lindenbaum, J., Stabler, S. P. & Allen, R. H. (1994) Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am. J. Med.* 96: 239-246.
55. Mason, J. B. & Miller, J. W. (1992) The effects of vitamins B12, B6, and folate on blood homocysteine levels. *Ann. N.Y. Acad. Sci.* 669: 197-204.
56. Selhub, J. & Rosenberg, I. H. (1996) Folic acid. In: Present Knowledge in Nutrition, 7th ed. (Ziegler, E. E. & Filer, L. J., Jr., eds.), pp. 206-219. ILSI Press, Washington, DC.
57. Frosst, P., Blom, H. J., Milos, R., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J. H., den Heijer, M., Kluijtmans, L. A. J., van den Heuvel, L. P. & Rozen, R. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* 10: 111-113.
58. Selhub, J., Jacques, P. F., Wilson, P. W. F., Rush, D. & Rosenberg, I. H. (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *J. Am. Med. Assoc.* 270: 2693-2698.
59. Gunter, E. W., Bowman, B. A., Caudill, S. P., Twite, D. B., Adams, M. J. & Sampson, E. J. (1996) Results of an international round robin for serum and whole-blood folate. *Clin. Chem.* 42: 1689-1694.
60. Molloy, A. M., Mills, J. L., Kirke, P. N., Whitehead, A. S., Weir, D. G. & Scott, J. M. (1998) Whole-blood folate values in subjects with different methylenetetrahydrofolate reductase genotypes: differences between the radioassay and microbiological assays. *Clin. Chem.* 44: 186-188.
61. Parkin, D. M., Pisani, P. & Ferlay, J. (1999) Estimates of the worldwide incidence of 25 major cancers in 1990. *Int. J. Cancer* 80: 827-841.
62. International Agency for Research on Cancer (1995) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Human Papillomaviruses. Int. Agency Res. Cancer, Lyon, France.
63. Di Lonardo, A., Marcante, M. L., Poggiali, F. & Venuti, A. (1998) HPV 16 E7 antibody levels in cervical cancer patients: before and after treatment. *J. Med. Virol.* 54: 192-195.
64. Ocke, M. C., Schrijver, J., Obermann-De Boer, G. L., Bloemberg, B. P. M., Haenen, G. R. M. M. & Kromhout, D. (1995) Stability of blood (pro)vitamins during four years of storage at -20°C: consequences for epidemiologic research. *J. Clin. Epidemiol.* 48: 1077-1085.
65. Tamura, T. (1990) Microbiological assay of folates. In: Folic Acid Metabolism in Health and Disease (Picciano, M. F., Stokstad, E. L. R. & Gregory, J. F., eds.), pp. 121-137. Wiley-Liss, New York, NY.
66. U.S. Food and Drug Administration (1996) Food standards. Amendment of standards of identity for enriched grain products to require addition of folic acid. 21 CFR part 136, 137, and 139. *Fed. Regist.* 61: 8781-8797.