# The influence of dietary folate supplementation on the incidence of teratogenesis in zinc-deficient rats

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Two studies were conducted to investigate the possibility that pteroylmonoglutamic acid supplementation would alleviate teratogenesis in zinc-deficient rats. Pregnant rats of the Wistar strain were fed on Zn-deficient ( < 0·5 mg Zn/kg) or Zn-supplemented (75 or 95 mg Zn/kg) diets from mating until day 18·5 of gestation. The basal level of pteroylmonoglutamic acid added to all diets (0·56 mg/kg) was supplemented with 30-200 mg/kg in selected diets. Dietary Zn deprivation resulted in fetal resorption, fetal growth retardation and reduced concentrations of Zn in fetuses and maternal plasma and tibia. Low maternal body-weight at conception emerged as an important determinant of risk of resorption in Zn-deficient rats. Dietary Zn deficiency resulted in reduced maternal plasma folate concentrations and these values were inversely correlated with litter size or weight in Zn-deficient rats. Pteroylmonoglutamic acid supplementation increased maternal plasma folate concentrations, but did not reduce the high incidence of teratogenesis which occurred in Zn-deficient rats. Supplementation of Zn-deficient rats with pteroylmonoglutamic acid significantly increased the incidence of clubbed foot and tended to increase the incidence of brain or meningeal abnormalities, or both, and cleft palate, but did not reduce maternal or fetal Zn status. Pteroylmonoglutamic acid supplementation also increased the weights of Zn-supplemented control fetuses.

Zinc deficiency: Folate supplementation: Teratogenesis: Rat

Dietary zinc deficiency (Hurley & Swenerton, 1966; Rogers *et al.* 1985), or low folic acid status (Kalter & Warkany, 1959) in pregnant rats results in teratogenesis, and a marked potentiation of teratogenesis occurs when both deficiencies are combined (Bremert *et al.* 1989). In addition, the results of several studies indicate that dietary Zn deficiency brings about a reduction in folic acid status. Williams *et al.* (1973) and Tamura *et al.* (1987) observed lower hepatic and plasma folate concentrations respectively in Zn-deficient than in control rats. Fuller *et al.* (1988) observed a lower concentration of folate in blood from Zn-deficient pregnant rats than in controls. In humans, studies in populations (Spring *et al.* 1979; Fehily *et al.* 1987) and pregnant women (Hambidge *et al.* 1983; Tuttle *et al.* 1985) report lower than recommended daily intakes of Zn. Low folate intakes have also been reported and folate deficiency is common in pregnant women, especially in low socio-economic groups and those living in developing countries (Iyengar, 1971; Herbert *et al.*

Table 1. *Composition of basal diets for Expts 1 and 2*\*

| Ingredient | g/kg |
|---|---|
| Chicken egg albumen | 260·0 |
| Sucrose | 592·4 |
| Maize oil | 80·0 |
| Vitamin mixture† | 20·0 |
| Mineral mixture‡ | 40·0 |
| Phytic acid§ | 7·6 |

\* 0·4 mg zinc/kg by atomic absorption analysis.

† Vitamin mixture (mg/kg diet): *myo*-inositol 471·4, ascorbic acid 94·28, calcium pantothenate 47·14, thiamin hydrochloride 28·28, pyridoxine hydrochloride 28·28, nicotinic acid 28·28, menadione sodium bisulphite 37·72, *p*-aminobenzoic acid 9·42, riboflavin 9·42, pteroylmonoglutamic acid 0·56, biotin (2%) 11·94, cyanocobalamin (0·1%) 28·28, vitamin A (as retinol equivalents) 4·24, cholecalciferol 0·0354, $\alpha$-tocopheryl acetate 226·28, choline chloride 942·8, glucose 18·00 (g/kg). Additional pteroylmonoglutamic acid was incorporated into the vitamin mixture at the required levels for folate-supplemented diets.

‡ Mineral mixture (g/kg diet): $K_2HPO_4$ 12·78, $CaCO_3$ 9·28, NaCl 6·61, $MgSO_4.7H_2O$ 3·28, $CaHPO_4$ 6·90, $FeSO_4.7H_2O$ 0·984, $MnSO_4.4H_2$ 0·119, KI 0·0316, $CuSO_4.5H_2O$ 0·0116. Zn was added to the mineral mixture as $ZnSO_4.7H_2O$ at the required levels for Zn-supplemented diets.

§ Sodium salt, 15·3% water.

1975; Rogozinski *et al.* 1983; Huber *et al.* 1988). Cherry *et al.* (1981) observed a significant correlation between the concentration of Zn and folic acid in plasma of pregnant women. Much emphasis in 'at risk' human pregnancies is placed on the prophylactic effects of pteroylmonoglutamic acid supplementation (Laurence *et al.* 1981; Wald & Polani, 1984), but Zn supplements are rarely prescribed.

The objective of the present study was to investigate the possibility that part of the teratogenic effect of reduced Zn status may be mediated by the adverse effect of Zn deficiency on folic acid status. This suggestion was evaluated in Zn-deficient pregnant rats by treatment with pharmacological doses of pteroylmonoglutamic acid.

### EXPERIMENTAL PROCEDURES
#### Animals
Virgin female rats of the Wistar strain were obtained from the Biological Services Unit, University College, Cork, for Expt 1, and Charles River (UK) Ltd, Margate, Kent, for Expt 2. For not less than 5 d before mating, female rats were acclimatized on the Zn-supplemented control diet. The day a vaginal plug was detected was considered to be day 0·5 of gestation. Pregnant rats were then weighed and randomized into groups of similar weights and fed on the experimental diets. In these experiments distilled water was available *ad lib.* from glass bottles fitted with melamine caps from which the rubber seals had been removed.

The animal room was fitted with a continuous filtered air-flow system and maintained at a temperature of $22 \pm 2°$, relative humidity of $50 \pm 2\%$; and a 12 h light – 12 h dark cycle was operated. Rats were individually housed in suspended plastic cages with stainless-steel fittings, including grid bottoms.

#### Diets and feeding regimen
Test diets were formulated as shown in Table 1. They were chosen to result in a high incidence of Zn-deficiency-induced teratogenesis and were based on the soya-bean-protein diets of Hurley & Swenerton (1971). In the present study, egg albumin replaced soya-bean
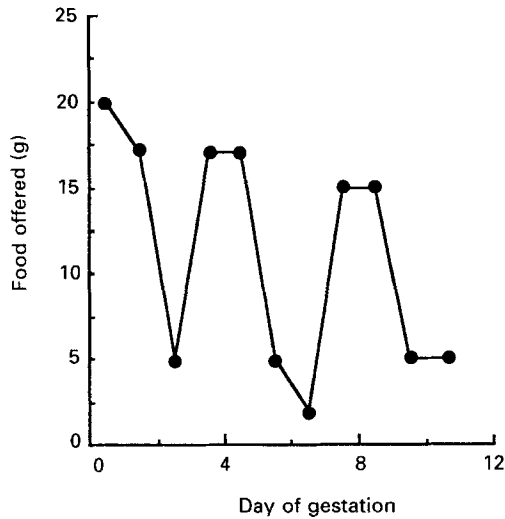
Fig. 1. Predetermined rat feeding schedule. Daily amounts of diet offered to pregnant rats from days 0·5 to 11·5 of gestation.

protein and, because the phytic acid content of soya bean reduces Zn availability, phytic acid was added at a level equivalent to that estimated in a soya-bean diet of similar protein concentration. With the exception of their Zn and folic acid contents, all diets were identical in composition. From day 0·5 to day 11·5 of gestation, Zn-deficient diets were not fed *ad lib.*, but to a predetermined cycle (Fig. 1), in which the feeding cycle of Zn-deficient rats (Williams & Mills, 1970) was synchronized to result in maximum teratogenesis. This feeding method was suggested by Record *et al.* (1985) and based on the idea that development of individual fetal organs is influenced by maternal serum Zn concentration at the time of organogenesis, which, in turn, is inversely related to recent food intake. Feeding rats on the Zn-deficient diet in a systematic cyclical manner to induce low maternal serum Zn concentrations on gestational days 8 and 9 led to a substantially higher incidence of teratogenesis when compared with feeding in the reverse cycle. Rats were fed *ad lib.* from day 11·5 to day 17·5 of gestation. At the conclusion of each experiment rats were anaesthetized with diethyl ether and killed by cervical dislocation.

### Expt 1

Five groups of pregnant rats were studied, each of mean body-weight 225 g on day 0·5 of gestation. Groups A, B, C and D were fed on the basal Zn-deficient diet supplemented with 0, 30, 100 and 200 mg pteroylmonoglutamic acid/kg respectively. Group E was fed on the basal diet supplemented with 95 mg Zn/kg, also to the predetermined cycle (Fig. 1) until day 11·5 of gestation and, thereafter, *ad lib.* until day 17·5. Food intakes were recorded daily throughout gestation and rats were fasted overnight before killing on day 18·5. Fetuses were removed by Caesarian section and resorbed litters were noted. Fetuses were stored in formal saline and subsequently examined for gross external malformations using a stereomicroscope.

### Expt 2

Four groups of pregnant rats were studied, each of mean body-weight 270 g on day 0·5 of gestation. Groups F and H consisted of eighteen rats each and were fed on the basal Zn-

deficient diet supplemented with 0 and 200 mg pteroylmonoglutamic acid/kg respectively. Groups G and J consisted of six rats each and were fed on the control diet (75 mg Zn/kg) supplemented with 0 and 200 mg pteroylmonoglutamic acid/kg respectively. Groups F and H were fed to the predetermined cycle (Fig. 1) from day 0·5 to day 11·5 of gestation and *ad lib.* thereafter. Groups G and J were pair-fed from day 0·5 to day 17·5 of gestation with six rats selected at random from each of groups F and H respectively. All rats were given 5 g food on the day before killing (day 17·5 of gestation) in order to minimize the elevating effect of fasting on plasma Zn concentration (Wallwork *et al.* 1981). On day 18·5 of gestation, rats were anaesthetized using diethyl ether and blood was withdrawn by retro-orbital puncture of the subretinal vein and collected in heparinized plastic tubes. Plasma was prepared within 3 h of withdrawal and stored at −20° for determination of Zn and folic acid concentrations. Maternal plasma folate concentration was measured because it represents the pool of folate readily available to the fetus, and according to Abab & Gregory (1987) is a good indicator of folate status in short-term bioassays. Litter size, and fetal and placental weights were recorded. One fetus per litter was selected at random from each of nine litters in the Zn-deficient groups and from each litter in the control groups. These fetuses, and placental tissue from all litters, were frozen in liquid nitrogen and stored at −20° for Zn analysis. The remaining fetuses were stored in formal saline and examined for gross external malformations.

## Analytical methods

Folate was assayed in undiluted plasma using a chloramphenicol-resistant strain of *Lactobacillus casei* by the method of Scott *et al.* (1974) as modified by Wilson & Horne (1982). Plasma was diluted 1:10 using high-performance liquid chromatography grade water and analysed for Zn by plasma emission spectrophotometry (ICP emission spectrophotometer model 6500; Perkin-Elmer). Diets (0·5 g) and oven-dried whole tibias, fetuses and placental tissue were wet ashed in 15 ml of a mixture (2:1, v/v) of Analar grade nitric and perchloric acids, and analysed for Zn by atomic absorption spectrophotometry (flame atomic absorption spectrophotometer model SP9; Pye Unicam).

Statistical analysis of malformation and resorption incidences were carried out by the Mann-Whitney *U* test using the Minitab statistical package (Ryan *et al.* 1985). All other values were analysed by two-way analysis of variance using the SPSS-X statistical package.

## RESULTS

In Expt 1, rats fed on diets adequate in Zn (group E) to the predetermined cycle exhibited a satisfactory pregnancy outcome (Table 2). Rats fed on the Zn-deficient diet (group A) exhibited a high rate of fetal resorption, 57% of the litters being totally resorbed. Folate supplementation did not reduce the incidence of litter resorption (groups B, C, D) incurred by Zn deficiency. Litter resorption tended to be increased in group D which was supplemented with 200 mg pteroylmonoglutamic acid/kg. Pregnancy outcome was also satisfactory in pair-fed Zn-replete rats in Expt 2 (group G). The incidence of litter resorption was 16·7% in Zn-deficient rats. Folate supplementation did not affect resorption incidence in Zn-deficient (group H) or Zn-replete (group J) rats. Analysis of body-weights within Expt 1 and between Expts 1 and 2 indicated that body-weight is an important determinant of resorption risk in Zn-deficient rats. Rats exhibiting a 100% resorption incidence in Expt 1 had significantly ($P < 0.001$) lower body-weights (mean 218·0 (SE 2.7) g) at conception than those with litters (mean 235·3 (SE 3.9) g). When rats of higher body-weights were used in Expt 2 (270 (SE 4.1) g), the incidence of litter resorption was substantially reduced.

Table 2. *Expts 1 and 2. Incidence of litter resorption and fetal malformation*

| | Expt 1 | | | | | Expt 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group... | A | B | C | D | E | F | G | H | J |
| Dietary zinc (mg/kg)† ... | 0 | 0 | 0 | 0 | 95·0 | 0 | 75 | 0 | 75 |
| Dietary folate (mg/kg)‡ ... | 0·56 | 30·56 | 100·56 | 200·56 | 0·56 | 0·56 | 0·56 | 200·56 | 200·56 |
| No. of pregnant dams | 14 | 15 | 15 | 17 | 5 | 18 | 6 | 18 | 6 |
| Starting body-wt (g) | 225 | 226 | 225 | 226 | 223 | 270 | 270 | 270 | 270 |
| Litters resorbed: $n$§ | 8 | 8 | 8 | 11 | 0 | 3 | 0 | 3 | 0 |
| % | 57·2 | 54·0 | 53·4 | 64·7 | 0 | 16·7 | 0 | 16·7 | 0 |
| No. of fetuses examined | 27 | 32 | 40 | 28 | 49 | 97 | 74 | 101 | 69 |
| Malformation (incidence; % of fetuses) | | | | | | | | | |
| Brain or meningeal abnormalities, or both | 40·7 | 62·5 | 70·0 | 46·4 | 0 | 45·5 | 0 | 54·5 | 0 |
| Umbilical hernia | 40·7 | 37·5 | 50·0 | 42·9 | 2·0 | 37·1 | 1·2 | 61·4 | 0 |
| Tail deformities | 77·8 | 87·5 | 90·0 | 82·0 | 0 | 94·9 | 0 | 91·9 | 2·9 |
| Adactyly or syndactyly | 85·2 | 75·0 | 87·5 | 71·4 | 0 | 88·7 | 0 | 80·2 | 0 |
| Micro-ophthalmia or anophthalmia | 51·9 | 59·4 | 60·0 | 46·4 | 0 | 25·8 | 1·2 | 25·7 | 0 |
| Micrognathia or agnathia | 18·5 | 18·8 | 35·0 | 3·6 | 0 | 43·3 | 0 | 40·6 | 0 |
| Cleft of primary palate | 0 | 6·3 | 27·5 | 7·1 | 0 | 5·2 | 0 | 5 | 0 |
| Cleft of secondary palate | 25·9 | 59·4 | 65·0 | 32·1 | 0 | 43·4 | 0 | 62·4 | 0 |
| Macroglossia | 14·8 | 15·6 | 15·0 | 21·4 | — | — | — | — | — |
| Club foot | — | — | — | — | — | 35·1 | 0 | 63·4* | 0 |

Value for group H was significantly higher than that for group F: * $P < 0.05$.

† By atomic absorption analysis all Zn- deficient diets contained < 0·5 mg Zn/kg and Zn-supplemented diets contained indicated concentrations.

‡ Added as pteroylmonoglutamic acid.

§ Total litter resorption, no fetuses on examination.

Table 3. *Expt 2. Effects of dietary zinc deficiency and folate supplementation on pregnancy outcome, maternal plasma folate concentrations and indices of maternal and fetal Zn status*

(Mean values with their standard errors)

| Group... | F 0 0·56 | | G 75 0·56 | | H 0 200·56 | | J 75 200·56 | | Statistical significance of difference between groups*: $P$ | |
| Dietary Zn (mg/kg).... Dietary folate (mg/kg)† | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Zn | Folate |
| Maternal rats | | | | | | | | | | |
| $n$ | 18 | | 6 | | 18 | | 6 | | | |
| Plasma Zn (mg/l) | 0·55 | 0·07 | 1·0 | 0·07 | 0·54 | 0·04 | 0·99 | 0·04 | <0·001 | NS |
| Plasma folate (μg/l) | 16·6 | 2·0 | 19·0 | 4·4 | 105·4 | 11·0 | 96·4 | 9·1 | NS | <0·001 |
| Tibia Zn‡ (μg/g) | 167·7 | 3·2 | 212·5 | 6·0 | 177·3 | 4·0 | 206·6 | 9·5 | <0·001 | NS |
| Fetal Zn‡ | | | | | | | | | | |
| $n$ | 9 | | 6 | | 9 | | 6 | | | |
| (μg/g) | 98·2 | 4·2 | 126·8 | 3·3 | 107·9 | 2·0 | 129·5 | 2·5 | <0·001 | <0·05 |
| Placental Zn‡ | | | | | | | | | | |
| $n$ | 15 | | 6 | | 15 | | 6 | | | |
| (μg/g) | 61·9 | 1·5 | 78·4 | 6·3 | 65·2 | 2·4 | 70·1 | 2·8 | <0·01 | NS |
| No of fetuses per litter§ | 7·70 | 1·31 | 13·33 | 1·02 | 7·13 | 0·98 | 12·83 | 0·15 | <0·001 | NS |
| Fetal wt‖ (g) | 0·65 | 0·07 | 1·12 | 0·11 | 0·61 | 0·03 | 1·32 | 0·23 | <0·001 | NS |
| Placental wt‖ (g) | 0·24 | 0·01 | 0·36 | 0·02 | 0·25 | 0·02 | 0·36 | 0·01 | <0·001 | NS |

NS, not significant.
* Significance due to Zn or folate by two-way analysis of variance; no significant interaction was observed.
† Added as pteroylmonoglutamic acid.
‡ Expressed on a dry-weight basis.
§ Mean values excluding total litter resorptions.
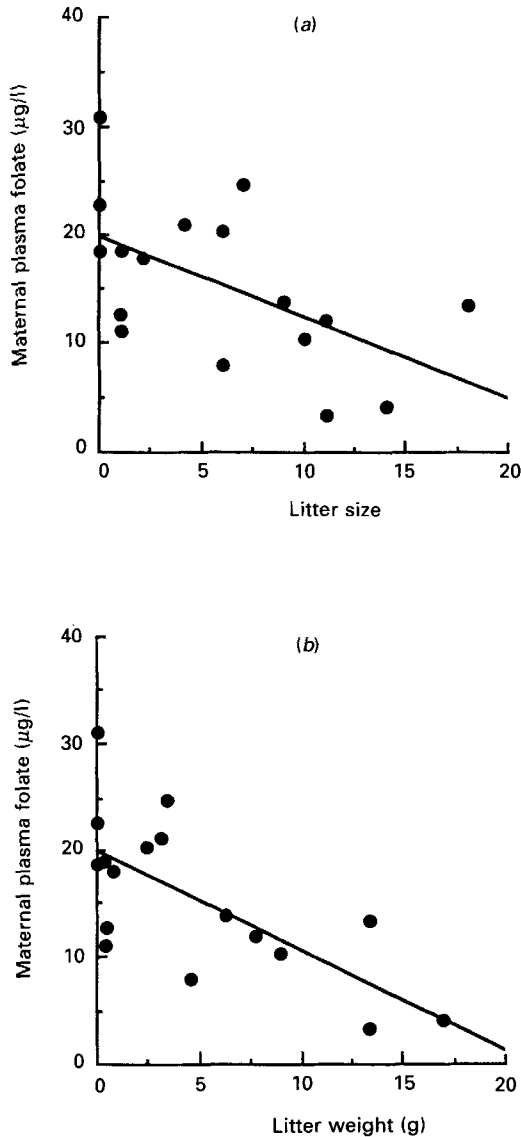‖ Mean litter values, fresh weights.

Fig. 2. Expt 2. Relationship on day 18·5 of gestation between maternal plasma folate concentration and (a) litter size and (b) litter weight of zinc-deficient (0·4 mg Zn/kg diet) animals in group F (for details of dietary regimen, see pp. 234–235). (a) $r$ −0·495, $P < 0.05$; (b) $r$ −0·630, $P < 0.01$.

The incidence of fetal malformations is also shown in Table 2. The occurrence of terata in Zn-supplemented rats was negligible in both experiments. In general, folate supplementation of Zn-deficient dams did not alleviate teratogenesis. The results of Expt 1 indicate that some malformations including brain and meningeal abnormalities, micrognathia or agnathia, and clefts of the primary and secondary palates may be increased by folate supplementation at 30 and 100 mg/kg diet. The incidence of terata varied slightly between experiments, but trends in Expt 2 were similar to those in Expt 1. Brain and meningeal abnormalities, umbilical hernia and cleft of the secondary palate were slightly

increased and the occurrence of club foot was significantly ($P < 0.05$) increased by folate supplementation of Zn-deficient dams. Food intakes were unaffected by folate supplementation and, therefore, did not contribute to the incidence of teratogenesis.

Dietary Zn deprivation resulted in a significant reduction in maternal tibia and plasma Zn concentrations (Table 3). Fetal and placental Zn concentrations, litter sizes and fetal and placental weights were also depressed by Zn deficiency. Maternal plasma folate concentration (group G) was not significantly ($P > 0.5$) decreased by Zn deficiency, but this lack of effect may have been due to the small litter sizes of Zn-deficient rats. Litter sizes and weights, and plasma folate concentrations varied from litter to litter within Zn-deficient (group F) rats. Regression analysis indicated significant inverse correlations between litter size ($r$ $-0.495$, $P < 0.05$) or weight ($r$ $-0.630$, $P < 0.01$) and maternal plasma folate concentrations (Fig. 2($a$, $b$)) within this group. Impositions of average control litter size or weight on Fig. 2($a$, $b$) indicate corresponding folate concentrations in Zn-deficient rats of 10.7 and 6.2 $\mu$g/l respectively. This compares with an actual average control (group G) value of 19.0 (SE 4.4) $\mu$g/l and a Zn deficiency (group F) value of 16.6 (SE 2.0) $\mu$g/l.

A positive effect of pteroylmonoglutamic acid supplementation on maternal plasma folate was observed in Zn-deficient (group H) and control (group J) rats. Folic acid supplementation resulted in a significant increase in fetal Zn concentration of the Zn-deficient group and a non-significant increase in fetal weight of the control group. No other effects of dietary folate were observed on variables of reproductive outcome, or fetal or maternal Zn status (Table 3).

## DISCUSSION

The results confirm the teratogenic effect of Zn deficiency and, with the exception of macroglossia, the incidence and pattern of malformations were similar to those previously reported (Hurley & Swenerton, 1966; Warkany & Petering, 1973; Rogers *et al.* 1985; Record *et al.* 1985). The sensitivity of a 20 g difference in maternal body-weight within Expt 1, or 50 g between Expts 1 and 2, highlights the critical nature of body-weight to litter resorption risk, an observation which concurs with that of Record & Dreosti (1988). Adverse effects of Zn deficiency on folic acid metabolism including folylpolyglutamate absorption have been observed in man (Tamura *et al.* 1978) and rats (Canton *et al.* 1989). Reduced concentration of folate in liver (Williams *et al.* 1973), whole blood (Fuller *et al.* 1988) and plasma (Tamura *et al.* 1987) have also been reported in rats. The results of the present study confirm previous findings in rat plasma. The effect is more evident when Zn-deficient and control rats with similar litter sizes or weights are compared, as severely reduced litter sizes in Zn-deficient rats tends to mask the effect. Folate supplementation reversed the reduction in plasma folate observed in Zn-deficient rats, but the teratogenic effects of Zn deficiency were not reduced.

Failure of folic acid supplementation to reduce teratogenesis may indicate that folic acid deficiency did not occur in Zn-deficient rats, or that folic acid metabolism was impaired by Zn deficiency rendering the supplement ineffective. A number of studies suggest that $\gamma$-glutamyl hydrolase (*EC* 3.4.22.12) requires Zn for activation (Silink *et al.* 1975; Wang *et al.* 1985; Chandler *et al.* 1986; Canton *et al.* 1989) and its activity may be decreased by Zn deficiency. In contrast, methionine synthetase activity is increased (Tamura *et al.* 1987) in Zn deficiency. Both enzymes may be important regulators of folate coenzyme distribution in tissues. In studies of uterine $\gamma$-glutamyl hydrolase activity during the oestrus cycle in the rat, Krumdieck *et al.* (1976) suggested that this enzyme may control uterine cell division by regulating the length of the $\gamma$-glutamyl side chain. It was suggested that chain length determines the ratio of metabolic inhibitor: active coenzyme form of folate for one carbon reaction and, thereby, tissue growth and differentiation. Failure of pteroylmonoglutamic

acid supplementation to ameliorate teratogenesis in Zn-deficient rats could, therefore, be caused by the inability of the Zn-deficient rats to convert the vitamin to the active metabolic form. Alternatively, because there was no evidence that Zn deficiency produced a severe folate deficiency, the induced reduction in folate status which did occur may not have been of sufficient severity to exacerbate the teratogenicity of the Zn deficiency. The use of grid-bottomed cages was the only precaution taken against coprophagy, and vitamin recycling may have occurred. Folate antagonists and antibiotics have been required to demonstrate the teratogenic potency of folate deficiency in rats (Nelson *et al.* 1955). Reproductive studies in which rats were fed on casein diets without added folate, or the use of antagonists or antibiotics, resulted in no adverse effects on pregnancy outcome (Fuller *et al.* 1988). Nevertheless, most interest focuses on the importance of folate nutriture in the prevention of neural tube defect recurrence in human pregnancy (Laurence *et al.* 1981; Smithells *et al.* 1981; Medico-Social Research Board, 1986). Comparison of the results of our study with those of the human investigations is difficult. The dietary Zn deficiency was extreme and with respect to folate the requirement of the pregnant rat is suggested to be 1 mg/kg diet (National Research Council, 1978) and the diets were supplemented to a level of 30–200 mg/kg.

In the current experiments, pteroylmonoglutamic acid supplementation tended to increase the incidence of teratogenesis in Zn-deficient groups, with the exception of group D. The lack of effect in this group may have been caused by the masking effect of litter resorption, as this group had the highest rate of resorption. Fetal distress in women taking prenatal vitamin supplements has been associated with low plasma Zn and high plasma folate concentrations (Mukherjee *et al.* 1984). A number of recent studies have drawn attention to a deleterious interaction between Zn and folic acid (Milne *et al.* 1984; McMaster *et al.* 1985; Ghishan *et al.* 1986; Simmer *et al.* 1987). These reports suggest that folic acid chelates intestinal Zn, thereby reducing the availability of dietary Zn. Others (Keating *et al.* 1987; Butterworth *et al.* 1988) failed to observe an effect. In the present study, neither the Zn status of the dams nor fetuses was compromised by folate supplementation. Failure to reduce Zn status may reflect the negligible concentration of Zn in the Zn-deficient diets. Fetal Zn is derived primarily from the breakdown of maternal tissues in Zn-deficient pregnancies. Masters *et al.* (1983) observed that two to three times more Zn was deposited in the products of conception of the rat than was consumed in the diet. The greater availability of tissue for catabolism and consequently Zn stores may also explain the lower rate of fetal resorption in Zn-deficient dams of higher body-weights. The higher incidence of malformations in Zn-deficient folate-supplemented rats may derive from the fact that folate has an avidity for a number of elements other than Zn (Albert, 1953), and in the absence of dietary Zn, binding of folate to other elements may be more important. In the present study the apparent teratogenic effects of Zn deficiency may have been exacerbated by reduced copper or manganase availability due to folate supplementation, as dietary deficiencies of these elements are also teratogenic. The observation that the Zn status of control rats was not compromised by folate supplementation is not surprising, as they were supplemented with 75 and 95 mg Zn/kg diet, quantities substantially greater than the requirement of the pregnant rat. Fuller *et al.* (1988) observed no effect of folate supplementation on maternal Zn concentration, fetal Zn concentration or pregnancy outcome of pregnant rats fed on marginal- or adequate-Zn diets. Fetal weight was enhanced by folate supplementation of control rats in the current study. Folic acid supplementation has also been reported to enhance human birth weight (Baumslag *et al.* 1970; Iyengar & Rajalakshmi, 1975; Blot *et al.* 1981). This effect may reflect the essential role of folic acid in cell multiplication and tissue growth (Morgan & Winick, 1978).

In summary, the results confirm the teratogenic effect of Zn deficiency. They also

demonstrate not only the inability of pteroylmonoglutamic acid supplementation to offset this effect, but indicate its possible potentiation. Maternal plasma folate concentration was inversely related to litter size or weight of Zn-deficient rats and pteroylmonoglutamic acid supplementation increased the size of offspring from Zn-supplemented rats. Low maternal body-weight at conception emerged as an important negative determinant of pregnancy outcome in Zn-deficient rats. The implications of this finding for pregnancy outcome in humans may be significant.

## REFERENCES

Abab, A. R. & Gregory, J. F. (1987). Determination of folate bioavailability with a rat bioassay. *Journal of Nutrition* **177**, 866–873.

Albert, A. (1953). Quantitative studies of the avidity of naturally occurring substances for trace elements. 3. Pteridines, riboflavin and purines. *Biochemistry Journal* **54**, 646–654.

Baumslag, N., Edelstein, T. & Metz. J. (1970). Reduction of incidence of prematurity by folic acid supplementation in pregnancy. *British Medical Journal* **1**, 16–17.

Blot, I., Papiernik, E., Kaltwasser, J. P., Werner, E. & Tchernia, G. (1981). Influence of routine administration of folic acid and iron during pregnancy. *Gynecology and Obstetrics Investigation* **12**, 294–304.

Bremert, J. C., Dreosti, I. E. & Tulsi, R. S. (1989). Teratogenic interaction of folic acid and zinc deficiencies in the rat. *Nutrition Reports International* **39**, 383–390

Butterworth, C. E. Jr, Hatch, K., Cole, P., Sauberlick, H. E., Tamura, T., Cornwell, P. E. & Soong, S. (1988). Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation. *American Journal of Clinical Nutrition* **47**, 484–486.

Canton, M. C., Cotter, B. M., Cremin, F. M. & Morrissey, P. A. (1989). The effect of dietary zinc deficiency on pancreatic $\gamma$-glutamyl hydrolase (*EC* 3.4.22.12) activity and on the absorption of pteroylpolyglutamate in rats. *British Journal of Nutrition* **62**, 185–193.

Chandler, C. J., Wang, T. T. Y. & Halsted, C. H. (1986). Pteroylpolyglutamate hydrolase from human jejunal brush borders. *Journal of Biological Chemistry* **261**, 928–933.

Cherry, F. F., Bennett, E. A., Bazzano, G. S., Johnson, L. K., Fosmire, G. J. & Batson, H. K. (1981). Plasma zinc in hypertension/toxemia and other reproductive variables in adolescent pregnancy. *American Journal of Clinical Nutrition* **34**, 2367–2375.

Fehily, D. F., Cremin, F. M., Grant, H., Flynn, A. & Jenkins, D. M. (1987). Zinc content and bioavailability in the Irish diet. *Irish Journal of Food Science and Technology*, **11**, 25–33.

Fuller, N. J., Evans, P. H., Howlett, M. & Bates, C. J. (1988). The effects of dietary folate and zinc on the outcome of pregnancy and early growth in rats. *British Journal of Nutrition* **59**, 251–259.

Ghishan, F. K., Said, H. M., Wilson, P. C., Murrell, J. E. & Greene, H. L. (1986). Intestinal transport of zinc and folic acid: a mutual inhibitory effect. *American Journal of Clinical Nutrition* **43**, 258–262.

Hambidge, K. M., Krebs, N. F., Jacobs, M. A., Favier, A., Guyette, L. & Ikle, D. N. (1983). Zinc nutritional status during pregnancy: a longitudinal study. *American Journal of Clinical Nutrition* **37**, 429–442.

Herbert, V., Colman, N., Spivack, M., Ocasio, E., Ghanta, V., Kimmel, K., Brenner, L., Freundlich, J. & Scott, J. (1975). Folic acid deficiency in the United States: folate assays in a prenatal clinic. *American Journal of Obstetrics and Gynecology* **123**, 175–179.

Huber, A. M., Wallins, L. L. & De Russo, P. (1988). Folate nutriture in pregnancy. *Journal of the American Dietetic Association* **88**, 791–795.

Hurley, L. S. & Swenerton, H. (1966). Congenital malformations resulting from zinc deficiency in rats. *Proceedings of the Society for Experimental Biology and Medicine* **123**, 692–696.

Hurley, L. S. & Swenerton, H. (1971). Lack of mobilization of bone and liver zinc under teratogenic conditions of zinc deficiency in rats. *Journal of Nutrition* **101**, 597–603.

Iyengar, L. (1971). Folic acid requirements of Indian pregnant women. *American Journal of Obstetrics and Gynecology* **111**, 13–16.

Iyengar, L. & Rajalakshmi, K. (1975). Effect of folic acid supplement on birth weights of infants. *American Journal of Obstetrics and Gynecology* **122**, 332–336.

Kalter, H. & Warkany, J. (1959). Experimental production of congenital malformations in mammals by metabolic procedure. *Physiological Reviews* **39**, 69–115.

Keating, J. N., Wada, L., Stokstad, E. L. R. & King, J. C. (1987). Folic acid: effect on zinc absorption in humans and in the rat. *American Journal of Clinical Nutrition* **46**, 835–839.

Krumdieck, C. L., Boots, L. R., Cornwell, P. E. & Butterworth, C. E. Jr (1976). Cyclic variations in folate composition and pteroylpolyglutamyl hydrolase (conjugase) activity in the rat uterus. *American Journal of Clinical Nutrition* **29**, 288–294.

Laurence, K. M., James, N., Miller, M. H., Tennant, G. B. & Campbell, H. (1981). Double blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural tube defects. *British Medical Journal* **282**, 1509–1511.

McMaster, D., Ewing, A. S., Ervin, C., McBriar, D. L. & Love, A. H. G. (1985). The influence of dietary vitamins on zinc uptake from the lumen of the perfused rat gut. *Nutrition Research* Supp. 1, 267–270.

Masters, D. G., Keen, C. L., Lonnerdal, B. & Hurley, L. S. (1983). Zinc deficiency teratogenicity: the protective role of maternal tissue catabolism. *Journal of Nutrition* 113, 905–912.

Medico-Social Research Board (1986). *Annual Report*, p. 25. Dublin: Health Research Board.

Milne, D. B., Canfield, W. K., Mahalko, J. R. & Sandstead, H. H. (1984). Effect of oral folic acid supplements on zinc, copper and iron absorption and excretion. *American Journal of Clinical Nutrition* 39, 535–539.

Morgan, B. L. G. & Winick, M. (1978). The effects of folic acid supplementation during pregnancy in the rat. *British Journal of Nutrition* 40, 529–533.

Mukherjee, M. D., Sandstead, H. H., Ratnaparkhi, M. V., Johnson, L. K., Milne, D. B. & Stelling, H. P. (1984). Maternal zinc, iron, folic acid and protein nutriture and outcome of human pregnancy. *American Journal of Clinical Nutrition* 40, 496–507.

National Research Council (1978). *Nutrient Requirements of Laboratory Animals*, no. 10, 3rd revised ed. Washington, DC: National Academy of Sciences.

Nelson, M. M., Wright, H. V., Asling, C. W. & Evans, H. M. (1955). Multiple congenital abnormalities resulting from transitory deficiency of pteroylglutamic acid during gestation in the rat. *Journal of Nutrition* 56, 349–370.

Record, I. R. & Dreosti, I. E. (1988). Teratogenesis as a function of maternal age in zinc deficient rats. In *Trace Elements in Man and Animals* 6, pp. 209–210 [L. S. Hurley, C. L. Keen, B. Lonnerdal and R. B. Rucker, editors]. New York: Plenum Press.

Record, I. R., Dreosti, I. E., Manuel, S. J., Buckley, R. A. & Tulsi, R. S. (1985). Teratological influence of the feeding cycle in zinc deficient rats. In *Trace Elements in Man and Animals 5*, pp. 210–213 [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Slough: Commonwealth Agricultural Bureaux.

Rogers, J. M., Keen, C. L. & Hurley, L. S. (1985). Zinc deficiency in pregnant Long-Evans hooded rats: teratogenicity and tissue trace elements. *Teratology* 31, 89–100.

Rogozinski, H., Ankers, C., Lennon, D., Wild, J., Schorah, C., Sheppard, S. & Smithells, R. W. (1983). Folate nutrition in early pregnancy. *Human Nutrition: Applied Nutrition* 37A, 357–364.

Ryan, B. F., Joiner, B. L. & Ryan, T. A. Jr (1985). *Minitab Handbook*, 2nd ed. Boston: Duxbury Press.

Scott, J. M., Ghanta, V. & Herbert, V. (1974). Trouble free microbiological serum and red cell folate assays. *American Journal of Medical Technology* 40, 125–134.

Silink, M., Reddel, R. Bethel, M. & Rowe, P. B. (1975). γ-Glutamyl hydrolase (conjugase) purification and properties of the bovine hepatic enzyme. *Journal of Biological Chemistry* 250, 5982–5993.

Simmer, K., Iles, C. A., James, C. & Thompson, R. P. H. (1987). Are iron-folate supplements harmful? *American Journal of Clinical Nutrition* 45, 122–125.

Smithells, R. W., Sheppard, S., Schorah, C. J., Seller, M. J., Nevin, N. C., Harris, R., Read, A. P. & Fielding, D. W. (1981). Apparent prevention of neural tube defects by periconceptional vitamin supplementation. *Archives of Diseases of Childhood* 56, 911–918.

Spring, J. A., Robertson, J. & Buss, D. H. (1979). Trace nutrients 3. Magnesium, copper, zinc, vitamin $B_6$, vitamin $B_{12}$ and folic acid in the British household food supply. *British Journal of Nutrition* 41, 487–493.

Tamura, T., Kaiser, L. L., Watson, J. E., Halsted, C. H., Hurley, L. S. & Stokstad, E. L. R. (1987). Increased methionine synthetase activity in zinc deficient rat liver. *Archives of Biochemistry and Biophysics* 256, 311–316.

Tamura, T., Shane, B., Baer, M. T., King, J. C., Margen, S. & Stokstad, E. L. R. (1978). Absorption of mono and polyglutamyl folates in zinc depleted man. *American Journal of Clinical Nutrition* 31, 1984–1987.

Tuttle, S., Aggett, P. J., Campbell, D. & MacGillivray, I. (1985). Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. *American Journal of Clinical Nutrition* 41, 1032–1041.

Wald, N. J. & Polani, P. E. (1984). Neural tube defects and vitamins: the need for a randomized clinical trial. *British Journal of Obstetrics and Gynaecology* 91, 516–523.

Wallwork, J. C., Fosmire, G. J. & Sandstead, H. H. (1981). Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat. *British Journal of Nutrition* 45, 127–136.

Wang, T. T. Y., Reisenauer, A. M. & Halsted, C. H. (1985). Comparison of folate conjugase activities in human, pig, rat and monkey intestine. *Journal of Nutrition* 115, 814–819.

Warkany, J. & Petering, H. G. (1973). Congenital malformations of the brain produced by short zinc deficiencies in rats. *American Journal of Mental Deficiency* 77, 645–653.

Williams, R. B. & Mills, C. F. (1970). The experimental production of zinc deficiency in the rat. *British Journal of Nutrition* 24, 989–1003.

Williams, R. B., Mills, C. F. & Davidson, R. J. L. (1973). Relationship between zinc deficiency and folic acid status of the rat. *Proceedings of the Nutrition Society* 32, 2A–3A.

Wilson, S. D. & Horne, D. W. (1982). Use of glycerol cryoprotected lactobacillus casei for microbiological assay of folic acid. *Clinical Chemistry* 28, 1198–1200.