

The effects of dietary folate and zinc on the outcome of pregnancy and early growth in rats

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1. The objective of the present study was to determine the effects of two levels of folic acid and two levels of zinc in the diets of rats during pregnancy and lactation. It addressed, among other things, the question of whether an inhibitory effect of folic (pteroylmonoglutamic) acid on Zn absorption might result in a secondary Zn deficiency in either the dams or the pups.

2. A purified diet was given to four groups of female DNL (Norwegian) Hooded rats, before and during pregnancy and during lactation. It contained the four possible combinations of: no added folic acid or 100 μg added pteroylmonoglutamic acid/g, and 6.6 or 20.2 μg Zn/g. Pups and dams were killed on day 20 of gestation or on day 20 postpartum. Measurements of body-weights, food intakes, blood folate and tissue Zn levels were performed.

3. The group with low Zn and low folate intake had a satisfactory reproductive outcome, and there were only minor effects of the supplements on body-weights.

4. Additional folate greatly increased blood (erythrocyte and plasma) folate levels, but did not compromise tissue Zn concentrations. Zn supplementation also enhanced blood folate levels, for reasons which are not yet clear.

5. There was a moderate enhancing effect of the Zn supplement on Zn levels in the livers and kidneys of pregnant dams, and the kidneys of lactating dams.

6. If the conclusions can be extrapolated to humans, then the results provide some reassurance that a high folate intake from prenatal supplementation need not necessarily cause Zn depletion, and hence functional Zn deficiency in pregnant women and their offspring.

Zinc deficiency is endemic in many countries, and pregnant and lactating women and the neonate are among the most vulnerable groups (Simmer & Thompson, 1985). Deficiency leads to growth retardation, together with functional and biochemical lesions. Folate deficiency is also widespread, especially during pregnancy, in many developing countries (Baumslag *et al.* 1970; Iyengar & Rajalakshmi, 1975). There exists, at present, a widespread belief that folate supplements are generally beneficial and desirable for pregnant women; consequently they are commonly prescribed, together with iron supplements, yet Zn is rarely included.

Some recent studies have drawn attention to an important deleterious interaction between Zn and folic acid at the intestinal level, such that high intakes of folate (i.e. pteroylmonoglutamic acid supplements) may interfere with Zn absorption (Albert, 1953; Milne *et al.* 1984; McMaster *et al.* 1985; Ghishan *et al.* 1986; Simmer *et al.* 1987). This interaction, which is thought to be a direct physico-chemical one within the intestinal lumen, may limit the availability of dietary Zn, but its possible results in terms of effects on Zn status have not yet been fully explored. Mukherjee *et al.* (1984) recorded fetomaternal complications and fetal distress associated with low plasma Zn and high plasma folate in human populations.

The purpose of the present study was to examine reproductive outcome, and anthropometric and biochemical indices of folate and Zn status, in pregnant and lactating rats fed on purified diets with two levels each of Zn and of folic (pteroylmonoglutamic) acid.

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Table 1. *Composition of diets*

Diet... Ingredients	A* (basal, low-Zn, low-folate)	B (low-Zn, high-folate)	C (adequate-Zn, low-folate)	D (adequate-Zn, high-folate)
New Zealand casein (g/kg)	200	200	200	200
Arachis oil (g/kg)	30	30	30	30
Cystine (g/kg)	1.5	1.5	1.5	1.5
Choline chloride (g/kg)	2.0	2.0	2.0	2.0
Sucrose (g/kg)	716.4	716.4	716.4	716.4
Salt mixture† (g/kg)	49.8	49.8	49.8	49.8
Vitamin mixture‡ (g/kg)	0.33	0.33	0.33	0.33
Added pteroylmonoglutamic acid ($\mu\text{g/g}$)	—	100	—	100
Added Zn ($\mu\text{g/g}$)	—	—	13.6§	13.6§

* Basal Zn content 6.6 $\mu\text{g/g}$ by analysis.

† Salt mixture (Greenfield *et al.* 1969) without zinc carbonate (g/kg diet): calcium carbonate 10.2, calcium hydrogen phosphate 16.2, disodium hydrogen phosphate 9.2, potassium chloride 10.2, magnesium sulphate 3.5, manganese sulphate 0.22, ferric citrate 0.22, copper sulphate 0.019, potassium iodate 0.0012.

‡ Vitamin mixture without folate (mg/kg diet): calcium pantothenate 19, thiamin hydrochloride 3.8, pyridoxine hydrochloride 8.5, nicotinamide 24, biotin 0.95, vitamin B₁₂ 0.05, riboflavin 14, retinyl acetate (as retinol equivalents) 2.1, vitamin D₂ 0.0075, menadione 9, α -tocopherol 250.

§ Added as the carbonate. Total dietary Zn 20.2 $\mu\text{g/g}$.

ANIMALS AND METHODS

Female Norwegian Hooded rats were maintained on a commercial laboratory diet until 110 d of age, when they were allocated to one of four dietary regimens (see Table 1). Each group consisted of animals of similar mean weight. They were housed singly in plastic and stainless-steel cages, thus avoiding Zn coatings. Wire-bottoms were used to reduce access to folate through coprophagy. Tap water for drinking was replaced by deionized water.

After 21 d acclimatization to the diets, the animals were mated, and from each dietary group nine animals were allocated to the pregnancy study and nine to the lactation study. A few of the animals in the lactation half of the study which had failed to become pregnant or had aborted or died were excluded from the study. These comprised: two in group A, two in group B and three in group D. There was no particular reason to attribute these occurrences to dietary factors. Body-weights, litter weights and food intakes were recorded daily.

The animals in the pregnant group were killed on day 20 of gestation and maternal blood was taken from the heart into a syringe containing heparin (10 units). Heparinized whole blood (0.5 ml) was preserved in 4.5 ml ascorbic acid (10 g/l) for folate assay. Plasma was separated from the remaining blood and 1 ml was preserved with 0.1 ml ascorbate (100 g/l) for folate assay; the remainder was stored frozen for subsequent Zn estimation. Maternal livers and kidneys were also stored frozen for Zn estimation. Fetal numbers and total fetal weights were recorded.

Lactating animals and their pups were killed at day 20 of lactation, and the dams' tissue samples were collected as for the pregnant animals. Pups' tissues from each litter were pooled for estimations of folate and Zn.

Whole blood and plasma folate concentrations were determined by radioassay (Fuller *et al.* 1983). Zn content of the diets, and of the livers, kidneys, plasma and fetal tissues, were determined by atomic absorption spectroscopy, using a Pye Unicam model SP9 atomic

absorption spectrometer with automatic standardization and an air-acetylene flame. Tissue and food samples were freeze-dried, and then digested in concentrated nitric acid (Spectrosol; BDH, Poole, Dorset) before analysis.

Two-way analysis of variance was used for the statistical analyses.

RESULTS

A large positive effect of raised dietary folate intake in whole blood and plasma folate levels in all groups (pregnant, lactating, and sucking pups) was observed (Table 2). There was also a highly significant positive effect of Zn on blood folate levels for pregnant and lactating rats, but not for sucking pups, when folate intakes were low (Table 2).

Pregnant rats receiving marginal folate had significantly ($P < 0.001$) higher whole-blood folate concentrations than lactating rats, in both Zn-treatment groups, but the reverse was true for both Zn groups on a high-folate intake. Plasma folate concentrations of the high-folate group were higher in the lactating than the pregnant animals, but in the low-folate-intake groups there were no detectable differences between pregnant and lactating animals.

The Zn content of the basal diet, analysed by atomic absorption, was $6.6 \mu\text{g/g}$, and the Zn-supplemented diets contained $20.2 \mu\text{g/g}$. In contrast to the very marked effect of the folate supplement on folate status, the effect of the Zn supplement on Zn status was less clearcut (Table 3). Significantly lower Zn concentrations were, however, observed in the livers of pregnant dams ($P < 0.05$) and sucking pups ($P < 0.05$), in the kidneys of pregnant ($P < 0.01$) and in lactating ($P < 0.05$) dams, and in whole fetuses ($P < 0.01$) when the dietary Zn was at the lower level. The highest tissue Zn levels were seen in the adequate-Zn, high-folate group (group D), e.g. in liver during pregnancy ($P < 0.05$), and in kidney during pregnancy ($P < 0.001$) and lactation ($P < 0.01$) (not shown). No intake-related differences were observed in plasma Zn or in hepatic Zn of the lactating animals, or in renal Zn of the pups.

The concentration of Zn in the livers of pregnant and lactating dams and pups showed no significant dietary folate-related differences. However, during pregnancy, renal Zn levels were significantly lower in the dams on the low-folate diets ($P < 0.01$). In the lactating rats, the low-folate diets again resulted in lower renal Zn levels ($P < 0.05$). No dietary folate effects could be detected on Zn in the sucking pups' kidneys; nor were plasma Zn levels affected. Fetal Zn levels were not significantly affected by folate intake.

Table 4 shows the effects of the four dietary regimens on body- and tissue weights. In pregnancy, high-folate intake decreased weight gain during the second trimester ($P < 0.05$), kidney wet weight ($P < 0.01$) and liver wet weight ($P < 0.001$). Fetal wet weight (total) was significantly increased in the high-folate-intake groups ($P < 0.002$), but this was partly offset by a (non-significant) decrease in fetal numbers in each litter, so that mean weights of individual fetuses were not significantly affected by the dietary folate level. During lactation, the high-folate intake had a beneficial effect ($P < 0.02$) in reducing body-weight loss in dams, but supplemental Zn had a somewhat deleterious effect ($P < 0.05$). Supplemental Zn lowered kidney wet weight ($P < 0.005$). When kidney weights were expressed relative to body-weight, supplemental Zn caused a marginal reduction during lactation ($P < 0.05$), as also did high-folate intakes during either pregnancy ($P < 0.05$) or lactation ($P < 0.005$). Supplemental Zn also increased the liver weights of the sucking pups ($P < 0.001$).

Food intakes (not shown) were, in general, not greatly influenced by diet group; however the low-folate-intake groups did have a significantly higher food consumption than the high-folate-intake groups, during pregnancy ($P < 0.05$).

Table 2. *The effect of folate and zinc intakes on blood folate concentrations ($\mu\text{g/l}$) of rats and their pups*
(Mean values with their standard errors)

Group	Low-Zn intake*						Adequate-Zn intake†						Statistical significance P	
	- Folate‡			+ Folate§			- Folate‡			+ Folate§				
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Folate	Zn
Pregnant rats														
Whole-blood folate	97	8	9	412	24	8	165	8	9	446	10	9	< 0.001	< 0.001
Plasma folate	4.0	2.5	8	48.8	2.9	9	2.1	0.6	9	46.1	4.9	9	< 0.001	NS
Lactating rats														
Whole-blood folate	42	7	7	498	7	5	64	7	9	513	3	6	< 0.001	< 0.001
Plasma folate	3.2	0.1	7	68.7	1.7	7	4.0	1.5	9	64.6	1.3	5	< 0.001	NS
Sucking pups														
Whole-blood folate	72	8	7	489	15	9	62	8	9	479	12	6	< 0.001	NS
Plasma folate	4.4	1.1	7	64.0	1.2	7	4.6	0.9	9	64.7	0.8	6	< 0.001	NS

NS, not significant.

* 6.6 $\mu\text{g Zn/g}$.

† 20.2 $\mu\text{g Zn/g}$.

‡ No added folate.

§ 100 μg pteroylmonoglutamic acid added/g.

|| Significant differences due to folate or Zn components of the diet with P values, based on two-way analysis of variance.

Table 3. The effect of folate and zinc intakes on organ ($\mu\text{g/g}$) and plasma (mg/l) Zn concentrations* of rats and their pups
(Mean values with their standard errors)

Group	Low-Zn intake†						Adequate-Zn intake‡						Statistical significance¶ P	
	- Folate§			+ Folate			- Folate§			+ Folate				
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Zn	Folate
Pregnant rats:														
Liver concentration	18.1	0.6	9	18.1	0.7	9	19.1	0.4	9	20.2	1.0	9	< 0.05	NS
Total liver Zn (μg)	45.7	3.1	9	49.4	2.5	9	53.9	2.3	9	54.8	2.6	9	< 0.02	NS
Kidney concentration	15.9	1.8	9	20.8	2.7	9	19.7	0.8	9	30.6	2.1	9	< 0.01	< 0.01
Plasma	—	—	—	—	—	—	0.58	0.03	9	0.59	0.05	9	NS	NS
Whole fetus	13.2	0.4	9	13.5	0.3	9	14.5	0.5	9	14.7	0.3	9	< 0.01	NS
Lactating rats:														
Liver concentration	19.4	1.1	7	20.5	1.7	7	19.0	0.4	9	19.7	1.5	6	NS	NS
Total liver Zn (μg)	50.6	2.4	7	60.2	6.1	7	55.4	2.3	9	46.6	1.9	6	I < 0.005	< 0.05
Kidney concentration	19.3	1.2	7	19.8	1.1	7	19.9	0.9	9	23.4	0.8	6	< 0.05	NS
Plasma	0.61	0.02	7	0.63	0.08	7	0.64	0.04	9	0.69	0.05	6	NS	NS
Sucking pups:														
Liver concentration	25.0	1.7	6	25.8	2.2	7	29.2	0.9	8	27.5	1.1	6	< 0.05	NS
Total liver Zn (μg)	58.1	5.5	6	56.1	4.7	7	74.2	3.8	8	74.8	5.7	6	< 0.001	NS
Kidney concentration	16.8	1.0	7	15.0	1.1	7	16.1	0.8	9	16.0	1.3	6	NS	NS
Plasma	0.81	0.08	7	0.74	0.04	7	0.87	0.06	9	0.92	0.08	6	NS	NS

NS, not significant.

* Concentrations per unit fresh weight.

† 6.6 $\mu\text{g Zn/g}$.

‡ 20.2 $\mu\text{g Zn/g}$.

§ No added folate.

|| 100 μg pteroylmonoglutamic acid added/g.

¶ Significant differences due to folate or Zn or interaction (I) between these components of the diet with P values based on two-way analysis of variance.

Table 4. *The effect of folate and zinc intakes on maternal, fetal and pup weights, and weights of maternal organs*
(Mean values with their standard errors)

Group	Low-Zn intake*						Adequate-Zn intake†						Statistical significance‡	
	-Folate‡			+Folate§			-Folate‡			+Folate§			Folate	Zn
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n		
Pregnant rats:														
Body-wt (g)¶	350	11	9	350	12	9	331	10	9	359	11	9	NS	NS
Wt gain (days 7-14) (g/d)**	3.8	0.16	9	3.5	0.2	9	4.3	0.16	9	3.7	0.16	9	< 0.05	NS
Wt gain (days 14-20) (g/d)**	7.0	0.7	9	6.4	0.6	9	6.8	0.5	9	6.4	0.3	9	NS	NS
Liver wt (g)††	13.8	0.5	9	11.1	0.5	9	13.0	0.4	9	11.9	1.1	9	< 0.001	NS
Kidney wt (g)††	1.89	0.06	9	1.74	0.04	9	1.86	0.07	9	1.71	0.05	9	< 0.01	NS
Kidney wt:body-wt ratio × 10 ²	0.54	0.02	9	0.53	0.01	9	0.53	0.02	9	0.48	0.02	9	< 0.05	NS
Fetal rats:														
Wt at day 20 (g)††	2.66	0.10	9	3.31	0.27	9	2.78	0.07	9	3.32	0.27	9	< 0.002	< 0.05
Fetal numbers at day 20††	12.1	0.8	9	10.6	0.9	9	10.9	1.2	9	9.9	0.5	9	NS	NS
Total fetal wt/dam at day 20††	32.6	2.9	9	34.3	3.2	9	30.4	3.6	9	32.8	3.0	9	NS	NS
Lactating rats:														
Body-wt (g)§§	212	7	7	228	9	7	212	6	9	218	4	6	NS	NS
Wt change (days 14-20) (g/d)‖‖	-2.1	1.1	7	-0.7	0.9	7	-3.7	0.5	9	-1.8	0.4	6	< 0.02	< 0.05
Liver wt (g)††	13.9	0.9	7	15.9	1.2	7	12.8	0.6	9	12.8	1.4	6	NS	NS
Kidney wt (g)††	1.80	0.03	7	1.81	0.05	7	1.72	0.03	9	1.67	0.04	6	NS	< 0.005
Kidney wt:body-wt ratio × 10 ²	0.85	0.03	7	0.80	0.02	7	0.81	0.02	9	0.77	0.01	6	< 0.005	< 0.05
Suckling pups:														
Body-wt (day 20) (g)§§	30.4	9.0	7	23.9	9.8	7	30.0	5.1	9	34.6	15.3	6	I	< 0.05
No. surviving (day 20)§§	8.9	0.8	7	9.0	0.6	7	8.7	0.9	9	9.3	1.1	6	NS	NS
Litter wt gain (days 12-20) (g/d)‖‖	11.2	1.0	7	11.5	3.1	7	11.6	1.4	9	11.5	0.6	6	NS	NS
Liver wt (g)††	2.24	0.15	7	2.19	0.13	7	2.56	0.08	9	2.71	0.13	6	< 0.001	NS
Kidney wt (g)††	0.191	0.015	7	0.171	0.011	7	0.176	0.010	9	0.163	0.009	6	NS	NS
Kidney wt:body wt ratio × 10 ²	0.75	0.05	7	0.73	0.05	7	0.75	0.09	9	0.57	0.05	6	NS	NS

NS, not significant. * 6.6 µg Zn/g. † 20.2 µg Zn/g. ‡ No added folate. § 100 µg pteroylmonoglutamic acid added/g.

‖ Significant differences due to folate or Zn or interaction (I) between these components of the diet with P based on two-way analysis of variance.

¶ Maximum value before parturition.

** Period after conception (d), corresponding to second and third trimesters of pregnancy.

†† Fresh weights at death.

‡‡ At day 20 of gestation, i.e. at death. (Means calculated first within each litter, then between litters, for overall means.)

§§ At day 20 of lactation, i.e. at death. (Means calculated first within each litter, then between litters, for overall means.)

‖‖ Period after parturition.

Significant interaction terms between folate-intake and Zn-intake groups generally were not encountered; exceptions being one ($P < 0.05$) for the sucking pups' body-weights, and one ($P < 0.005$) for the total Zn content of the lactating rats' livers.

DISCUSSION

Folic acid is required for cell multiplication and tissue growth (Morgan & Winick, 1978). It is therefore not surprising that body-weight changes exhibited a beneficial effect of high-folate intake in the fetuses and in lactating dams. The folate content of the basal diet unfortunately is not precisely known, and indeed it is very difficult, by existing analytical techniques, to obtain really-reliable estimates of very minute amounts of folate in diet components. On the basis of other recent studies (Richardson *et al.* 1979; Akesson *et al.* 1982; Hakim *et al.* 1984), it is likely that the folate content of a purified diet, where the only source of folate is casein, present at 200 g/kg, is in the range 10–50 μg folate/kg. However, the rat is known to obtain considerable amounts of folate through intestinal floral synthesis and refection which, unless antibiotics are used, may greatly increase the supply of absorbed folate. This, and other details of diet design, may influence the outcome of studies of experimental folate deficiency in a rat model (Walzem *et al.* 1983). Nevertheless, the observation from the present study, that the folate present in a 200 g casein/kg diet can support a perfectly satisfactory outcome of reproduction in Norwegian Hooded rats, helps to place in perspective parallel observations on folate requirements of rats during reproduction (Morgan & Winick, 1978; Baker *et al.* 1984).

Paradoxically, body-weight gains and liver and kidney weights in the pregnant dams were somewhat smaller in the high-folate-intake than the low-folate-intake groups. However, this difference was not seen in the fetal weights; it was no more evident in the low-Zn-intake group than the adequate-Zn-intake group, and it was not accompanied by reduced tissue Zn levels in the high-folate-intake groups (Table 3).

A heavy drain on maternal Zn stores occurs during pregnancy (Herzfeld *et al.* 1985). By comparison with several other studies in the rat, the Zn content of the basal, un-supplemented diet in the present study, might have been insufficient for optimum outcome of reproduction, since the requirement has been reported as being greater than 6 $\mu\text{g}/\text{g}$ diet, and usually in excess of 10 $\mu\text{g}/\text{g}$ (Williams *et al.* 1973*b*; Fosmire *et al.* 1977; Halas *et al.* 1982; Herman *et al.* 1985; Herzfeld *et al.* 1985). One study (Fairweather-Tait *et al.* 1985) has, however, recorded paradoxically increased fetal weights in litters of rats fed on a marginal Zn diet during the later stages of pregnancy. In addition, variations in fetal survival can complicate the interpretation of pregnancy-outcome studies (Cunnane, 1982). In the present study, 20 μg Zn/g diet was chosen as an 'adequate' level, on the basis of literature values for rodent Zn requirements (Greenfield *et al.* 1969; Clarke *et al.* 1977). It was not our intention to provide a generous excess of Zn, since any competitive interaction with folate seems more likely to become evident at relatively low Zn intakes. There was no clearcut advantage of 20 μg Zn over 6 $\mu\text{g}/\text{g}$ diet, either for pregnancy weight gain, or for fetal weights or numbers at day 20 of gestation. Effects of Zn on organ weights were variable and difficult to interpret. The Zn concentration in these organs was not grossly depressed in the marginal-Zn-intake groups but it was, nevertheless, significantly enhanced by supplementary Zn for the following tissues: pregnant rats' livers and kidneys; lactating rats' kidneys, and sucking pups' livers. The beneficial effect of the higher Zn intake on tissue Zn concentration appeared, however, to be smaller than that recorded in other similar studies (Fosmire *et al.* 1977; Halas *et al.* 1982; Herman *et al.* 1985). Sparing effects on fetal growth and fetal Zn have been described at very low maternal Zn intakes (Fosmire *et al.* 1977; Masters *et al.* 1983) but not at marginally-sufficient intakes, comparable to the

conditions of the present study. It is clear, nevertheless, that Norwegian Hooded rats can achieve generally satisfactory tissue Zn levels, pregnancy outcome and pup development, even when dietary Zn (from the casein component of the diet) is present at only 6 $\mu\text{g/g}$ diet.

The absence of evidence, in the present study, for any deleterious interaction between dietary pteroylmonoglutamic acid and dietary Zn, either at the level of maternal, fetal or pup growth, or at that of tissue Zn concentrations, clearly contrasts with the evidence, from human studies, of an inhibitory effect of large doses of pteroylmonoglutamic acid on intestinal Zn absorption (Milne *et al.* 1984; Ghishan *et al.* 1986; Lukaski *et al.* 1986; Sandstead *et al.* 1987; Simmer *et al.* 1987). The concentration of pteroylmonoglutamic acid in the supplemented diet of the present study was large enough to have provided the equivalent of at least 100–300 mg/d to a human, which would clearly be a very high intake. It is conceivable that the rat is, for some reason, better able to counteract an intralumen interaction of these two nutrients than human subjects are. Whereas two published rat studies from other laboratories have documented an inhibitory effect of folate on Zn absorption (McMaster *et al.* 1985; Ghishan *et al.* 1986), two others (Pallauf & Kirchgessner, 1972; Wada *et al.* 1986) failed to detect such an interaction in rats' intestine.

More importantly, an apparently deleterious interaction at the lumen level may fail, for reasons associated with the efficient homeostasis of nutrients *in vivo*, to be translated into a significant effect on tissue Zn concentrations and functional indices of Zn status. Apart from a single report (Simmer *et al.* 1987), it is not clear to what extent a Zn–folate interaction in the human intestine can affect Zn economy at the tissue level, and indeed no functional results of the interaction have yet been documented. The magnitude of the requirement for Zn during human pregnancy also remains uncertain (Tuttle *et al.* 1985).

The observation of a positive effect of supplementary Zn on whole-blood folate is an intriguing but unexplained observation. In this context, Williams *et al.* (1973*a*) found that Zn-deficient sucking rat pups accumulate less folic acid in their livers than Zn-replete ones; Tamura *et al.* (1978) showed that intestinal γ -glutamyl hydrolase (conjugase; EC 3.4.22.12) in man is a Zn-dependent enzyme and Tamura *et al.* (1987) found that hepatic methionine synthase activity is increased in Zn-deficient rats. These observations point towards altered folate metabolism in Zn deficiency, which may affect tissue folate levels. It will be important to determine whether the Zn effect on erythrocyte folate, seen in the present study, is confined to one or two tissues, or whether it represents a generally positive effect on whole-body folate status.

In practical terms, the fact that folate deficiency during human pregnancy apparently can compromise the development of the fetus, at least with respect to birth weight (Baumslag, 1970; Iyengar & Rajalakshmi, 1975; Rolschau *et al.* 1979; Blot *et al.* 1981; Tchernia *et al.* 1982), is a strong argument for ensuring that folate deficiency during pregnancy is not allowed to remain untreated. This may, of course, require maternal supplementation.

The results of the present study, if they can be extrapolated to human populations, provide some degree of reassurance that, despite the obviously serious implications of a reaction between Zn and pteroylmonoglutamic acid in the intestine, the use of folate supplements during pregnancy may, in practice, be harmless. With the present state of knowledge, however, it would still appear to be prudent to ensure that those pregnant women who receive prophylactic folate supplements should receive appropriate monitoring, and if necessary, Zn supplementation, to eliminate any possibility of an iatrogenic Zn deficiency, and to ensure that optimum Zn supplies are provided to the vulnerable growing fetus.

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