

Foetal mortality in moderately zinc-deficient rats is strictly related to the process of parturition: effect of maternal essential fatty acid supplementation

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(Received 22 September 1981 – Accepted 28 October 1981)

1. Although disrupted parturition and high foetal losses have previously been reported in pregnant rats maintained on zinc-deficient diets this is the first report to differentiate between the effects of reduced Zn intake and the effects of reduced food intake on the outcome of pregnancy in the rat.

2. Rats maintained on a 0.5 mg Zn/kg diet for the last 7 d of gestation or on a 5 mg Zn/kg diet throughout gestation did not consume significantly less food than rats given 10 or 20 mg Zn/kg diets except during the last 2 d of gestation. Pair-feeding of Zn-adequate rats (20 mg/kg) to those given low-Zn diets for the last 2 d of gestation did not affect the outcome of pregnancy in these rats.

3. In the rats maintained throughout gestation on 5 mg Zn/kg or on 0.5 mg Zn/kg in the last 7 d of gestation, parturition onset and duration were not significantly altered. Foetal survival was very significantly reduced but only from day 22 onwards; before the onset of parturition, foetal survival was not significantly affected by Zn deficiency during gestation.

4. Subcutaneous injection of evening primrose (*Oenothera biennis*) oil into rats throughout gestation enhanced foetal and neonatal survival in rats given 5 mg Zn/kg but reduced foetal survival in rats given 0.5 mg Zn/kg in the last 7 d of gestation.

5. The results suggest three points: (1) subtotal Zn deficiency during gestation in the rat jeopardises foetal survival at parturition without affecting the onset or duration of parturition, (2) foetal death in rats maintained on Zn-deficient diets occurs only in relation to parturition itself, (3) provided that Zn intake near term is at least 5 mg/kg, supplemental essential fatty acids (evening primrose oil) will reduce foetal mortality during parturition and in the neonatal period.

Previous research on the effects of zinc deficiency on the process of parturition has shown that rats maintained on less than 2 mg Zn/kg throughout gestation have prolonged and possibly delayed parturition and that foetal mortality is very significantly increased above normal (Apgar, 1968*a, b*; O'Dell *et al.* 1977). These investigators have used the method of pair-feeding to try to compare more directly the Zn-deficient and control groups because of the significantly reduced food intake in the former group. Unfortunately, there is evidence that reduced maternal food intake for a prolonged period during gestation has detrimental effects on foetal nutrition and survival (Rosso, 1977; Lederman & Rosso, 1980). This point is, in fact, evident in one of the reports on Zn deficiency and parturition (Apgar, 1968*a*).

This study was therefore undertaken with the intention of determining whether subtotal Zn deficiency during gestation would affect either food intake or parturition. Less than 12 mg Zn/kg intake reduces foetal and neonatal survival in rats (Williams *et al.* 1973) whereas 15 mg Zn/kg, although not optimal, has been shown to be adequate for foetal survival and postnatal development (US National Research Council, 1979).

The groups in this study were therefore given 5, 10 or 20 mg Zn/kg diet. A fourth group was maintained on 10 mg Zn/kg for the first 15 d of gestation and changed to 0.5 mg Zn/kg for the remainder of gestation. This group was included to assess the effect of acute total Zn deprivation late in gestation.

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Another aspect of interest was whether essential fatty acid supplementation would have any effect on foetal survival at parturition in Zn-deficient rats. Evening primrose (*Oenothera biennis*) oil (EPO) supplementation (84% essential fatty acids) has previously been demonstrated to inhibit the onset of Zn-deficiency symptoms in rats (Cunnane & Horrobin, 1980; Huang *et al.* 1981) by making available prostaglandin precursors, the synthesis of which may be disrupted by Zn deficiency. EPO supplementation of Zn-deficient rats during gestation was therefore also examined.

The results demonstrate that subtotal Zn deficiency during gestation adversely affects foetal survival at term independently of any effect of reduced food intake and support the observations that adequate Zn intake during gestation and particularly at term is critical to successful parturition. The reduced foetal mortality in rats given 5 mg Zn/kg diet and supplemented with EPO supports the hypothesis that Zn deficiency significantly alters essential fatty acid metabolism.

METHODS

Second parity female Hooded Lister rats (Rowett Strain) were kept individually in polypropylene cages with stainless-steel gridded bottoms and tops and housed under 'barrier-maintained' conditions at all times. They were fed *ad lib.* on a semi-synthetic diet based on egg albumin, sucrose, arachis oil, inorganic salts, minerals and vitamins, a diet which has been fully described and validated elsewhere (Williams & Mills, 1970). Double-distilled water was also available *ad lib.* Following successful mating with males of the same strain the pregnant rats were divided into four groups of five each: 20 mg Zn/kg (control), 10 mg Zn/kg, 5 mg Zn/kg, 10 mg Zn/kg for the first 15 d of gestation followed by 0.5 mg Zn/kg to term (10/0.5 mg Zn/kg).

Additional groups of rats were maintained under identical conditions to that described previously in order to assess foetal mortality before and after normal parturition, effects of pair-feeding and the effects of supplementation with essential fatty acids (EPO). Three groups of female rats were mated and placed on diets containing 20, 5 or 10/0.5 mg Zn/kg. Each group was subdivided into two with half receiving a daily subcutaneous injection of EPO (84% essential fatty acids) which was increased from 500 μ l/kg per d in the first week of gestation to 600 μ l/kg per d in the second week and to 700 μ l/kg per d in the final week of gestation. The caging was as previously described and food and water were available *ad lib.* In all experiments daily weight gain, food intake, length of gestation and parturition, weight loss at parturition and foetal weights and survival were measured. Subjective aspects of the effects of Zn deficiency at parturition such as the extent of haemorrhaging, skin lesions and alopecia were also noted. Statistical analysis between groups was done using Student's *t* test.

RESULTS

Because the rats were housed under barrier-maintained conditions, dermal infection was minimal and the development of dermal lesions and oral incrustation rarely occurred. Alopecia was likewise an inconsistent feature of the Zn-deficient rats. Hence the severity of Zn deficiency could not adequately be assessed by these factors.

Effect of suboptimal Zn intake on maternal weight gain, organ weights and food intake during gestation

A typical daily growth curve for the last 12 d of gestation and the first 2 d of lactation is shown in Fig. 1. The rats in the control group (20 mg Zn/kg) gained weight steadily to day 22 and lost 18.5% of their body-weight at term as the total products of conception. The rats given 10 mg Zn/kg gained weight similarly but lost 21% of their final body-weight as

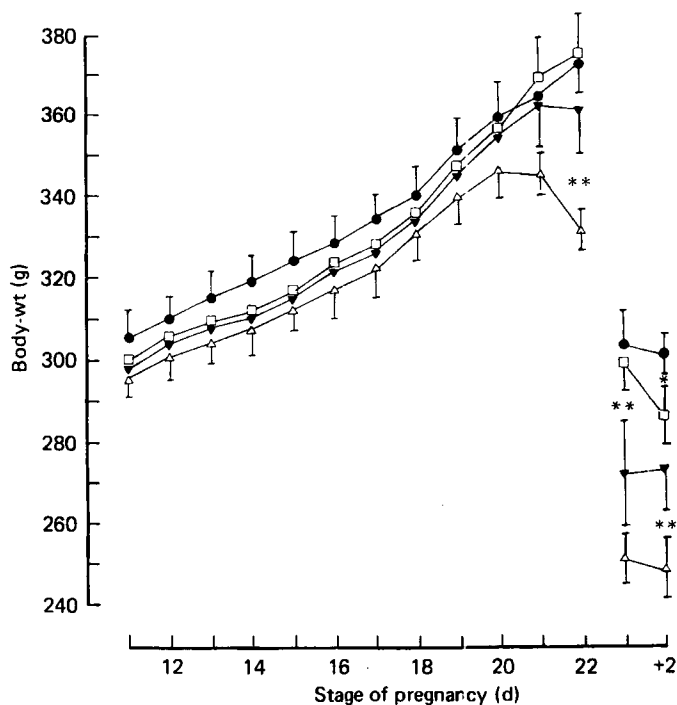


Fig. 1. The effect of diets containing various amounts of zinc on maternal weight gain during pregnancy and the first 2 d of lactation. Points represent mean values with their standard errors for five rats: (●—●), 20 mg Zn/kg; (□—□), 10 mg Zn/kg; (▼—▼), 5 mg Zn/kg; (△—△), 10 mg Zn/kg to day 15 of pregnancy followed by 0.5 mg Zn/kg thereafter (10/0.5 mg Zn/kg). Body-weight gain of the rats given 10 mg Zn/kg did not differ from that of the rats given 20 mg Zn/kg except on day 2 of lactation (day of pregnancy +2). Significance values compared to group fed 20 mg Zn/kg: * $P < 0.05$, ** $P < 0.01$.

the products of conception. The rats given 5 mg Zn/kg had identical weight gains to those given 20 or 10 mg Zn/kg up to day 21 but lost 25% of their final body-weight as the products of conception. The rats given 10/0.5 mg Zn/kg gained weight normally to day 20 but lost weight thereafter to day 22 and lost 24% of their final body-weight on delivery. The increased weight loss of the latter two groups at parturition was significantly greater than in the 20 mg Zn/kg diet ($P < 0.05$).

Values for liver, uterine, and peri-uterine fat weight:body-weight were determined at the time of killing (day 22). Liver weight:body-weight and uterine weight:body-weight values were not significantly different between groups. The peri-uterine fat weight:body-weight was very significantly decreased only in the group given the 10/0.5 mg Zn/kg diet.

Food intake in the control rats was consistently observed to increase to day 4 of gestation, remain constant at 19–21 g until day 12–14 and then decrease steadily thereafter to an intake of 10–14 g at term (Fig. 2). Food intake in the other three groups followed the same pattern as in the controls up to day 20 after which the intake of the rats in the groups given 5 mg and 10/0.5 mg Zn/kg diet dropped significantly below that of those given either the 10 or 20 mg Zn/kg diet ($P < 0.01$). Over the 22 days of gestation the total food intake of the four groups did not vary significantly from each other. Thus it was only in the last 48 h of gestation that the rats on the low-Zn diets had significantly lower food intakes than the control group (20 mg Zn/kg). During the first two d of lactation the food intakes of the low-Zn rats were significantly below that of the groups given 10 or 20 mg Zn/kg diet (Fig. 2).

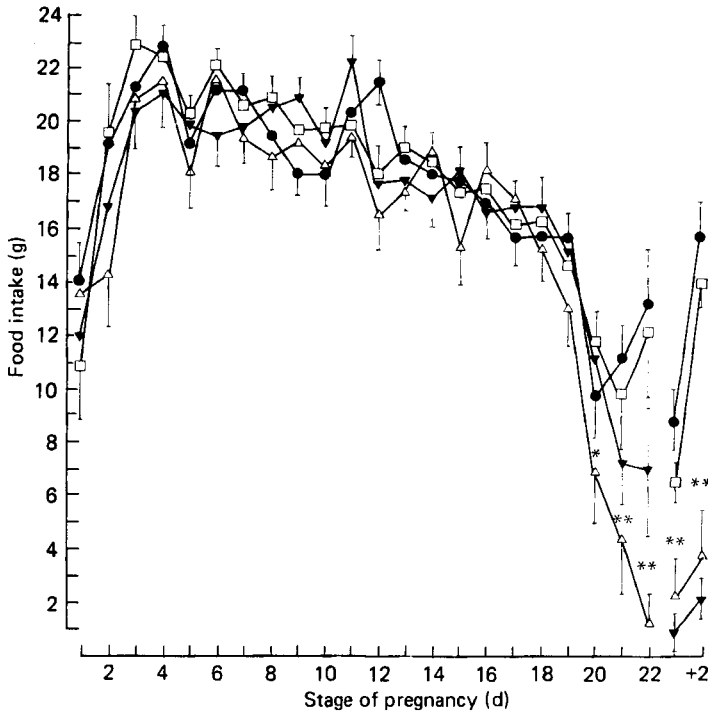


Fig. 2. Maternal food intake throughout pregnancy and including the first 2 d of lactation in rats given diets containing various levels of zinc. Points represent mean values with their standard errors for five rats: ●—●, 20 mg Zn/kg; □—□, 10 mg Zn/kg; ▼—▼, 5 mg Zn/kg; △—△, 10 mg Zn/kg for the first 15 days of pregnancy and 0.5 mg Zn/kg thereafter (10/0.5 mg Zn/kg). Significance values compared to group fed 20 mg Zn/kg: * $P < 0.05$, ** $P < 0.01$.

The trend to lower food intake in the low-Zn groups (5 mg and 10/0.5 mg Zn/kg) developed in the last 4 d of gestation but only became significant in the last 2 d. The weight gain: food intake value, which is an indication of the efficiency of digestion and absorption was continually highest in the control group (20 mg Zn/kg), and lowest in the group given 10/0.5 mg Zn/kg. In terms of weight gain/mg Zn intake, the group given the 5 mg Zn/kg diet was significantly more efficient than those given either the 10 or 20 mg Zn/kg diet, suggesting that an intake of 20 mg Zn/kg is more than adequate for optimal growth during pregnancy in the rat. Zn excretion was not measured but on the basis of over-all weight gain/mg Zn intake, the efficiency of Zn absorption varied inversely with available Zn, particularly in the last trimester.

Effect of suboptimal Zn intake on events at parturition

Parturition time was measured by checking every 15 min at the expected time of delivery for the emergence of the foetuses. Once parturition had started the progress of delivery was noted every 5 min. Constant observation was not maintained because of the noticeable stress effect it had on the dams at parturition. Parturition times for the four groups are shown in Table 1. In general, parturition time bore no relation to foetal number in any of the groups. Although the trend was towards longer parturition time in the lowest-Zn group (10/0.5 mg Zn/kg): 54 ± 11 min v. 72 ± 13 min, the effect was not significant.

Foetal number at birth was not significantly affected by Zn deficiency (Table 1). However, foetal weight and mortality varied directly and significantly with dietary Zn intake. Foetal

Table 1. *The effect of various levels of dietary zinc intake during gestation on parturition time, foetal characteristics at birth, and maternal fluid loss at parturition*

(Mean values are shown with their standard errors for five rats/group)

Dietary Zn level (mg/kg)...	20		10		5		10/0.5†	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Parturition time (min)	53.8	11.1	73.8	11.2	63.3	15.3	72.2	12.6
Foetal no.	11.8	1.5	10.4	1.4	10.8	1.7	9.9	0.9
Foetal wt (g)	5.24	0.35	5.16	0.08	5.10	0.12	4.23	0.58*
Foetal mortality (%)								
d 22 – gestation	0.6	0.6	0.8	0.4	1.4	0.5	2.1	1.0
at birth	4.2	5.0	12.1	9.4	48.6	7.0*	65.1	13.7**
d 3 – lactation	6.4	3.2	14.9	11.6	68.1	10.6***	92.4	11.2***
Maternal fluid loss (g)‡	6.3	1.7	8.4	0.9	19.5	3.8**	21.1	4.2**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† 10 mg Zn/kg for the first 15 d of gestation and 0.5 mg Zn/kg thereafter.

‡ Maternal weight loss at parturition not attributable to foeto-placental weight as a percentage of the total weight loss at parturition.

weights at birth were 20% less in the 10/0.5 mg Zn/kg group than in the other groups (Table 1). The percentage of foetuses still alive within 1 h of birth decreased dramatically with decreasing dietary Zn (Table 1). This trend continued post-natally due to maternal neglect. However, before the onset of parturition, i.e. up to and including day 22 of gestation, foetal mortality in all groups was negligible (Table 1). Thus, foetal mortality was associated strictly with the process of parturition itself. Occasional resorption sites were noted in the low-Zn groups but did not exceed the normal rate (0.5–1.0%). In the low-Zn groups oedematous foetuses in the process of being resorbed were also occasionally present.

The comparison of foetal survival to 48 h post partum with maternal food intake between day 22 and 2 days post-partum is shown in Fig. 3. In spite of the trend towards increasing foetal survival with higher maternal food intake, there was no significant correlation between maternal food intake and foetal survival. This was due in part to the large variability in maternal food intake at term particularly in the low-Zn groups. Of the thirty-seven dams making up the values in Fig. 3, only five had foetal survival rates between 1–75%. In the group given 10/0.5 mg Zn/kg, litters were either 90% live-born or 0% were live-born with no intermediate survival values occurring. Survival of the litters at birth would therefore appear to be an 'all-or-nothing' phenomenon, i.e. either survival is very low for the entire litter or more than 75% of the foetuses survive. This would suggest that each member of a given litter has an equal chance to survive maternal Zn deficiency and that preferential blood and nutrient supply to some foetuses does not occur.

The other feature of parturition to correlate directly with Zn deficiency during gestation was weight loss at parturition which was not accounted for by total foetal and placental weight. This difference is largely attributable to blood loss in the perinatal period. Dissection of the uterus on day 22 immediately before parturition invariably revealed moderate to extensive intrauterine haemorrhaging in the dams fed low-Zn diets, which invariably continued during parturition. Both the subjective extent of haemorrhaging in utero and the non-foeto-placental weight loss at parturition correlated with the extent of maternal Zn deficiency during gestation (Table 1).

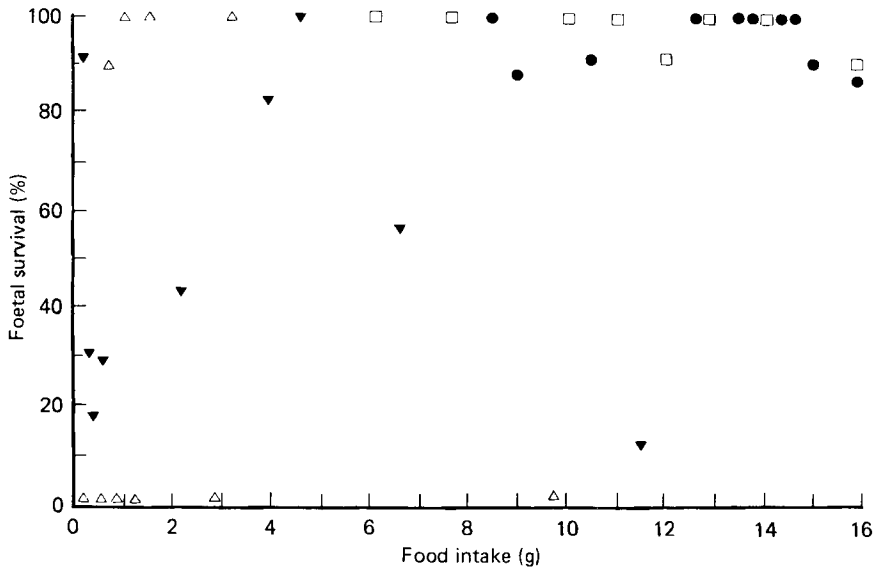


Fig. 3. Correlation between maternal food intake (mean of the intakes from day 22 of pregnancy to day 2 of lactation) and foetal survival (to day 2 of lactation) in rats given diets containing various levels of zinc. Points represent values for single rats: ●—●, 20 mg Zn/kg; □—□, 10 mg Zn/kg; ▼—▼, 5 mg Zn/kg; △—△, 10 mg Zn/kg for the first 15 days of pregnancy followed by 0.5 mg Zn/kg thereafter (10/0.5 mg Zn/kg).

Effect of pair-feeding on foetal mortality at parturition

The maternal food intake in previous experiments was always *ad lib.* for each group regardless of dietary Zn content. As shown in Fig. 2, food intake in the various groups did not vary significantly from control (20 mg Zn/kg) up to day 20 of gestation, after which food intake in the low-Zn groups was significantly decreased. Two additional groups of pregnant rats, one given 20 mg Zn/kg and the other given 5 mg Zn/kg were pair-fed to another group given 10/0.5 mg Zn/kg over the last 7 d of gestation. Foetal number, weight and survival to day 3 of lactation is shown in Table 2 for each of the groups. Foetal survival in rats pair-fed 20 and 5 mg Zn/kg diets was not significantly different than in the *ad lib.* fed rats. This suggests that food intake in the 48 h period immediately preceding parturition is not a limiting factor to foetal survival.

Effect of essential fatty acid supplementation on foetal mortality at parturition

EPO injection throughout gestation did not significantly affect daily weight gain or food intake in any of the groups. However, non-foeto-placental weight loss at parturition, which approximates maternal blood loss, was significantly less in the groups given 20 or 5 mg Zn/kg diet and injected with EPO than in the corresponding non-injected rats (Table 3). EPO had no effect on weight loss at parturition in the group given the 10/0.5 mg Zn/kg diet. Litter size in the groups given the 20 and 5 mg Zn/kg diets and injected with EPO was significantly lower than in the non-injected rats (Table 3). Foetal weights at birth or up to 3 d of age were not affected by EPO injection. However, survival of the neonates in the EPO-injected groups was significantly different than in the non-injected controls. There were no foetal deaths from birth up to 3 d of age in the group given 20 mg Zn/kg diet and treated with EPO, whereas in the untreated group given the 20 mg Zn/kg diet, mortality to 3 d of age was 10% (Table 3). In the group given the 5 mg Zn/kg diet but not treated

Table 2. Effect of pair-feeding rats maintained on 5 or 20 mg Zn/kg diets over the last 2 d of gestation to the daily intake of rats given 10/0.5 mg Zn/kg†, on foetal number, weight and survival to day 3 of lactation

(Mean values are shown with their standard errors for five rats/group)

Dietary Zn level (mg/kg)...	Ad lib.-fed						Pair-fed			
	20		5		10/0.5†		20		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Foetal no. per litter	11.3	1.1	10.9	1.5	10.3	0.6	11.7	2.1	11.8	1.3
Foetal weight at birth (g)	5.31	0.27	5.19	0.32	4.51	0.41*	5.11	0.5	4.85	0.19
Foetal mortality (%)										
day 22 of gestation	0.4	0.5	1.9	0.7	1.9	1.5	0.7	0.5	1.8	1.3
At birth	3.7	3.1	41.1	8.7**	69.4	15.2**	5.1	3.9‡	53.7	7.2***
day 3 of lactation	4.9	3.0	56.4	10.3**	88.2	16.9**	6.5	4.1‡	69.0	12.5***

* $P < 0.05$ with respect to the group fed 20 mg Zn/kg, ad lib.

** $P < 0.01$ with respect to the group fed 20 mg Zn/kg, ad lib.

*** $P < 0.01$ with respect to the group fed 20 mg Zn/kg, pair-fed.

† 10 mg Zn/kg for the first 15 d of gestation and 0.5 mg Zn/kg thereafter.

‡ Not significantly different with respect to the group fed 20 mg Zn/kg, ad lib.

|| Not significantly different with respect to the group fed 5 mg Zn/kg, ad lib.

Table 3. Effect of subcutaneous injection of evening primrose (*Oenothera biennis*) oil into dams given various levels of Zn during gestation, on maternal fluid loss, foetal weight and foetal mortality

(Mean values are shown with their standard errors for five rats/group)

Dietary Zn level (mg/kg)...	Evening primrose oil-injected‡						Untreated					
	20		5		10/0.5†		20		5		10/0.5†	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Maternal fluid loss (g)	7.3	1.0	8.8	2.9	16.7	3.4	10.0	1.2*	15.6	1.2	19.6	1.6
Foetal no. per litter	9.3	1.6	7.3	1.0	8.7	1.5	11.5	1.3	10.2	1.6	8.9	1.2
Foetal mortality (%)												
At birth	0	—	19.5	5.7	80.1	12.5§	0	—	51.5	14.3**	86.5	9.4
day 3 of lactation	0	—	24.5	7.3	100	—	10.0	5.0*	69.2	15.1**	100	—

* $P < 0.05$ with respect to group given 20 mg Zn/kg, and injected with evening primrose oil.

** $P < 0.01$ with respect to group given 5 mg Zn/kg, and injected with evening primrose oil.

† 10 mg Zn/kg for the first 15 d of gestation and 0.5 mg Zn/kg thereafter.

‡ 500 μ l/kg per d increasing to 700 μ l/kg per d at parturition.

|| Maternal weight loss at parturition not attributable to foeto-placental weight, as a percentage of the total weight loss at parturition.

§ Maternal mortality was 80% in this group (4 out of 5).

with EPO, mortality at birth was 50% and increased to 70% at 3 d post-partum. In the group given the 5 mg Zn/kg diet and treated with EPO, mortality both at birth and at 3 d of age was 40% less than that of the untreated group given the 5 mg Zn/kg diet. EPO injection of the dams in the group given the 10/0.5 mg Zn/kg diet did not affect foetal survival at birth but did cause significant maternal mortality shortly after parturition.

DISCUSSION

This study was designed to investigate the effect of moderate, subtotal Zn deficiency during gestation on the outcome of pregnancy with a view to dissociating the effect of Zn deficiency and food intake on foetal survival at parturition. Thus the true effects of Zn deficiency, uncomplicated by reduced food intake during gestation, might then be assessed. The complication of reduced food intake during Zn deficiency is normally accepted as inevitable and pair-feeding of Zn-adequate controls is considered the only alternative. However, in pregnancy studies, protein-energy depletion through decreased food intake is itself deleterious to foetal survival (Rosso, 1977; Lederman & Rosso, 1980), but apparently not through decreased blood flow to the placenta (Rosso & Kava, 1980). Hence a dietary Zn deficiency to a level sufficient to affect parturition but not to affect food intake significantly was sought. Zn intakes of either 5 mg/kg diet throughout gestation or 10 mg/kg diet for the first 2 weeks followed by 0.5 mg/kg diet for the remainder of gestation were found suitable for this purpose. Under these conditions the experimental results demonstrate that, although Zn is an inevitable determinant of food intake, moderate Zn deficiency does have clinically relevant effects on parturition which do not depend entirely on food intake at the end of gestation.

Under these conditions of Zn deficiency two important observations were made: (1) the duration of gestation and parturition were not significantly altered, (2) foetal mortality was almost exclusively limited to the period of imminent parturition. These observations suggest that in pregnancy in the rat, which is uncomplicated by reduced food intake, the major effect of Zn deficiency is to threaten foetal survival at parturition. Significant haemorrhaging both in utero and during parturition is also associated with the increase in foetal mortality.

The mechanism by which Zn deficiency affects foetal survival at parturition may involve prostaglandins in both the placenta and uterus. In vitro contractility of uterine strips from Zn-deficient parturient rats has been shown to be significantly reduced (Cunnane, 1981*a*) and may be associated with the decreased conversion of labelled arachidonic acid to prostaglandins E_2 , $F_{2\alpha}$ and I_2 in the uterus (Cunnane, 1981*b*). A significant reduction in placental perfusion at term (Cunnane *et al.* 1982) has recently been noted and may be related to an increase in placental conversion of labelled arachidonic acid to prostaglandins E_2 , $F_{2\alpha}$ and I_2 which also occurs in Zn-deficient rats at term (Cunnane, 1981*c*). Therefore altered uterine and placental prostaglandin synthesis from exogenous arachidonic acid suggests disrupted endogenous prostaglandin metabolism may contribute to uterine hypocontractility at a time when placental blood flow is also reduced. This in turn may be responsible for the high incidence of foetal mortality in Zn-deficient rats.

Supplementation with essential fatty acids (EPO treated groups) significantly reduced foetal mortality at parturition in rats maintained on 5 mg Zn/kg diet throughout gestation. Although the EPO supplementation was given subcutaneously, its beneficial effect was similar to that seen clinically with intravenous infusion of a fat emulsion (Intralipid) in pregnancy (Elphick *et al.* 1978). They reported a possible beneficial effect of the linoleic acid (18:2 *n* 6)-rich Intralipid in a patient at term whose baby had foetal growth retardation. Parenteral fat supplementation reportedly also increases uterine contractions, particularly near parturition (Heller, 1972). Since uterine hypocontractility has been demonstrated in Zn-deficient rats at term (Cunnane, 1981*a*), a reversal of this situation may be an additional effect of EPO injection.

The effectiveness of EPO in inhibiting the onset of Zn deficiency symptoms has been suggested to be due to the presence of the essential fatty acid γ -linolenic acid (18:3 *n* 6) (Cunnane & Horrobin, 1980; Huang *et al.* 1981). γ -linolenic acid is synthesized from the

parent essential fatty acid linoleic acid by the Δ^6 desaturase enzyme, the activity of which has been shown to be increased in the uterus in Zn-deficient rats at term (Cunnane & Wahle, 1981). Essential fatty acid supplementation, however, will tend to normalize such a change (Holman, 1981). Thus, in animals with marginal Zn intake (i.e. 5 mg Zn/kg diet), EPO supplementation may counter the effect of Zn deficiency in animals by making '1' series prostaglandin precursors directly available and by reducing the increased activity of the Δ^6 desaturase, hence decreasing arachidonic acid synthesis from linoleic acid.

It would appear that the effects of essential fatty acid supplementation in Zn-deficient rats depend on the amount of Zn in the diet. Thus, although it is effective at a Zn intake of as low as 2 mg/kg diet (Cunnane & Horrobin, 1980), in the rats in this experiment which were maintained on 0.5 mg Zn/kg diet in the last trimester of pregnancy, EPO supplementation did not affect foetal survival and actually increased maternal mortality at parturition. High maternal mortality near parturition has also been shown to occur in vitamin E-deficient pregnant rats given high amounts of peroxidized fats including linoleic acid (Stamler, 1959). Death was related to the development of oedema and thrombi in the kidneys, adrenals and spleen and occurred in the terminal stage of pregnancy (day 22). It was also associated with eclampsia-like convulsions. In view of the fact that in the pregnant rats in the present experiments which were maintained on 0.5 mg Zn/kg diet during the last 7 d of pregnancy, maternal death occurred only on day 22 in close association with parturition, and only in those rats also given EPO (72% linoleic acid), suggests that diets deficient in vitamin E or Zn and containing high amounts of polyunsaturated fatty acids have similar detrimental effects on the outcome of pregnancy. This view is further supported by the fact that like vitamin E, Zn may have a significant function in the control of lipid peroxidation (Bettger & O'Dell, 1981). Although the results are as yet incomplete, it is not unreasonable to suggest that extreme Zn deficiency may contribute to the development of eclampsia-like syndromes in the rat.

Considering the fact that in Zn-deficient pregnant rats: (1) pair-feeding of Zn-adequate pregnant rats to very low food intakes in the last 2 d of gestation had no significant effects on foetal growth or survival, (2) peri-uterine fat was very significantly decreased in relation to body-weight, (3) Zn is closely related to essential fatty acid metabolism, suggests that foetal lipid intake near term may be more of a limiting factor to foetal growth and survival than protein intake. The present results, showing a reduction of foetal mortality in EPO-supplemented pregnant rats, further supports this possibility.

In conclusion, it is apparent that subtotal Zn deficiency during gestation in the rat not only has pathophysiological effects on the foetus which are of clinical relevance but also can be dissociated from effects complicated by food intake reduction. The fact that a Zn intake at 25% of the adequate level was sufficient to increase significantly foetal mortality makes Zn deficiency in human high-risk pregnancies which are associated with poor nutrition much more of a reality than has previously been suggested. This is true in spite of the fact that no adequate measure of human Zn status in health or disease is yet available.

S.C.C. is very grateful to the Lalor Foundation of Wilmington, Delaware, USA for a post-doctoral fellowship and grant. Excellent dietary management of the rats was provided by Mrs A. Watt. The EPO was kindly supplied by Efamol Limited, London.

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