Maternal administration of folic acid diminishes the risk of neural tube defects (NTDs) in offspring, but whether folic acid exerts a similar effect in diabetic pregnancy is unknown. The aim was to investigate whether maldevelopment in rat embryos caused by exposure to diabetes in vivo or high-glucose concentrations in vitro is affected by subcutaneous administration of folic acid to the pregnant mother or by adding the compound to the culture medium, respectively. Exposure of embryos to maternal diabetes in vivo or 30 mmol/l glucose in vitro yielded an increased malformation rate (71 and 88% NTD, respectively) and lowered somite number and crown-rump length compared with control embryos. When we injected folic acid into the diabetic pregnant rat, or added 2 mmol/l folic acid to the culture medium with high glucose, the embryonic parameters improved (3 and 5% NTD, respectively). The present work shows that administration of folic acid can diminish diabetes-induced maldevelopment. This suggests that folic acid supplementation may have a role in the prevention of malformations in diabetic pregnancy. *Diabetes* 54:546–553, 2005

Diabetic pregnancy is associated with increased risk for congenital malformations (1,2), including neural tube defects (NTDs) (3). Both genetic and environmental factors are involved in the induction of NTDs and other malformations in diabetic pregnancy. Similarly, the induction of NTDs in normal pregnancy is affected by genetic and environmental factors, one of which is the availability and metabolism of folic acid. It has been shown that periconceptional supplementation with folic acid can prevent the incidence of NTDs (4,5), by 72% in a recent study (6). It has also been shown that folic acid administration fails to completely prevent the developmental damage (7). However, the antiteratogenic mechanism of folic acid is still unclear. A favored notion is that the addition of extra folic acid corrects subtle alterations in the folic acid metabolism. Folic acid supplementation would thereby normalize disturbances in several intracellular metabolic pathways that critically depend on an optimal level of intracellular folic acid, e.g., the methylation and remethylation of homocysteine (8,9).

However, some studies show no correlation between folic acid levels in the mother and incidence of NTDs in infants (10,11). In addition, supplementation of folic acid does not reduce the NTD rate to zero in any clinical study (4,5), which suggests the existence of nonfolate deficiency–dependent NTDs. This notion is supported by several studies of animal NTD models where folic acid supplementation fails to completely prevent the developmental damage (12,13).

The cellular uptake of folate (as 5-methyltetrahydrofolate) is mediated by clusters of membrane-bound transport proteins, folate binding proteins (folbps), of which two isoforms have been identified in mice: folbp-1 and folbp-2. In rats and humans, only one folbp has been described so far. The regulation of the folbps is not completely known, but it has been observed that folate deficiency leads to an upregulation of the number of folbps bound to the membrane (14–17).

The aim of this study was to examine in a rat model of diabetic embryopathy whether folic acid supplementation would affect the teratological impact of maternal diabetes in vivo and whether such supplementation in vitro would also alter the effect of one major teratogen in diabetic pregnancy—hyperglycemia. In addition, we wanted to investigate whether diabetes in vivo or high-glucose concentration in vitro regulates gene expression of embryonic folbp and folic acid levels in this rat model and whether folic acid addition affects gene expression of folbp and folic acid concentration in the embryos.

For this purpose, we injected diabetic rats with 15 mg/kg folic acid daily during gestation and evaluated the putative antiteratogenic effect of the drug on gestational day 10 and 11. In addition, we cultured rat embryos at gestational day 9 for 24 or 48 h (corresponding to gestational day 10 and 11, respectively) in 10 or 30 mmol/l glucose with or without folic acid in different concentrations. We examined the effect of folic acid addition on embryonic growth and malformations. We also collected embryos from normal and diabetic rats with or without folic acid injections, as well as embryos from in vitro low-
or high-glucose culture with or without folic acid supplementation, for evaluation of folbp gene expression and folic acid concentration on gestational day 10 and 11 and after 24 and 48 h of culture, respectively.

RESEARCH DESIGN AND METHODS

Embryos were obtained from a local outbred Sprague-Dawley rat strain with an increased incidence of congenital malformations in diabetic pregnancy (18,19). All rats were fed a commercial pelleted diet (AB Analyizen, Lidköping, Sweden) and had free access to food and tap water. They were maintained at an ambient temperature of 22°C with a 12:12 light/dark cycle. Female and male rats were caged together overnight. The morning that conception was verified by the presence of sperm in a vaginal smear was designated gestational day 0.

Induction of diabetes. Induction of diabetes was performed in a subset of female rats by injecting 40 mg/kg streptozotocin (Sigma, St. Louis, MO) into the tail vein 1 week before mating commenced, i.e., 1–3 weeks before conception. A state of manifest diabetes was confirmed 1 week after the injection by the presence of a serum glucose level >20 mmol/l (Glucose Analyzer II; Beckman Instruments, Fullerton, CA) in the female rat. Non-streptozotocin-injected female rats served as nondiabetic normal controls.

Injection of folic acid. Control and manifest diabetic pregnant rats were given 15 mg/kg folic acid (Sigma) by daily subcutaneous injections in the neck for folic acid determination (see MEASUREMENT OF FOLIC ACID below). Pregnant nondiabetic rats were killed by cervical dislocation on gestational day 9, and the embryos in their intact yolk sacs were processed for total RNA isolation (see PREPARATION OF TOTAL RNA below), and some embryos were used for folic acid determination (see MEASUREMENT OF FOLIC ACID below).

Whole-embryo culture. Pregnant nondiabetic rats were killed by cervical dislocation on gestational day 9, and the embryos in their intact yolk sacs were cultured (four to five embryos per Falcon 50-ml culture tube) in 5 ml of 80% immediately centrifuged rat serum (20) and 20% saline in a roller incubator at 45 rpm at 37°C or 38°C. The 5% difference in temperature was used to achieve different compounds from stock solutions yielding an end concentration of 10 or 30 mmol/l glucose (Apoteksbolaget, Stockholm, Sweden), 0.25-2 mmol/l folic acid (Sigma-Aldrich Sweden). These concentrations were obtained from previous pilot experiments where different doses of the compounds were tested, such as 0.1–5 mmol/l folic acid.

After 24 or 48 h of culture, the embryos were dissected out of their yolk sacs and examined under a stereo microscope. A normal embryo was fully rotated with a closed neural tube, whereas malformed embryos were divided into embryos with a single malformation or embryos with multiple malformations (21). Embryos with a single malformation had an open neural tube in the rhombencephalic area (i.e., NTD) or a tail twist, whereas embryos with multiple malformations frequently exhibited NTDs, rotational defects, and/or heart enlargement. In addition, we determined the crown-rump length and somite number of each embryo. Some embryos were processed for total RNA isolation (see PREPARATION OF TOTAL RNA below), and some embryos were used for folic acid determination (see MEASUREMENT OF FOLIC ACID below).

Preparation of cDNA. One microgram of total RNA was used for reverse transcription. First-strand cDNA synthesis used first-strand beads (Ready To Go; Pharmacia Biotech) according to the manufacturer’s description. The resulting cDNA was diluted threefold with water previously treated with diethyl pyrocarbonate.

Analysis of folbp mRNA levels. One microliter of the cDNA purified from embryos was amplified and measured with real-time PCR using LightCycler (Roche Diagnostics, Mannheim, Germany). Cybergene (Novum Research Park, Huddinge, Sweden; Table 1) made specific primers for folbp. After the LightCycler protocol, 1 μl of the cDNA was amplified in a final volume of 10 μl containing 0.2 μl RNase-free water, 1 μl FastStart DNA Master SYBR Green I (Roche Molecular Biochemicals, Mannheim, Germany), 2 μmol/l MgCl₂, and 0.5 μmol/l of the sense and antisense primers of folbp. In addition, glucose-6-phosphate dehydrogenase (G6PDH) sense and antisense primers (TIB Moliob, Berlin, Germany) were used in the same manner as for folbp. We have previously assessed the stability of expression of various housekeeping genes and found the G6PDH gene to be constant in day 10–11 embryos exposed to high glucose in vitro or diabetes in vivo (data not shown); therefore, here we chose the G6PDH gene as a reference in the real-time PCR protocol.

The LightCycler Run version 5.32 program was used with the following parameters: 1) denaturation at 95°C for 10 min and 2) amplifying with a total of 45 cycles, each cycle with a denaturation temperature of 95°C for 15 s, annealing temperature of 60°C for 10 s, and elongation temperature of 72°C for 15 s.

Controls were included in each run of the real-time PCR assay; for each primer pair, one sample with no cDNA (containing only RNase-free water) was included. Furthermore, to exclude the possibility of remaining DNA fragments in the samples, 10 ng total RNA of each sample was amplified in the LightCycler. We found no PCR product in the water or in the total RNA samples. Furthermore, we excluded the avian myeloblastosis virus–RT enzyme in the cDNA preparation and found that no PCR product could be amplified.

Results were analyzed for each sample with relative quantification comparing the difference between sample and control crossing point (Cp) values. To render a true value for each mRNA level, the calculated difference was transformed according to the following:

\[
2^{-\Delta\Delta Cp_{\text{sample}}}
\]

to yield the ratio folbp/G6PDH.

Measurement of folic acid. Whole day 10 or 11 embryos (or cultured embryos of analogous age) were submerged in 400 μl PBS containing 1% sodium ascorbate (Merck Eurolab), sonicated, and analyzed with a competitive immunoanalysis (ADVIA Centaur; Bayer Health Care, Gothenburg, Sweden) in accordance with the manufacturer’s instructions. Briefly, the sonicated samples were diluted 10-fold by addition of PBS-ascorbate buffer, and 150 μl was subjected to analysis where the folic acid in the sample competes with acridine ester–labeled folic acid for a limited number of folic acid binding proteins (the latter are labeled with biotin). The amount of acridine ester–labeled folic acid bound to the protein is then estimated by measuring the emitted chemiluminescence of each sample, which is inversely proportional to the amount of embryonic folic acid in the sample. In each sample, total protein content was determined by the method of Lowry et al. (22) using BSA as the standard. The final result was expressed as amount folic acid per amount embryonic protein.

Ethical and statistical evaluation. The Animal Ethical Committee of the Medical Faculty of Uppsala University approved the research protocol, including all experimental procedures involving animals. The comparisons between different experimental groups were based on individual embryos. Differences between means were evaluated by ANOVA, where the applied post hoc test was Fisher’s protected least-square difference at the 95% significance level. The malformation rates were compared between groups with χ² statistics (test of homogeneity with 2 × 2 contingency tables) (23). In particular, differences in malformation rate were evaluated by comparing the number of embryos that were normal (showing no malformations, third column, Table 2) and malformed (showing at least one malformation, second

### Table 1

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Cycles</th>
<th>Annealing temperature (°C)</th>
<th>Base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PDH forward</td>
<td>5’-ATTGACCACCTACCTGGGCA-3’</td>
<td>28</td>
<td>60</td>
</tr>
<tr>
<td>G6PDH reverse</td>
<td>5’-GGATACATCTTACACATTTTGCCT-3’</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Folbp forward</td>
<td>5’-TCCTGGCCTACCTTACCTCATC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folbp reverse</td>
<td>5’-AGAACCTGGCAACACCTTCGT-3’</td>
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<td></td>
</tr>
</tbody>
</table>
RESULTS

Embryos exposed to a diabetic environment in vivo and high glucose in vitro showed an increased number of severe malformations (Table 2). In particular, we found a very high rate of NTDs caused by maternal diabetes in vivo or high-glucose concentration in vitro, most often combined with other somatic malformations (Table 2). In addition, we found decreased crown-rump length and decreased number of somites in embryos from manifest diabetic rats and high-glucose–cultured embryos compared with control embryos (from a normal mother and cultured at a low-glucose concentration) (Fig. 1).

Subcutaneous injection of folic acid to the manifest diabetic rats during gestation diminished malformation rate in the fetuses (Table 2). The folic acid injections also largely normalized crown-rump length and somite number in the offspring of manifest diabetic rats (Fig. 1). Supplementation of 1–2 mmol/l folic acid to the culture medium with 30 mmol/l glucose concentration decreased the malformation rate—in particular, the incidence of NTDs (Table 2). The folic acid supplementation also increased crown-rump length and somite number compared with embryos cultured in 30 mmol/l glucose concentration (Fig. 1). Supplementation of 0.25 mmol/l folic acid did not affect the glucose-induced malformations (Table 2) or the lowered morphological parameters (Fig. 1).

In particular, both diabetes in vivo and high glucose in vitro increased the incidence of NTDs in the embryos—in vivo from 4 to 71% and in vitro from 0 to 88% (Table 2). Injection of folic acid to pregnant manifest diabetic rats lowered the NTD rate to 3% (Table 2). Likewise, supplementation of 1 mmol/l folic acid to the 30-mmol/l glucose cultures decreased the incidence of NTDs to 29%, and addition of 2 mmol/l folic acid further decreased the rate of NTDs to 5% (Table 2).

The gene expression of folbp was decreased in the offspring of manifest diabetic rats compared with the offspring of control rats, both on day 10 and 11 (Fig. 2A). The same pattern was found in embryos cultured in 30 mmol/l glucose compared with embryos cultured in 10 mmol/l glucose after 24 h of culture (Fig. 2B). When folic acid was injected in pregnant control and manifest diabetic rats, the folbp gene expression in the embryos was decreased and normalized, respectively, at gestational day 10. On gestational day 11, folic acid injections did not affect follic gene expression in embryos of control rats, whereas it normalized the suppressed folbp gene expression in embryos of diabetic rats (Fig. 2A). Furthermore, when 2 mmol/l folic acid was added to the medium, we found decreased and normalized folbp gene expression, respectively, in embryos cultured for 24 h (Fig. 2B). In contrast, after 48 h of culture, there were no differences in folbp gene expression between embryos cultured in 10 or 30 mmol/l glucose with the addition of folic acid (Fig. 2B).

The folic acid concentration was decreased in embryos of manifest diabetic rats compared with embryos of control rats on gestational day 10, but not on day 11 (Fig. 3A). In the embryos cultured in 30 mmol/l glucose, there was no change in folic acid concentration compared with embryos cultured in 10 mmol/l glucose on gestational day 10 and 11 (Fig. 3B). Supplementation of exogenous folic acid in vivo and in vitro, however, markedly increased the folic acid concentration in day 10 and 24-h–cultured embryos, respectively (Fig. 3). On gestational day 11 and in 48-h–cultured embryos, folic acid supplementation only increased embryonic folic acid concentration in diabetic and 30-mmol/l–cultured embryos, not in control and 10-mmol/l–cultured embryos (Fig. 3).

### TABLE 2

Morphology of day 11 rat embryos

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Normal embryos</th>
<th>Open neural tube (single)</th>
<th>Open neural tube (combined)</th>
<th>Tail twist (single)</th>
<th>Somatic malrotation</th>
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<tr>
<td>N</td>
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<td>25</td>
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<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>N+F</td>
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<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>MD</td>
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<td>5*</td>
<td>0</td>
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<td>17</td>
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<tr>
<td>MD+F</td>
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<td>29†</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10G</td>
<td>31</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10G+2F</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>30</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>30G</td>
<td>34</td>
<td>3‡</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>30G+0.25 F</td>
<td>3</td>
<td>0‡</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>30G+1F</td>
<td>14</td>
<td>8§§</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>30G+2F</td>
<td>21</td>
<td>14§</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

Morphology of day 11 embryos from normal (N) and diabetic (MD) rats, some of which were supplemented with folic acid (N+F and MD+F). Morphology of embryos cultured in vitro for 48 h in 10 or 30 mmol/l glucose (10G or 30G) and cultured with the addition of 0.25, 1, or 2 mmol/l folic acid (+0.25F, +1F, or +2F). The malformation categories are not mutually exclusive. Open neural tube: a single malformation or combined with other malformations. Tail twist: tail not completely curled. Somatic malrotation: straight tail or nonrotated body. These embryos always have open neural tube; therefore, there is an overlap with open neural tube (combined). Differences in malformation rate were evaluated by comparing the numbers of embryos, which were normal (showing no malformations, third column) and malformed (show at least one malformation, second minus third column) in one experimental group with the analogous numbers in another group in a 2 × 2 table format. The resulting significances are displayed in the third column: *P < 0.05 vs. N; †P < 0.05 vs. MD; ‡P < 0.05 vs. 10G; §P < 0.05 vs. 30G (χ² statistics).
FIG. 1. Crown-rump length (A) and somite number (B) in day 11 embryos from normal (N) and diabetic (MD) rats, some of which were supplemented with folic acid (NF and MDF). Crown-rump length (A) and somite number (B) in embryos cultured for 48 h in 10 or 30 mmol/l glucose (10G or 30G) and cultured with the addition of 0.25, 1, or 2 mmol/l folic acid (+0.25F, +1F, or +2F). *P < 0.05 vs. N; †P < 0.05 vs. MD; ‡P < 0.05 vs. 10G; §P < 0.05 vs. 30G (ANOVA).
The most important finding in the present study was diminished diabetes- and glucose-induced teratogenicity in rat embryos by folic acid supplementation in vivo and in vitro. Most diabetes- and glucose-induced embryonic damage included NTDs, and, indeed, this malformation was corrected by the folic acid addition. Furthermore, we found decreased mRNA levels of the folic acid transporter

**FIG. 2.** A: Relative expression of folbp mRNA in embryos of normal (N) and diabetic (MD) rats on gestational day 10 and 11, some of which were supplemented with folic acid (NF and MDF). B: Folbp expression in embryos cultured in 10 or 30 mmol/l glucose (10G or 30G) for 24 h (corresponding to gestational day 10) or 48 h (corresponding to day 11) and cultured with the addition of 2 mmol/l folic acid (10GF 10, 30GF 10, 10GF 11, 30GF 11). *P < 0.05 vs. N 10 (A) and 10G 10 (B); **P < 0.05 vs. MD 10 (A) and 30G 10 (B); ***P < 0.05 vs. N 11 (A); ****P < 0.05 vs. MD 11 (A) (ANOVA).
folbp in the embryos exposed to diabetes in vivo and to high-glucose concentration in vitro, and decreased embryonic concentration of folic acid in day 10 embryos of diabetic rats. These findings suggest that a diabetes-like environment affects folate metabolism in the developing embryo and, furthermore, that a primary cellular consequence of maternal diabetes may be diminished folbp-mediated uptake of folate due to a decreased number of
Folic acid transporters in the embryo. In pregnant diabetic animals, the transport deficiency helps create a state of intracellular folic acid deficiency in the embryo, whereas the embryonic exposure in the in vitro culture is not long enough to decrease the folic acid levels in the embryo. We suggest, however, based on our findings, that both diabeties in vivo and high glucose levels in vitro create a state of functional folic acid deficiency in the embryo. In line with this concept, supplementation of large amounts of exogenous folic acid may overcome the functional deficiency and thereby rectify the disturbed embryonic development.

Decreased folic acid transport has been shown to be teratogenic in mice with a null mutation of folbp-1. Embryos homozygous for the knockout folbp-1 gene were shown to lack folbp-1 function, and the offspring displayed excess intrauterine mortality, exencephaly, and NTDs (24). However, mouse embryos with a null mutation of the other folate binding protein, folbp-2, displayed no abnormal phenotype, despite impaired folbp-2 function, thereby indicating a low functional importance of folbp-2 in embryogenesis, at least in mice (24). In addition, mice with inactivated folbp-2 gene developed more NTDs in response to in utero exposure to arsenic than did control mice exposed to the same teratogen (25). In this study, exencephaly incidence was 41% in folbp-2/- embryos compared with 24% in control folbp-2+/+ embryos (25). These results suggest that a major inhibition of folate transport may directly induce NTDs in the offspring, whereas a less pronounced disturbance of folate transport may act in a synergistic manner to enhance the effect of other teratogens.

Diabetes in vivo and high glucose in vitro decreased folbp gene expression in day 10 embryos and in the corresponding 24-h–cultured embryos, which suggests that a decreased folate uptake during organogenesis may be a component of diabetes-induced teratogenesis. This notion was supported by the concomitant findings of decreased folic acid concentration of the day 10 embryos of diabetic rats. The handling of folate in the developing conceptus, however, appears to be affected by several intra- and extra-embryonic factors. Our data suggest that the time of gestation (more active folic acid metabolism on day 10 compared with day 11) affects folic acid levels and handling. The length of exposure to, and the severity of, the diabetes-like milieu may also be of importance, since decreased folbp gene expression was also apparent in the day 11 embryos of diabetic rats, which have been exposed to a diabetic environment for 11 days, in contrast to the embryos that had been cultured in vitro for 48 h in high-glucose concentration, i.e., exposed to a diabetes-like environment for a shorter period of time. Also, day 10 embryos of diabetic rats showed marked folic acid deficiency, whereas their high-glucose–cultured age-matched counterparts, embryos cultured in 30 mmol/l glucose for 24 h, did not show decreased folic acid levels, again illustrating the importance of the duration of embryonic exposure to a diabetic environment.

Supplementation of folic acid decreased folbp gene expression and increased folic acid concentration in control and 10-nmol/l glucose–cultured embryos both in vivo and in vitro on gestational day 10. This was interpreted as a downregulation of the folbp receptor gene as a result of abundance of the ligand in this period of organogenesis. Notably, 1 day later, on gestational day 11, the folbp mRNA and folic acid levels in control and 10-nmol/l glucose–cultured embryos were lower than on day 10 and mainly unresponsive to folic acid supplementation, both in vivo and in vitro. Furthermore, we found that injection of folic acid to manifest diabetic animals and addition of folic acid to high-glucose culture resulted in an upregulation and normalization of the folbp gene expression in day 10 and day 11 embryos of diabetic rats and 24-h high-glucose–cultured embryos, as well as markedly increased folic acid tissue levels in these embryos, actually exceeding the folic acid levels in the control embryos. This finding, which was somewhat surprising, shows that the relation between ligand and transport protein mRNA levels is complex and affected by the intra- and extracellular milieu of the embryo. The full explanation of these relations will have to await further studies.

NTDs have been reported in a number of mouse KO gene models, where the exact role of folate metabolism has been difficult to clarify. For instance, embryos and fetuses of mice with the Cited2 gene knocked out (Cited2−/− mutants) exhibit heart defects and exencephaly and die at late gestation (26). Cited2 is a member of a gene family of nuclear transactivators and is evidently required for the survival and normal development of the neuroepithelial cells. Treatment with folic acid reduced the exencephalic phenotype in the Cited2−/− embryos both in vivo and in vitro without affecting folate homeostasis (26), thereby suggesting that abolished Cited2 function affects embryonic development by mechanisms other than disturbed folate metabolism. In contrast, in a recent study of Splotch mice with a defect Pax-3 function and a high incidence of NTDs, folic acid supplementation did not protect the fetuses from neural tube malformations (27). It appears reasonable to conclude that folate metabolites are involved in a number of cellular pathways and that changes in folate metabolism other than plain disturbance of cellular uptake may be of teratological importance and, consequently, that there are NTDs resistant to folate supplementation.

The relation between diabetes and folate metabolism in general, and between maternal diabetes and maternal-fetal handling of folate in particular, has not been studied in detail. In streptozotocin-induced diabetic rats, deranged folate metabolism has been reported, as evidenced by decreased intracellular folate levels and increased urinary folate excretion (28), supporting the view of diabetes-induced disturbance of folate metabolism. On the other hand, there was no detectable abnormal metabolism of folate in pregnant women with diabetes, as estimated by unchanged folate levels in serum, erythrocytes, and urine compared with normal pregnant women (11). Given our results, however, it may be argued that the authors did not assess embryo-fetal folate metabolism and that a diabetes-induced change in embryonic folate uptake caused by diminished number of folate transporters may have a teratological impact on the offspring.

We postulate, based on our findings, that a diabetic environment induces a state of functional folic acid deficiency in the embryo during early organogenesis. This deficiency is only partly a transport and concentration deficiency, since the high-glucose–cultured embryos did...
not show decreased levels of folic acid, despite the presence of marked developmental dysmorphogenesis, which was diminished by folic acid supplementation. The postulated deficiency may instead be caused by an increased demand for folic acid and related to a recently recognized beneficial aspect of folic acid supplementation, the antioxidative activity of the compound (29–31). Because diabetes-induced developmental damage in vivo and glucose-induced embryonic dysmorphogenesis in vitro have been associated with enhanced oxidative stress and antioxidative treatment has been shown to be beneficial (32–38), it may be relevant to consider the possibility that some of the antiteratogenic effects of the drug in the present study are due to antioxidative effects of folic acid in the exposed embryos.

Nevertheless, supplementation of folic acid before conception and during pregnancy has been shown to reduce the incidence of NTDs in women with a previous NTD-affected pregnancy (6). Our results suggest that supplementation with folic acid may have a preventive antiteratogenic role also in diabetic pregnancy.

ACKNOWLEDGMENTS

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