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REVIEW

Zinc Deficiency and the Developing Embryo

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ABSTRACT

The effect of *in utero* zinc deficiency on fetal development in rats is reviewed. Attention is paid to the primary biochemical lesion associated with zinc-related teratogenesis and special consideration is given to the central nervous system. Evidence is presented that the thymidine kinase salvage pathway, used for the synthesis of thymidine monophosphate in DNA synthesis, is depressed more in fetal brain tissue than in the liver. In addition, greater reliance appears to be placed on this pathway than on *de novo* synthesis in the fetal brain than in other tissues. Some consideration is given to the use of in vitro embryo culture in studies relating to neurogenesis, but evidence is presented of a greater capacity of explanted rat embryos to obtain zinc from maternal serum than occurs in vivo.

The rapid onset of a teratogenic zinc deficiency following dietary zinc restriction is again highlighted and further studies are described which demonstrate the critical impact of a single feeding cycle, of 4 d duration, on maternal plasma zinc levels and on the extent and nature of the observed fetal abnormalities. Evidence is presented that by shifting the timing of the high dietary intake/low plasma zinc peak to

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coincide with a particular 48 h period between days 6 and 10 of pregnancy, the pattern of malformations thus obtained reflected the coincidence of the high dietary intake of zinc-deficient diet and the critical time of morphogenesis of several organ systems.

Whereas diminished plasma zinc levels at term in zinc-deficient animals are generally well correlated with reduced growth and dysmorphogenesis of the offspring, the same is not always found in human studies. In some cases, elevated plasma zinc levels at parturition are found in mothers with growth-retarded children, or vice versa. Experimental studies with rats are reported that suggest that maternal zinc status at term may be higher in dams bearing pups stunted by exposure to a transient zinc deficiency early in pregnancy, which in turn may have reduced the demand for maternal zinc in the later stages of gestation.

The protective effect of zinc on cadmium-induced teratogenesis is discussed, particularly in relation to findings concerning an interaction of these metals in the embryonic yolk sac and thus on preplacental embryonic nutrition. Possible interactions between alcohol and zinc deficiency are also considered and data are presented pointing to increased fetotoxicity and teratogenesis in the presence of both treatments and to a more specific interaction with respect to reduced cell numbers in the developing rat hippocampus. Malondialdehyde levels, which reflect the extent of lipid peroxidation in tissue, are reported to be substantially higher in microsomes from fetal rat livers when *in utero* deficiency and gestational alcoholism are combined. The suggestion is made that alcohol and zinc deficiency act independently in the body, but overlap to some extent at the common biochemical locus of membrane lipid peroxidation.

Index Entries: Zinc deficiency, and the developing embryo; embryo, zinc deficiency and the developing; developing embryo, and zinc deficiency; alcohol, and zinc in teratogenesis; teratogenesis, alcohol, cadmium, and zinc in; cadmium, and teratogenesis.

I. INTRODUCTION

Interest in the effects of zinc deficiency on fetal development began in 1966 following the work by Hurley and Swenerton with rats (*1*) and has spread over the last decade to include several significant studies on humans (*2–4*). Recent recognition that zinc deficiency occurs as a substantial nutritional problem in humans (*5*) and that zinc levels appear to fall as rapidly in humans as in animals (*6*) following dietary zinc impoverishment, has highlighted this concern and has led to considerable interest in the importance of zinc for optimum pregnancy outcome in women.

In animals, several workers have confirmed the potent teratogenicity of maternal zinc deprivation (*7–9*), even when the deficit is imposed for only a few days during gestation (*10*). Most organ systems are affected, but the central nervous system appears to be particularly vulnerable (*11*).

II. TERATOGENIC MECHANISM

Although the involvement of zinc in general metabolism can be readily attributed to its wide association with many enzymes (*12*) and to its role in membrane stability (*13,14*) and in the immune response (*15*), the precise mechanism underlying the teratogenicity of zinc deficiency is less clear. Many workers consider the primary defect to lie in impaired synthesis of the nucleic acids (*8,16*) and to a resulting asynchrony in mitosis and consequent distortion of normal morphogenesis (*17*). Data from several workers suggest that diminished activity of the zinc-dependent enzyme thymidine kinase (EC 2.7.1.21) may be an important factor responsible for reduced mitotic activity (*18–20*), but some studies indicate that the effect of zinc deficiency on RNA and/or protein synthesis (*21*), or the involvement of the metal in gene activation and/or repression (*22*), may be of greater biochemical consequence. Possibily several mechanisms are affected and the outcome may vary depending on conditions. Clearly, more work is needed to define precisely the teratogenic lesion of zinc deficiency.

III. ZINC DEFICIENCY AND DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM

Zinc restriction during early neurogenesis affects the closure and development of many derivatives of the primitive neural tube (*9,11,23*). Many of these early brain malformations appear to be consistent with impaired mitosis during embryonic development, and the involvement of zinc in cell division offers an acceptable mechanism to account for the wide range of abnormalities seen in the brains of zinc-deficient fetuses. Of particular interest in this context are the autoradiographic studies of Eckert and Hurley (*24*), who demonstrated that *in utero* zinc deficiency reduced the incorporation of ^{3}H-thymidine into DNA, and the total DNA content, more in the head region of 12-d-old rat fetuses than in the body. This reduction could be reversed by zinc replacement and autoradiographs revealed that the recovery occurred mainly in the developing central nervous system. These findings suggested a greater sensitivity to zinc deficiency with respect to cell division in the fetal brain than in other organs.

Studies in our laboratory (*25*) indicated that the activity of thymidine kinase fell more in the brains (53%) than in the livers (34%) of zinc-deficient 20-d-old rat fetuses when compared with restricted-fed controls. Subsequent investigations were carried out on fetal rat liver and brain to determine the relative contribution of the two pathways involved in the synthesis of the deoxythymidine monophosphate used for DNA synthesis (*26,27*). The first pathway, which is dependent upon the

enzyme thymidine kinase, represents a mechanism for salvaging existing thymidine, whereas the second utilizes the enzyme thymidylate synthetase (EC 3.1.3.35) and permits manufacture of the metabolite *de novo* (Fig. 1). This latter pathway can be inhibited by methotrexate, a folate analog and inhibitor of the enzyme dihydrofolate reductase (EC 1.5.1.4) that is necessary for the activity of thymidylate synthetase.

In control animals, the salvage pathway contributed more to the supply of thymidine monophosphate in the fetal brain (43%) than it did in the fetal liver (18%). Both pathways were reduced by zinc deficiency, as also was total DNA synthesis measured by the incorporation of $^{32}PO_4$. However, in the liver, total DNA synthesis was reduced by 69% and the contributions of the salvage and *de novo* pathways by 69 and 70%, respectively, whereas in the brain, total DNA synthesis declined by 40% and the salvage and *de novo* pathways decreased by 70 and 16%, respectively. Thus it appears that, although DNA synthesis is spared in the zinc-deficient fetal brain relative to the liver, the greater contribution of the salvage pathway reflects more reliance upon the activity of thymidine kinase in the central nervous system and a higher requirement for preformed nucleotides.

Some caution is necessary, however, in attributing the reduced synthesis of DNA in zinc-deficient fetal brains entirely to diminished activity of thymidine kinase, as flux of ^{3}H-thymidine through the salvage pathway increased following treatment with methotrexate in both replete (1.9-fold) and zinc deficient (4.4-fold) animals. Possibly other enzymes, including DNA polymerase (EC 2.7.7.7), may be involved, as has been suggested for whole fetal tissue by Duncan and Hurley (*20*). Further studies are needed, especially in relation to the differences that appear to exist between tissues in their relative use of the two pathways for the synthesis of thymidine monophosphate. Also, it should be noted that because, in the liver, the contribution of the *de novo* and salvage pathways were maintained constant relative to one another during zinc deficiency,

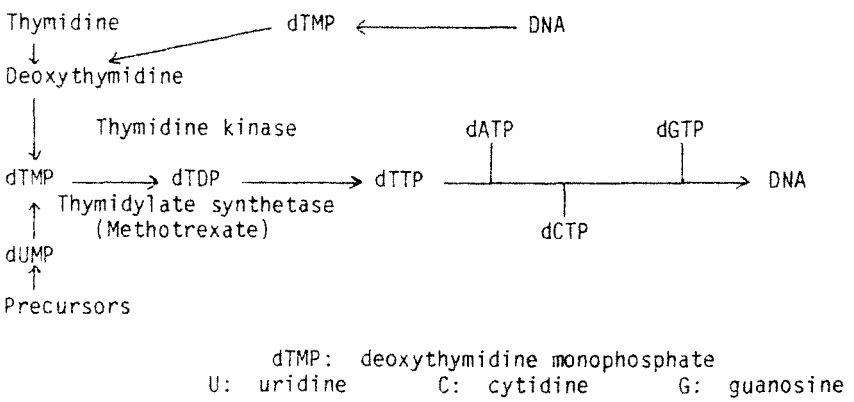

Fig. 1. Pathways of DNA synthesis.

total DNA synthesis can adequately be estimated by the incorporation of ^{3}H-thymidine into DNA. In the brain, however, where the contribution of the salvage pathway was disproportionately reduced, it appears that estimates of DNA synthesis based on incorporation of ^{3}H-thymidine into DNA may be inclined to overestimate the extent of the reduction, as would also the appearance of most autoradiographs.

Zinc Deficiency and Embryo Culture

The deleterious effects of zinc deficiency on the development and closure of the neural tube prompted further studies in this laboratory, using explanted rat embryos in culture, which provided a high degree of control and precise manipulation of the embryonic environment during this critical 48 h (days 9.5–11.5) of neurogenesis (*28*). Our principal aims were to examine the development of normal 9.5-d-old embryos grown for 48 h on zinc-deficient serum, as well as morphologically imperfect embryos taken from zinc-deficient dams and grown on normal and zinc-deficient serum. Particular attention was paid to the formation and development of the neural tube, but other major malformations were also noted.

Normal embryos grew and developed normally in either zinc-deficient or zinc-replete serum. Embryos from dams that had been fed a zinc-deficient diet since mating fell into two broad morphological categories, those appearing normal (Fig. 2a) and those with substantially retarded embryonic development (Fig. 2b). The first group grew normally in either serum (Fig. 2c), whereas those that appeared abnormal on day 9.5 became grossly malformed and frequently exhibited open neural tubes after 48 h of culture on both zinc-deficient or replete serum (Fig. 2d).

Thus it appears that the rapid teratogenic effects of zinc deficiency observed in vivo cannot be induced by direct culture of zinc-replete embryos in zinc-deficient serum. The reasons underlying this anomaly are not clear, but it seems that when grown in vitro the embryo is able to extract sufficient zinc, even from zinc-deficient serum, for normal cell growth. Possibly, zinc bound to the large α_2-macroglobulin protein is taken up and digested by the yolk sac of the explanted embryo following the removal of Reichert's membrane, which in vivo might act as an ultrafilter and exclude such large molecules from the embryonic environment. Other possibilities exist that have been discussed in detail elsewhere (*28*).

Of further interest is the observation that abnormal embryos taken from zinc-deficient dams on day 9.5 of pregnancy grew abnormally even in zinc-replete serum, which demonstrates that much of the teratogenicity associated with maternal zinc deficiency is established prior to the period of greatest organogenesis. These findings are in accord with the report by Hurley and Shrader (*29*) in relation to abnormally

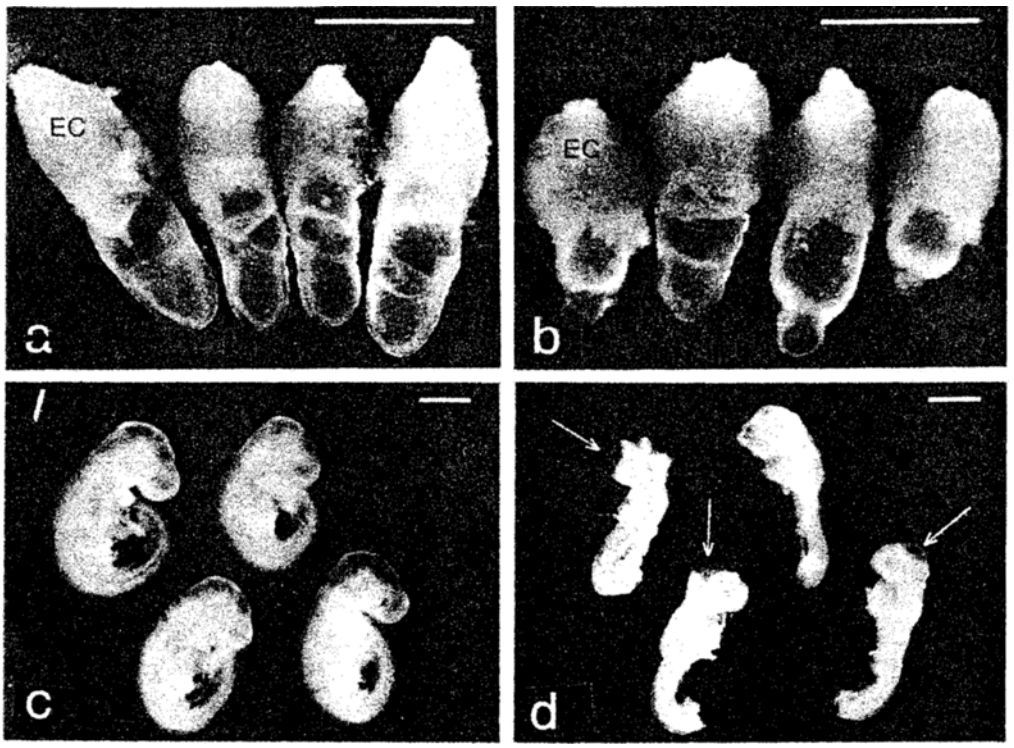

Fig. 2. Zinc-deficient morphologically normal (a) abnormal (b) egg-cylinders removed on day 9.5 of gestation; 11.5-d-old embryos grown from zinc-deficient egg cylinders that were apparently normal (c), morphologically abnormal (d). E. C. ectoplacental cone. Bar = 1 mm. Reprinted with permission University of Adelaide, South Australia. (28).

developing blastocytes and with our studies presented later in this review, in connection with the effect of the maternal feeding cycle on fetal development.

IV. WHOLE ANIMAL TERATOLOGY STUDIES MID-WAY THROUGH PREGNANCY

With a few notable exceptions, most teratology studies concerning *in utero* zinc deficiency have centred on an examination of fetuses taken from dams towards the end of pregnancy. Comparatively little is known about earlier changes that occur before or during the time of organogenesis and that may, or may not, be evident in the surviving offspring at the end of gestation. Investigations were accordingly undertaken in our laboratory on the morphological and histological development of 11-d-old rat fetuses taken from dams deprived of dietary zinc from day 0 of gestation (*30*). Removal of embryos at this stage of organogenesis precluded examination of several later-developing organ systems, but did allow the

recovery of most or all of the conceptuses, since it included many embryos that would subsequently have perished *in utero* and would have led to the increased number of fetal resorptions associated with gestational zinc deficiency.

No evidence was found of impaired implantation in the zinc-deficient dams, nor was there any increase in the intrauterine death rate at that stage. The incidence of malformations in the 11.5-d-old zinc-deficient embryos was around 50%, with evidence of a strong litter effect. Abnormalities include failure of the embryos to rotate, lack of fusion of the allantois with the chorion, craniofacial defects, anophthalmia, and open anterior neural tubes. Histological examination indicated reduced cell numbers in both the mesodern and the neuroepithelium in many zinc-deficient embryos, and light microscopic examination of serial coronal sections taken through zinc-deficient embryos showed definite derangements of cells in the neural tube and mesoderm, whereas the ectoderm remained apparently normal.

Zinc-deficient embryos were considerably smaller than the controls and it was noted that within the zinc-deficient group, maternal serum zinc levels on day 11 were inversely related to growth and to the prevalence of abnormalities. At the time, the physiological explanation for this correlation was not clear, but it was considered that it may be associated with periods of maternal anabolism and catabolism, resulting from the cyclical feeding pattern that accompanies zinc deficiency (*31*). Also of interest was the fact that the experimental studies with rats reflected a similar, but weaker, association we had noted earlier in women, between increased infant birth weight and low maternal serum zinc levels at 32 wks (*32*). Reference will be made to these studies later in this review.

V. CYCLICAL FEEDING AND *IN UTERO* ZINC DEFICIENCY

Subsequent studies (*30*) that monitored the feeding patterns of individual pregnant rats confirmed that zinc-deficient animals rapidly entered a cyclical feeding pattern, each with a period of about 4 d, but without any real synchrony. Maternal serum zinc levels on the morning of day 11 were found to correlate inversely with food intake the previous night ($r = -0.95$, $P < 0.001$), which demonstrated that consumption of the zinc-deficient diet led to a rapid lowering of maternal serum zinc levels, whereas fasting resulte in values rising towards normal. Mean 11.5-d-old embryonic protein levels were considerably reduced ($P < 0.001$) in zinc-deficient animals and major malformations (including anophthalmia and neural tube defects) occurred in about 30% of cases. A pronounced litter effect was again evident. Comparison of the feeding cycles with the established times of organogenesis suggested that peak consumption of the zinc-deficient diet during days 8 and 9 of gestation, and hence low maternal serum zinc levels at that time, were related to the production of several malformations of the central nervous system

and to an overall prevalence of terata. By day 11, however, the feeding cycle had entered a period of low food intake, resulting in the established dysmorphology then appearing to be associated with high maternal serum zinc levels.

This suggestion received experimental support (*30*), when pregnant dams were fed the zinc-deficient diet systematically to represent cycles of feeding that induced either low maternal serum zinc levels on days 8 and 9 of gestation, or adequate levels on these days, but decreased levels on days 6 and 7.

Induction of low maternal serum zinc levels on gestational days 8 and 9 further decreased the size of the 11.5-d-old embryos and increased the incidence of open neural tubes from 12.5 to 32%, with a total of 72% of embryos exhibiting at least one major malformation. Dams fed the zinc-deficient diet in the "reverse" cycle had larger 11.5-d embryos, with only 2% showing major malformations, although loss around the time of implantation increased, which corresponded with reduced maternal zinc levels accompanying peak feeding on days 6 and 7.

Studies just completed in our laboratory have examined the effect of timed, 4-d feeding cycles (Fig. 3) imposed on pregnant Sprague Dawley dams (4–5 animals/group) to induce low maternal zinc levels either on days 7 and 8 or on days 9 and 10 of gestation. Rats treated in this way received stock colony diet (W. Charlick Ltd., Adelaide, Australia) containing approximately 100 μg zinc/g until day 4 of pregnancy and from day 13 to day 21. The zinc-deficient, soybean-based diet (<0.5 μg/g) was fed to follow a particular 4-d cycle, flanked on either side by several days at 10 g/d. In this latest study, fetuses were examined on day 21 of gestation, which enabled soft tissue and palatal defects to be observed, that would not have been evident in 11-d-old embryos. Offspring from dams with depressed serum zinc levels on days 7 and 8 exhibited substantial teratogenesis (48.6%), especially with respect to skeletal development. Pups from dams consuming a high dietary intake on days 9 and 10 were also severely malformed (66.7%), but in this group a considerable number (28.3%) of palatal and soft-tissue abnormalities were evident. This latter study again highlights the potent effect of a single feeding cycle on fetal development and points to the facilitation of future studies concerning zinc-related teratogenesis using a single 4-d treatment period, imposed at the critical time of morphogenesis of the organ system under examination.

Recently, other workers (*33,34*) have drawn attention to the severely detrimental effects of force-feeding on anorexic, zinc-deficient rats, which possibly relates to the induction of an anabolic state in the force-fed dams. During the feeding cycles described in our studies, it seems likely that the animals alternated between a fasting, catabolic state, when some maternal zinc may have become available to the embryo, and a feeding, anabolic state when circulating zinc was lost in digestive enzymes and used for maternal anabolism, and was thus less available to

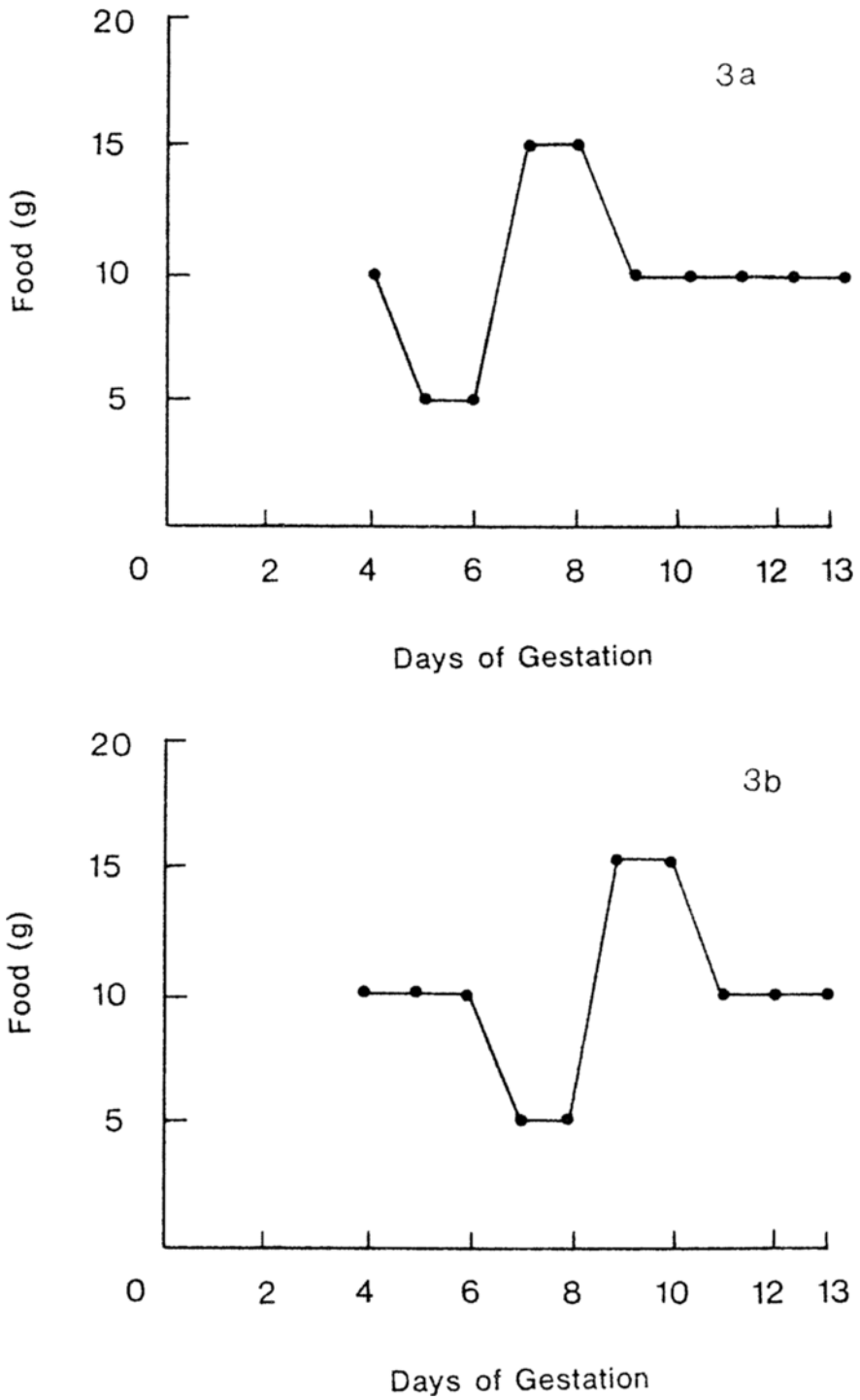

Fig. 3. Feeding cycles presented to dams to induce peak consumption and hence low serum zinc levels on (a) days 7 and 8, and (b) days 9 and 10.

the conceptus. During gestation, in rats feeding *ad libitum* on a zinc-deficient diet, the offspring will be exposed to 4 or 5 cycles of low maternal plasma zinc levels, each probably exerting an effect at a particular time of development. Because of the random nature of cycling in *ad libitum*-fed animals, such cycles will result in widespread adverse pregnancy outcomes, ranging from pre-implantation loss associated with the early cycles to behavioral anomalies arising from late gestational zinc depletion.

VI. SERUM ZINC AND FETAL GROWTH IN HUMANS AND RATS

Although in most experimental studies on zinc deficiency in animals, reduced birthweight and fetal dysmorphogenesis are directly correlated with diminished maternal serum zinc levels at term, in humans the association is less clear. Thus, while in several cases low zinc status has been observed in women with growth-retarded or dysmorphic offspring (*4,35–38*), in others no such association has been observed, though on occasion an opposite relationship has emerged (*2,3,39–41*). These contrasting observations have led to speculation that, although in experimental studies and in poorly nourished human populations, a correlation between impaired fetal development and low maternal zinc status at term will generally reflect a persistent zinc deficiency, in some human settings the opposite association may obtain, such as in adequately nourished individuals, when transfer of zinc to a large fetus is above average and serum zinc levels are low. Alternatively, serum zinc levels at term may be raised in some mothers with growth-retarded infants arising from an earlier embryological insult and subsequently reduced fetal growth (*32,39,42*).

The suggestion was studied experimentally in our laboratory with a group of four pregnant Sprague-Dawley rats subjected to a single zinc-deficient feeding cycle, timed to induce low serum zinc levels on days 8 and 9 of gestation. Thereafter, animals were fed the stock colony diet (W. Charlick Ltd., Adelaide, Australia, 100 μg zinc/g) until day 21 of gestation, when fetal development and maternal serum zinc levels were compared with a control group of four animals receiving the stock diet throughout pregnancy. Fetuses from dams subjected to the zinc-deficient feeding cycle were substantially reduced in body weight (28%) and length (12%) relative to the controls and many (40%) were severely malformed, mainly with respect to the central nervous system. Maternal plasma zinc levels were appreciably higher ($0.1 > P > 0.05$) in the group exposed to an early zinc deficiency (1.27 ± 0.13 μg/mL) than in the controls (0.96 ± 0.13 μg/mL). These data offer a measure of support for our earlier hypothesis and demonstrate that growth-retarded or abnormal fetuses affected early in pregnancy by a transient zinc deficiency may be

associated at term with a normal or elevated maternal zinc status. Taken together with our earlier studies concerning the severely teratogenic consequences of even brief episodes of dietary zinc impoverishment, the present findings emphasise the problems that may arise in interpretation when zinc status at the end of pregnancy is linked causally with events that may have occurred very much earlier. Certainly, the recent report by Breskin et al. (*43*), in which first trimester serum zinc levels were found to be lower in 7 out of 25 women who aborted spontaneously, reinforces the view that maternal zinc status is best investigated in relation to embryological events occurring at the time the analyses are made. Moreover, the high incidence of aborters in their study, who exhibited a low zinc status, suggests that the impact of zinc nutriture on pregnancy outcome in humans might be of considerably greater quantitative significance than the relatively few reported cases of zinc-associated birth defects.

VII. ZINC AND CADMIUM-INDUCED TERATOGENESIS

Cadmium-induced teratogenesis is well established in animals (*44,45*) and includes abnormalities of most organ systems. Several workers have suggested that the mode of action of cadmium as a teratogen lies in an accompanying, induced fetal zinc deficiency (*46*) or in antagonism by cadmium of several fetal and placental zinc metalloenzymes (*47*). Certainly, some protection is afforded by zinc against cadmium-induced teratogenesis (*48*) and increased cadmium-related fetotoxicity has been reported in zinc-deprived rats (*49*).

Considerable attention has been paid to the placenta in relation to cadmium embryopathy as the metal is accumulated to some extent in this organ (*50*). No consideration has yet been given to the yolk sac although, like the later-developing placenta, it may provide some protection to the early embryo against cadmium in the maternal circulation (*47,51*). Yolk sac morphology (*52*) and yolk sac function (*53*) were accordingly studied in this laboratory in cultured, explanted rat embryos and in isolated rat yolk sacs. DNA synthesis was markedly reduced in rat embryos grown in serum containing various levels of cadmium and the yolk sacs appeared thick and aplastic. Zinc, added to the medium, increased the synthesis of DNA and improved the physical appearance of the yolk sac. Yolk sac function, as measured in vitro by pinocytic uptake of the synthetic substrate ^{125}I-polyvinyl pyrrolidone, was severely depressed by cadmium, but activity was largely restored on the addition of zinc (Fig. 4) (*54*).

Together, these studies point to an interaction between zinc and cadmium at the level of the yolk sac and suggest that the effect of cadmium on yolk sac endocytosis and embryonic nutrition may be a factor of some significance in cadmium-related teratogenesis. The protective effect

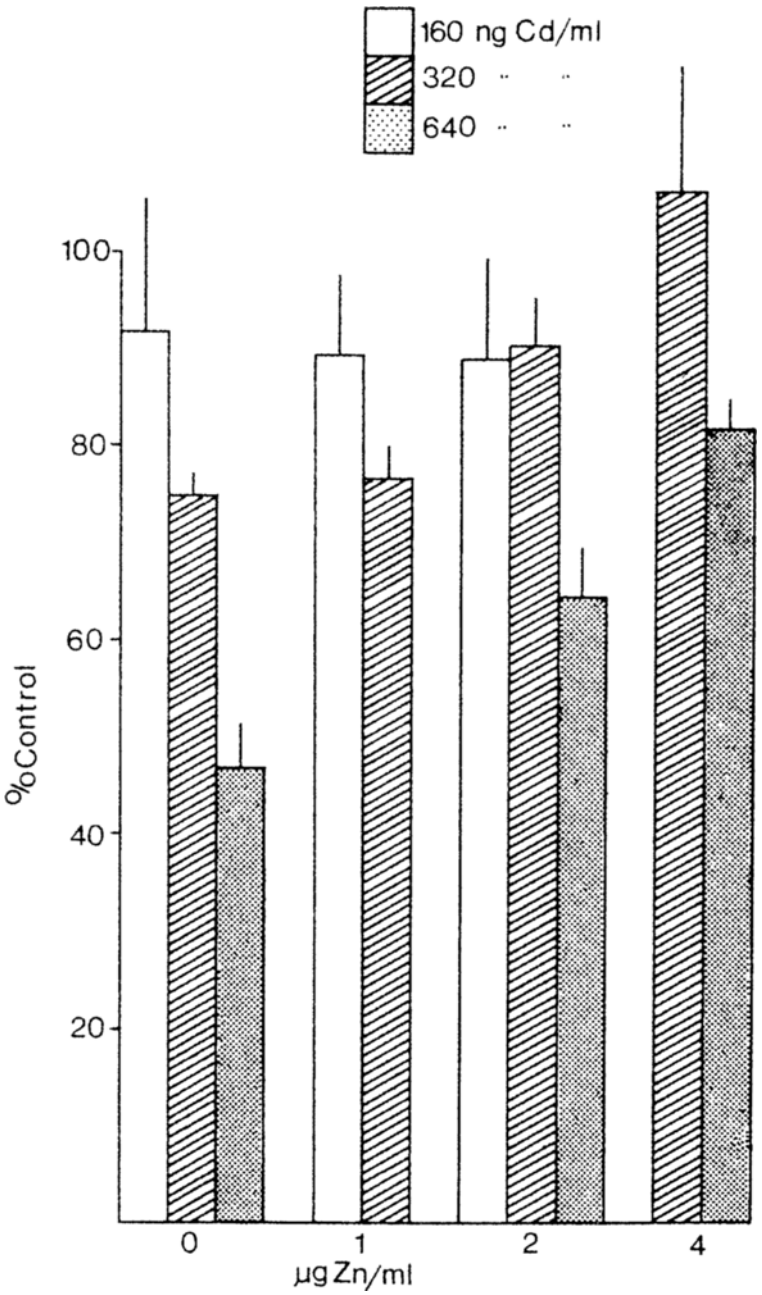

Fig. 4. Interactions of cadmium and zinc on the uptake of ^{125}I-polyvinylpyrrolidone by rat yolk sacs. Reprinted with permission from The Nutrition Society of New Zealand (*54*).

of zinc in connection with cadmium embryopathy and the increased sensitivity of zinc-deprived embryos to cadmium toxicity suggest that zinc status should always be considered in studies relating to cadmium-induced fetal damage (*54*).

VIII. ZINC, ALCOHOL INTERACTIONS, AND TERATOGENESIS

Apart from cadmium, possible potentiation has been suggested between zinc deficiency and other teratogens in relation to 6-mercaptopurine (*55*), acetazolamide (*56*), and with respect to a thalidomide analog (*57*). In humans and animals both, zinc deficiency and hyperexposure to alcohol disturb general organogenesis and especially development of the central nervous system. In humans, the possibility that nutrient imbalances may accompany prolonged alcohol abuse has been recognized for several decades and animal studies now link the consumption of alcohol to changes in the body status of several essential trace elements (*58*).

The situation with regard to zinc is particularly interesting, since it has been suggested that the hyperzincuria and low serum and liver zinc levels often reported to accompany prolonged alcohol abuse (*59*) may reflect an induced zinc deficiency, which in turn might contribute to the development of the chronic alcohol syndrome (*60*) and possibly to the morphological abnormalities of the fetal alcohol syndrome (FAS) (*15,61–63*). Low fetal zinc levels have been reported to accompany gestational alcoholism in several animal studies (*64,65*), but not in all (*66,67*). Four studies on animals (*58,68–79*) concerning a possible link between zinc status and FAS indicate increased embryotoxicity, growth retardation, and teratogenicity when gestational alcoholism and *in utero* zinc impoverishment were superimposed.

Though recognizing the emerging association between zinc status and alcohol embryopathy, it seems unlikely that the relationship exists entirely because of an alcohol-induced zinc deficiency (*71*). A more likely explanation may be found in the possibility that each factor exerts its influence independently in the body, but that their effects overlap to some extent at a common biochemical locus. Such considerations have led to the suggestion (*58,72*) that at least some aspects of alcohol and zinc deficiency-related toxicity may lie in the role played by zinc in the scavenging of free radicals released during the metabolism of ethanol, and in membrane antioxidant mechanisms. Certainly, studies in our laboratory pointing to increased levels of superoxide dismutase (EC 1.15.1.1) in adult and fetal rat livers exposed to gestational alcoholism (*73*) suggest enhanced cellular free radical defense in response to treatment with alcohol, although the greater involvement of manganosuperoxide dismutase, rather than the cupro,zinc form of the enzyme, points to a possibly wider participation of several trace metals in cellular free radical defense, than zinc alone (*58,74*).

Pursuing this hypothesis further, we studied the endogenous levels of the lipid peroxidation product malondialdehyde in several rat tissues exposed to a nutritional zinc deficit and/or oral consumption of ethyl alcohol (*58,70*). Elevated levels of malondialdehyde were found in the

microsomal fraction of livers from adult rats receiving either a zinc-deficient diet or 20% alcohol for several weeks. However, no potentiation of peroxidation was noted when the treatments were superimposed, which was in accord with the separate finding of Sullivan et al. (*72*), but appeared inconsistent with our notion that the zinc/alcohol interaction stems from the role of zinc in protecting membranes against alcohol-related free radical damage.

However, studies just completed in our laboratory have demonstrated a considerable degree of potentiation with respect to *in utero* zinc deficiency and alcohol in the microsomal fraction of fetal rat livers. Thus groups of Sprague-Dawley dams were fed either a zinc deficient (<0.5 μg/g) soybean-based diet or a zinc-supplemented (100 μg/g) diet on a restricted-fed basis, from day 8 to day 21 of pregnancy. Half the dams received alcohol (4 g/kg as a 25% aqueous solution) by gavage throughout pregnancy. Controls were dosed with an isocaloric amount of glucose. On day 21, fetuses were removed by caesarean section and fetal livers were collected and pooled to provide one or two samples from each dam. Endogenous malondialdehyde levels were determined (Table 1) by the thiobarbituric acid method of Misra and Gorsky (*75*).

Malondialdehyde levels in microsomes from the zinc-deficient fetuses were significantly higher ($P < 0.05$) than in the controls. In alcohol-treated pups, malondialdehyde levels were unchanged but, when in utero alcoholism and zinc deficiency were superimposed, the levels rose to be more than threefold above those in zinc-supplemented, alcohol-treated pups ($P < 0.005$) and a little more than double the levels ($0.1 > P > 0.05$) in zinc-deficient pups not exposed to maternal alcoholism. Taken together with our earlier data concerning increased fetal superoxide dismutase following gestational alcoholism, the present findings suggest an involvement of the fetus in the metabolism of alcohol and a degree of zinc-sensitive pathology in fetal livers that is probably related to free radical damage of membrane lipids. Whether this alcohol-induced lipid peroxidation is connected in any way to fetal embryopathy as expressed in FAS is not clear, but a nexus between zinc and alcohol-related free radical damage could be expected to have some deleterious consequences on normal fetal development.

TABLE 1
Effect of Gestational Alcoholism and Maternal Zinc Status on Endogenous Levels of Malondialydehyde in Fetal Rat Liver Microsomes

Treatment	No. of dams	Concentration of malondialdehyde,[a] μmol/mg protein
Control	5	0.55 ± 0.03
Zinc-deficient	4	0.69 ± 0.01
Controls + alcohol	5	0.49 ± 0.04
Zinc-deficient + alcohol	5	1.65 ± 0.60

[a]Values are means ± SEM of 6–10 fetal liver samples/treatment.

IX. ZINC, ALCOHOL, AND THE CENTRAL NERVOUS SYSTEM

In animals, zinc deficiency and alcohol individually affect morphological development of the central nervous system. Furthermore, even in the absence of overt teratology, behavioural and learning deficits are a common feature of both conditions (*76,77*). Little is known, however, concerning the possibility of an interaction of these factors at more subtle levels of neural organization, although a case exists for consideration of an alcohol/zinc interaction specifically involving the hippocampus and possibly localized in the mossy fiber pathway (*78*).

Thus, recently, loss of hippocampal pyramidal cells and cells of the dentate gyrus have been reported in adult rats consuming alcohol for an extended period (79), whereas prenatal exposure to ethanol has been associated with the development of an aberrant band of infrapyramidal mossy fibers in the hippocampal subfield CA3a (*80*). Zinc deficiency too has been associated with reduced hippocampal weight in rat pups exposed to late prenatal and/or early postnatal zinc impoverishment (*81,82*) and with abnormal synaptic responses in the mossy fibers of adult rats (*83*). In human alcoholics, an apparent association was noted between ethanol damage and hippocampal zinc levels, as evidenced by marked degeneration of the granule cell layer, which was accompanied by a reduction of similar magnitude in the level of hippocampal zinc (*84*). The existence of an alcohol–zinc interaction in the hippocampus would be of interest because of the role played by this region of the brain in memory processing and behavior (*85*), and because of the possibility that the interaction may underlie some of the cognitive deficits referred to earlier. However, little is known concerning the effect of ethanol or zinc deficiency on prenatal development of the hippocampus and no studies have considered the possibility of an interaction in this brain region.

Investigation in our laboratory examined the cellular development of the hippocampus in 20-d-old rat fetuses exposed to either gestational alcoholism or to maternal zinc deficiency, and in some cases to both (*86*). Zinc deficiency reduced cell numbers and cell density in both the granule cell layer of the dentate gyrus and the pyramidal cell layer of the Horn of Ammon (Table 2; Fig. 5). The data with regard to cell numbers are in accord with those of Kawamoto and Hallas (*87*), whose findings appeared simultaneously, but differ with respect to cell density.

Ethanol alone reduced the total number and the density of cells in the granule cell layer and, when imposed concurrently with zinc deficiency, the deleterious effects of both treatments appeared to be additive. The possibility is thus raised that some aspects of the behavioral deficits associated with *in utero* zinc impoverishment and with gestational alcoholism in animals may arise from impaired development of the fetal hippocampus, with an apparent interaction occurring between treatments in this region of the brain.

TABLE 2
Effect of Zinc Status and Ethanol on the Granule Cell Layer of the Dentate Gyrus in Fetal Rats

	Histological characteristics[a]				
Treatment[b]	No. cells/mitotic section	No. index, figures	Mitotic cells, %	Area of cells/ $\times 10^2 \mu m^2$	Cell density 250 μm^2
Control (9)	2330 ± 93	5.6 ± 0.8	0.22 ± 0.02	1171 ± 39	4.2 ± 2.1
+Ethanol (6)	1540 ± 118[c]	4.7 ± 0.7	0.30 ± 0.04	1009 ± 83	3.2 ± 0.3[d]
−Zn (11)	1538 ± 70[c]	4.2 ± 0.4	0.27 ± 0.02	1132 ± 38	2.8 ± 0.1[c]
+Ethanol −Zn (10	988 ± 61[e,g]	4.2 ± 0.4	0.44 ± 0.05[c]	1061 ± 61	2.0 ± 0.1[e,g]

[a]Values represent the means ± SEM.
[b]Numbers of hippocampi studied given in parentheses.
[c]$P < 0.05$ relative to control.
[d]$P < 0.01$ relative to control.
[e]$P < 0.01$ relative to control.
[f]$P < 0.01$ relative to −Zn group.
[g]$P < 0.001$ relative to −Zn group.
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X. CONCLUSIONS

The emergence of zinc deficiency as a substantial nutritional problem in humans, coupled with the established teratogenicity of the condition during pregnancy, have contributed to the considerable current interest concerning the possibility of zinc-related congenital defects in humans. Further studies are needed to quantify the extent of the problem and these should take into account the experimental evidence with rats concerning the potent teratogenicity associated with high intakes of low-zinc food by the dam, even for very brief periods during gestation. Also, consideration should be given to the wide-ranging effects of zinc deficiency on pregnancy outcome in animals, so that in human studies attention is not focused exclusively on gross teratology as the principal consequence of *in utero* zinc impoverishment. With respect to overt dysmorphogenesis, the acute sensitivity of the developing central nervous system invites special attention as, in most human studies to date on infants deemed to have suffered *in utero* zinc deficiency, terata of the brain and spinal cord have been particularly evident. More probing experimental studies are required to identify the underlying biochemical lesions associated with abnormal development in zinc-deprived embryos and special attention should be given to the pathways of DNA synthesis in brain in relation to other body tissues. Also of growing importance must be the identification of other factors that, together with reduced zinc status, act to amplify the individual teratogenic potential of each

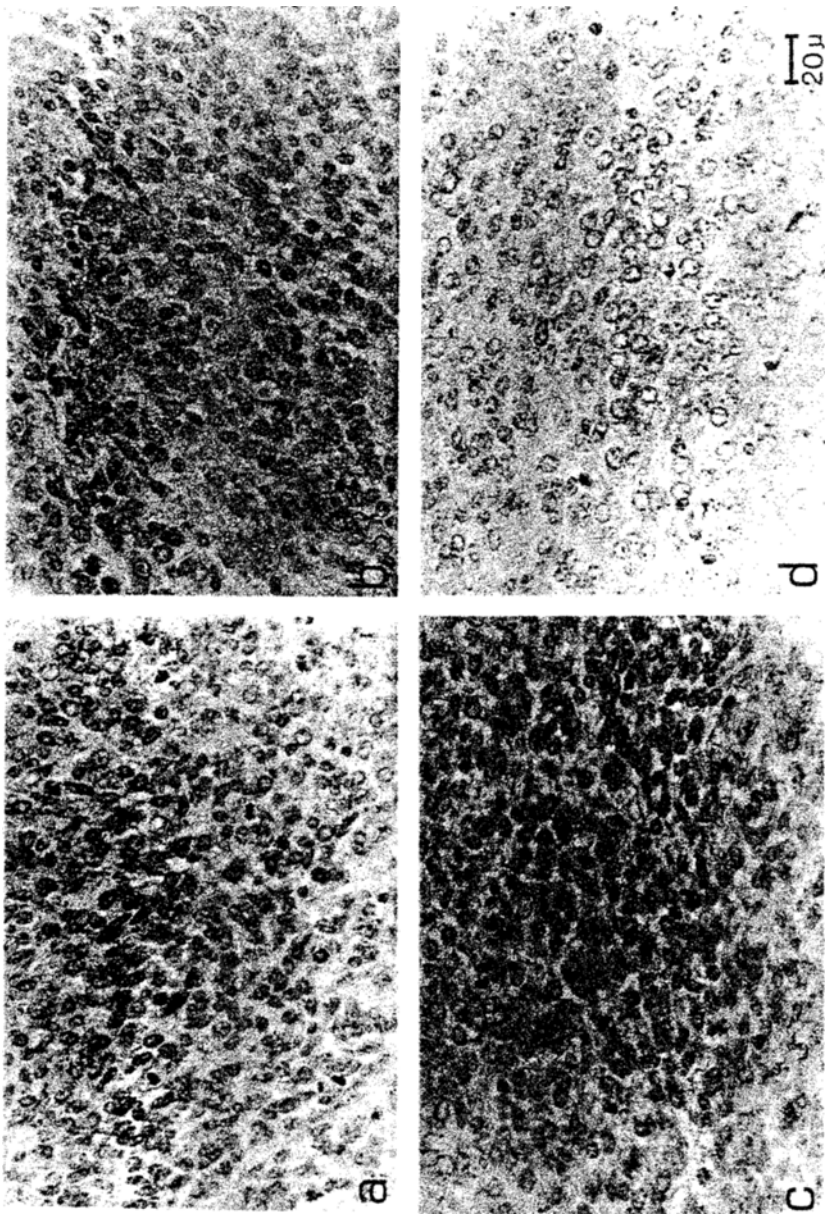

Fig. 5. Photomicrograph of sagittal sections of fetal (20 d) hippocampi from control (a), alcohol-treated (b), zinc-deficient (c) and alcohol-treated/zinc deficient (d) dams. Reprinted with permission from Liss, New York (86).

treatment. In the human setting, where a serious, uncomplicated dietary zinc deficiency is probably not widely encountered, such multifaceted interactions may be of greater significance to public health, especially in developed nations. Alcohol would seem to be important in this regard as several interactions have been reported between ethanol and trace elements in man and animals and recent evidence points to enhanced alcohol-related lipid peroxidation in the livers of zinc-deficient rat fetuses. In all studies, wider attention should be paid to the involvement of other trace metals, as their complex interactions with zinc may modify the outcome of a primary zinc deficit with respect to abnormal fetal development.

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