

ZINC DEFICIENCY DURING PREGNANCY

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## INTRODUCTION

In 1979, Prasad (1) published a review of the role of zinc in nutrition. The review covered the period from the first establishment of a need for zinc in *Aspergillus Niger* in 1869 to the present. It included the establishment of the need for zinc in rats by Todd et al. in 1934 and the discovery of evidence of zinc deficiency in humans in 1961 by Prasad himself. Much research has been done on the effects of zinc deficiency in animals, especially the rat. Many roles of zinc have been identified, but few of the mechanisms are understood.

The recommended dietary allowances (NRC-RDA) for zinc were first established in 1974 as 15 mg per day for adult men and women (2). This amount, which remained unchanged in the 1980 revision, was based on the turnover of body zinc that had been calculated from radioisotope studies to be 6 mg per day. With this amount of turnover, and allowing for absorption of about 40% of dietary zinc, an intake of 15 mg/day is recommended for adults.

An additional 5 mg per day are recommended during pregnancy (2). The increased recommendation for pregnant women is based on the calculated additional .75 mg of zinc required daily for growth of fetus and placenta and a liberal safety allowance assuming only 15% of the dietary zinc is absorbed.

The major role of zinc is for growth. This may be explained by the fact that the DNA dependent RNA polymerase and DNA polymerase of numerous prokaryotic and eukaryotic organisms are zinc enzymes which are important in the replication and transcription of DNA during cell division (3). Thus a major detrimental effect of a deficiency of zinc is a decrease in growth functions. This is multiplied by anorexia, that normally accompanies zinc deficiency, and can cause malnutrition. These effects are seen quite soon after dietary zinc is limited to below required amounts. The fast onset of adverse effects is due to the body's limited stores of zinc available for use when compared to the need for zinc. The average human body contains 1.4

to 2.5 grams of zinc, but 70% of this zinc is in the skeleton and is not readily available for use. The other 30% is relatively available for release from the tissues that it is in, but this small amount is depleted quickly.

The availability of zinc from the diet is critical because a deficiency can occur so quickly, and because an estimated double or triple amount must be consumed in order to absorb enough zinc from the diet. Several factors can influence negatively the absorption of zinc. Availability of zinc in vegetarian diets, typical diets of low income people, and diets of alcoholic women may be dangerously low.

The increased requirements for all nutrients during pregnancy are particularly critical for trace elements such as zinc. With the key role that zinc plays during pregnancy, and the increased requirements for zinc during pregnancy, the severity of both the risk and the results of zinc deficiency are increased. This has been established through many studies done with laboratory animals, particularly rats, and several human studies.

Zinc has been found to be involved, either as a constituent or as a catalyst, in 59 enzymes (1). Zinc is therefore essential for many of the body functions, and a deficiency can affect many parts of the body. Some examples are:

<u>Part of body</u>	<u>Factor effected</u>	<u>Functions effected</u>
red blood cells	- carbonic anhydrase	- maintenance of acid base balance
pancreas	- cocarboxypeptidase	- digestion
bone	- alkaline phosphatidase	- bone metabolism
liver	- alcohol dehydrogenase	- detoxification of ethanol
epidermis	- methionine to protein	- the healing process
pancreas	- insulin	- glucose tolerance
gustatory system	- hypogeusia	- taste and smell acuty

Because zinc has so many critical roles, the resulting effects on fetuses of zinc deficient females vary from slight congenital physical abnormalities



to compositional changes in organ development to severe birth defects. There are also lasting effects on learning ability and behavior of the fetus after birth.

Although the majority of information about zinc deficiency comes from animal studies, some human studies also have been done on the relationships between zinc deficient diets of certain groups of people, levels of zinc in various portions of the body, and abnormalities known from animal studies to result from zinc deficiency. Many positive correlations have been found, and the evidence indicates that zinc is as critical to human reproduction as it is to rats and other animals.

This paper will include a review of the effects of vegetarian or low income diets, and alcohol consumption on the zinc status of pregnant women. The availability of zinc to the body may be dangerously low in women in such commonly occurring circumstances. Other topics to be discussed will include the metabolic and biochemical aspects of zinc during pregnancy, the effects of zinc deficiency on the pregnant and lactating female and correlation of human and animal studies of zinc deficiency during pregnancy.

## ZINC STATUS IN PREGNANT WOMEN

Maternal plasma concentration of zinc decreases in pregnancy, due to the use of zinc by the growing fetus and the development of the other products of conception (4). It has been estimated that pregnant women must retain approximately 750 ug of zinc per day for growth of the products of conception during the last two-thirds of pregnancy.

Some common dietary sources of zinc are listed in table 1. Wheatgerm and nuts are high in zinc, but dietary zinc in the United States is obtained primarily from meat and other expensive animal food (5). Presence of fiber and phytate in cereal grains and other plant foods may influence the absorption and retention of zinc and thus its availability from the diet. Therefore vegetarian diets and diets of lower income people may be in danger of being zinc deficient. In addition, the presence of alcohol in the blood of alcoholics induces an increase in the urinary excretion of zinc and subsequent zinc deficiency.

TABLE 1

Zinc (mg/100 g) in various foods and food groups (5)

Food group	zinc	Food group	zinc
Nuts group	3.4	Seafood	1.8
Meat group	3.1	Oyster	149.0
Eggs	2.1	Vegetable group	1.1
Cereal group	1.9	Dairy products	0.86
Wheat germ	6.7	Fats and oils	0.84

Vegetarians. As more people turn from animal to vegetable protein sources for economical, ecological, or health reasons, it becomes critical to evaluate the adequacy of meatless diets for meeting nutritional needs. One of the nutrients of concern in a vegetarian diet is zinc. Although dry

beans, nuts, and whole grains are high in zinc content, the availability of this zinc has been questioned because of the high phytic acid and fiber content of these foods and losses during processing. The World Health Organization (6) has estimated that only about 10% of the dietary zinc is available from a diet made up exclusively of vegetable protein sources but as much as 40% may be available from mixed protein sources.

In 1960, O'Dell and Savage (7) reported that phytate, which is present in cereal grains, markedly impairs the absorption of zinc. Likuski and Forbes (8) showed that phytic acids depressed the availability of zinc whether the protein source was pure amino acid or casein. Reinhold (9) reported that unleavened bread, consumed in large amounts by the Iranian villager, contained significantly more phytate than urban leavened breads because leavening resulted in the destruction of phytate. He also observed a correspondingly higher zinc deficiency in the Iranian villager than in the urban person who consumed leavened bread.

Reinhold (9) indicated that high fiber intake, which is common in subjects consuming high cereal protein, is also detrimental to zinc availability. High fiber foods, already low in zinc content, have extremely low zinc availability due to the binding of zinc to the fiber. Fiber is not degraded by digestive secretions, so zinc remains attached to the fiber and is transported through the small intestine without being absorbed and is ultimately lost in the feces. Zinc from other food sources consumed at the same time as the high fiber foods also can be bound to the fiber and lost.

Since zinc requirements are increased during pregnancy, pregnant vegetarian women may be at greater risk of developing a poor zinc status than pregnant nonvegetarian women. King et al. (10) evaluated the zinc status of twelve pregnant ovolacto vegetarians, six pregnant nonvegetarian women, and five nonpregnant ovolacto vegetarian women during the last trimester. Zinc intake was calculated from a 3-day dietary recall. Hair, urinary, and

nonfasting plasma samples were collected. None of the women consumed diets which provided the recommended amounts of zinc. The mean zinc intake of each group, and their zinc status indicators are listed in table 2.

TABLE 2  
Zinc status indicators by group (10)

	Pregnant Vegetarians	Nonpregnant Vegetarians	Pregnant Nonvegetarians
Zinc intake (% RDA/mg)	62%/12.6	43%/6.4	72%/14.4
Plasma zinc (ug/100g)	60	80	66
Urinary zinc (mg/g)	0.39	0.17	0.41
Hair zinc (ug/g)	177	179	178

Four of the pregnant vegetarians and one pregnant nonvegetarian took a vitamin-mineral supplement which provided additional zinc and brought the average total zinc intake of these women up to 46 mg per day. Even though the nonpregnant women consumed significantly less zinc than the pregnant women, the plasma zinc levels of the nonpregnant women were significantly higher than those of the pregnant women. Plasma zinc was about 21% lower in the pregnant women than in the nonpregnant women (63 vs. 80 ug per 100 g, respectively). Urinary zinc excretion was about twice as high in pregnant women as in nonpregnant women. The average urinary zinc concentration per gram creatinine also was about twice as high for the two pregnant groups as for the nonpregnant group. The hair zinc concentrations were essentially the same in all three groups, 178 ug per g. Consumption of an ovolacto vegetarian diet did not appear to alter zinc status in this group of pregnant women. Even though the pregnant vegetarians consumed about twice as much crude fiber as the nonvegetarians (8.9 vs. 4.8 g/day), the differences in zinc

status were not significant. Thus, these results suggested that the zinc status of ovolacto vegetarians was not at risk when their diets contained less than 9.0 g per day crude fiber.

In Swanson and King's study (11), pregnant and nonpregnant women were fed identical amounts of zinc, but urinary zinc levels were higher in the pregnant group. Swanson and King concluded that urinary zinc may be elevated in pregnant women due to the increased glomerular filtration rate, or a reduction in tubular reabsorption, or an increase in the excretion of amino acids which bind zinc.

Hambidge and Droegemueller (12) have suggested that plasma zinc levels below 50  $\mu$ g per dl, urinary zinc levels below 150 mg per day and hair zinc concentrations of less than 100 mg/g in pregnant women are indicative of unacceptable zinc status. Using these criteria, one pregnant vegetarian in the King et al. study (10) had an unacceptable plasma zinc and two others had unacceptable urinary zinc levels. The data suggest that the parameters of zinc status studied were not significantly affected by ovolacto vegetarian dietary habits.

Low income pregnant women. It is known that the best sources of dietary zinc are the more expensive foods, particularly meats. Therefore, persons subsisting in the low income class are at particular risk of low blood zinc concentration.

Cavdar et al. (13) determined serum zinc concentrations in 101 pregnant women and 40 nonpregnant healthy controls. The pregnant women were divided on the basis of socio-economic background and nutritional status. The 30 poorly nourished women were those from low socio-economic background whose diet consisted mostly of carbohydrates and also contained very little or no protein. The 71 well nourished women were from high and upper-middle socio-economic classes whose diets contained animal protein in the form of meat and/or eggs on a daily basis. The well nourished women also had been taking

supplemental vitamins and iron during their pregnancy. As shown in table 3, serum zinc levels as determined by atomic absorption spectrophotometry were significantly lower in 30 poorly nourished pregnant women than in the controls, whereas there was no statistical difference between serum zinc values of well-nourished pregnant women and those of the control women, and furthermore this was true for each trimester of pregnancy. The results of serum zinc determination in the poorly-nourished pregnant women reflected significantly lower values in each trimester compared to those of the controls, and a striking decrease in serum zinc level between the first and the third trimester.

TABLE 3

Comparison of serum zinc levels (ug/100ml) in two groups of pregnant women (13)

Group	<u>1st trimester</u>			<u>2nd trimester</u>			<u>3rd trimester</u>		
	n	$\bar{X}$	$S\bar{X}$	n	$\bar{X}$	$S\bar{X}$	n	$\bar{X}$	$S\bar{X}$
Poorly nourished	10	88	3.8	10	80	2.7	10	68	4.0
Well nourished	11	117	6.9	22	108	4.0	38	105	3.5

Hunt et al. (14) estimated the zinc intake of low income pregnant women of Mexican decent by use of 24-hour dietary recalls obtained during the first two trimesters for 344 women and again during the third trimester for 279 of the same women. The daily mean zinc intake was calculated to be  $9.4 \pm 3.8$  mg during the first two trimesters and as  $10.0 \pm 4.3$  mg during the third trimester. For about 85% of the women, the reported intakes were below 2/3 of the Recommended Dietary Allowance for zinc.

In some of the women studied by Hunt et al. (14), no significant correlation was shown between low dietary zinc intakes and low serum zinc levels during either early or late pregnancy. Zinc intake and the energy value of the diets were fairly well correlated; step-wise regression analysis showed that this relationship was due to the protein in the diets and not to

energy per se. During the third trimester, the simple correlation coefficient between zinc and energy was 0.73 but the partial correlation coefficient between zinc and energy with the linear effect of protein removed was only 0.08. An equation to estimate the intake of zinc from the intakes of protein and iron was developed:

$$\text{Zinc(mg)} = 0.283 + 0.094 \text{ Protein(g)} + 0.0260 \text{ Iron(mg)}.$$

Since the inclusion of iron did not markedly increase the multiple correlation coefficient, the following equation could be used for a rough estimate of the intake of zinc :

$$\text{Zinc(mg)} = 0.735 + 0.134 \text{ Protein(g)}.$$

Although this equation would apply only to groups of people who eat foods that have a nutrient composition similar to the foods eaten by the Mexican women in this study, it still follows that zinc intake is correlated closely to protein content of the diet. The foods which are high in protein or iron that can provide needed zinc are generally expensive and may not be readily available to people with limited incomes such as those in the Mexican study.

Alcoholic women. Another group that is subject to zinc deficiency is alcoholics. Jackson and Schmacher (15) proposed that any agent given during the period of organogenesis which is capable of decreasing the zinc levels, or of altering its activity, should theoretically act as a teratogenic or fetal toxic agent. Alcohol is such an agent in that it increases urinary excretion of zinc and decreases availability of zinc to both the mother and the fetus. Although many studies have been done, the mechanism of this increased excretion is unknown. Kahn et al. (16) proposed that a possible cause of hyperzincuria is the direct effect of alcohol on the renal tubular epithelium. Prasad (1) showed that this direct effect was evident when a complete urine collection was analyzed for zinc during the first 3 hour and second 3 hour periods following ingestion of 6 oz of chilled vodka.

There are many studies documenting the relationship of zinc deficiency

to chronic alcoholism in both animals and man. Martier et al. (17) studied the zinc status of 25 alcoholic and 25 nonalcoholic pregnant women and correlated it with fetal outcome. Alcoholic women were defined by the volume index of alcohol consumption and the Michigan Alcoholic Screening Test. The alcoholic women had significantly lower plasma zinc levels than the nonalcoholic women (50.7 vs. 72.2 ug/dl). Fetal cord plasma zinc levels also were lower in the offspring of alcoholic women (65.5 vs. 81.3 ug/dl). Infants from alcoholic mothers had slightly more defects than infants from nonalcoholic mothers, as shown by table 4.

TABLE 4

Congenital birth defects and birth weights in  
infants of alcoholic and non alcoholic women (17)

Defect:	Alcoholic	Non-alcoholic
Craniofacial:		
Nose	12	7
Eyes	6	2
Lips	4	2
Mandible	1	2
Ears	0	2
Maxilla	0	1
Tongue	1	0
Cutaneous	5	2
Hernias	3	1
Heart	1	0
Genital	0	1
Neurological	4	2
Other	2	0
Total birth defects	39	22
Children affected	18/25	14/25
Birth weight(g)	3090±410	3180±420

Although the increase of birth defects with alcoholism in the Martier et al. study (17) was only slight, 18 of 25 vs. 14 of 25, maternal zinc



deficiency does not have to be severe to cause fetal defects. Hurley and Shrader (18) pointed out that a mild zinc deficiency produced teratogenic effects, whereas severe deficiency resulted in fetal mortality.

Epidemiological factors of alcohol-related fetal dysmorphogenesis also can be correlated with the zinc status of the mother. In a study at Cleveland Metropolitan General Hospital (19), dietary zinc intake by alcoholic and nonalcoholic pregnant women appeared to be similar, but plasma zinc values for alcoholic pregnant women were low, which may be a clue to the mechanism of alcohol induced fetal dysmorphogenesis. Zinc continues to be absorbed by the alcoholic mother, but due to hyperzincuria, it may not be available as plasma zinc, and thus both mother and fetus suffer zinc deficiency.

#### METABOLIC ASPECTS OF ZINC DURING PREGNANCY

Maternal-fetal zinc metabolism. The mammalian embryo contains little or no internal nutritive material and must depend on the maternal organism for nutrition. Since the only maternal source of nutrients for the embryo is the oviductal or uterine fluid, dietary zinc deficiency produces a rapid decrease in the zinc concentration of these fluids and provides the conceptus with inadequate zinc for normal development (20).

Henkin et al. (21) examined human maternal-fetal interrelationships of zinc at term and demonstrated differences in zinc binding in the serum of mother and fetus to explain the transfer of zinc from mother to fetus. At delivery, venous blood was obtained from 15 normal mothers and from the umbilical cords of their 15 normal babies; and amniotic fluid was obtained. Total concentration of zinc in blood serum was measured by atomic absorption spectrophotometry. Ultrafilterable zinc (free zinc) was obtained by ultrafiltration. The mean concentration of total zinc in maternal serum (48 ug/100 ml) was about half of the normal nonpregnant adult female value (90 ug/100 ml). Mean concentration of free zinc was 2-1/2 times the normal non-

pregnant value. Mean concentration of the total zinc in the fetal serum was 85 ug per 100 ml, with free zinc 32% of its total concentration. Mean concentration of zinc in amniotic fluid was 32 ug per 100 ml, with free zinc 92% of its concentration. Henkin et al. concluded that the lowered levels of total zinc in maternal serum at term are primarily due to significant decreases in zinc binding, and either diminished quantities of zinc binding proteins or alterations in binding affinities. This decrease in total zinc in maternal serum at term is an example of one of the few instances wherein binding may diminish during pregnancy. However, in spite of the decrease in serum zinc, absolute levels of free zinc in the pregnant woman at term remain relatively unchanged from the nonpregnant state. Mean fetal serum zinc concentrations, both bound and free, are not significantly different from adult, nonpregnant levels. Thus, those factors which alter zinc metabolism in the mother at term apparently are not active in the fetus. Henkin et al. (21) showed that zinc moves across the placenta by passive transport. Since only free metals were available for transfer, there were maternal-fetal gradients for zinc of 5 ug per 100 ml (maternal free zinc 32 ug/100ml, fetal free zinc 27 ug/100 ml).

Swanson and King (11) fed 8 pregnant and 10 nonpregnant women the recommended dietary allowance for zinc to measure differences in parameters of zinc status and to study differences in zinc utilization due to pregnancy. The participants were confined to a metabolism unit for 21 days. A constant, semi-purified, formula diet containing 20 mg of zinc as zinc sulfate, and the RDA for pregnancy all other nutrients were fed. Serum, saliva, hair, urine, and feces were analyzed for zinc content. Retention and apparent absorption of zinc were greater in the pregnant than in the nonpregnant group (2 vs. 1 mg /day retention and 15 vs. 9% apparent absorption). Serum zinc was significantly greater in the nonpregnant than in the pregnant group (92 vs. 68 ug/dl.). Urinary zinc excretion was greater in the pregnant

group. Serum and urinary zinc increased from day 1 to day 21 in the non-pregnant women; the increase in serum zinc was correlated with an increase in urinary zinc, due to a renal regulation of zinc homeostasis.

Effects of low zinc concentrations in oviductal and uterine fluid.

Hurley and Shrader (18) noted that the onset of adverse effects of severe maternal zinc deficiency in rats was very rapid. After only 3 days of maternal deprivation of dietary zinc, abnormal development was seen in preimplantation embryos. Live eggs flushed from the oviducts of controls were in the 8 or 16 cell stages, while many of those from zinc-deprived females had failed to cleave and were undergoing necrosis. Similarly, live eggs removed on day 4 from controls were normal blastocysts, while most of those from rats fed the zinc deficient diet from day 0 of pregnancy were abnormal with cells of unequal size and small number. Hurley and Shrader (18) hypothesized that the abnormal development of preimplantation embryos in rats fed a zinc deficient diet results from an insufficient amount of zinc available to them from their surroundings, the oviductal and uterine fluids.

Gallaher and Hurley (20) studied the effect of a zinc deficient diet on the uterine fluid in pregnant rats. The rats were fed a purified diet either complete (100 ppm zinc) or zinc deficient (0.4 ppm zinc) from the day of mating. On day 4 of pregnancy, rats were anesthetized and uterine fluid was collected and plasma zinc concentration and uterine fluid protein concentration were determined. The concentration of zinc in the uterine fluid (3.9 ug/g) on day 4 in the pregnant rats fed a zinc deficient diet from the day of mating was only about half that of the controls (7.6 ug/g). The plasma zinc concentration of rats fed the zinc deficient diet was much lower than that of the controls, 85 ug/100 ml compared to 157 ug/100 ml. However, there was no difference between the two groups in the concentration of protein in the uterine fluid, approximately 42 mg/ml, suggesting that no dilution of uterine fluid had occurred because of zinc deficiency.

Gallagher and Hurley (20) suggested that the zinc concentration of uterine fluid and plasma undergo a rapid decline under conditions of inadequate dietary intake. Thus uterine and oviductal fluids may be in equilibrium with blood plasma and derive their zinc content from it. The concentration of zinc in the uterine fluid of rats is much higher than that of blood plasma. The result of Gallagher and Hurley's study supported the hypothesis suggested by Hurley and Shrader (18) that the abnormal development of preimplantation embryos in rats fed a zinc deficient diet results from an insufficient amount of zinc available to them from their surroundings, the oviductal and uterine fluids.

#### BIOCHEMISTRY ASPECTS OF ZINC IN ZINC DEFICIENCY

The first concrete demonstration of a specific biological function that was critically dependent on the presence of zinc came with the discovery of Keilin and Mann (22) that carbonic anhydrase contained zinc that was essential to its mechanism of action. Zinc metalloenzymes exhibit the greatest diversity of catalytic functions among all enzymes and they participate in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis or degradation (1). In zinc metalloenzymes, the zinc is located at the active site and so tightly bound that it does not dissociate from the protein during the isolation procedure (23). Enzymes in which the zinc is loosely bound, are chemically and functionally more tenuous, and are given the name "metal-enzyme complexes" to distinguish them from tightly bound metalloenzymes. In the past few years zinc has been found in both DNA and RNA polymerases, and the activity of RNase also is regulated by exogenous zinc (24).

Enzyme activity. Zinc is needed for many enzymes, so the level of zinc in cells controls the physiological processes through the formation and/or regulation of activity of zinc dependent enzymes (25). Prasad (1) reported that the activity of various zinc dependent enzymes was reduced in the testes,

bones, esophagus, and kidneys of zinc deficient rats in comparison to their pair-fed controls. Prasad also found positive correlations between the decreased zinc content, the decreased zinc enzyme activity, and the clinical manifestations of testicular atrophy, reduced growth rate, and esophageal parakeratosis. This suggested that the likelihood of detecting any biochemical changes is greatest in tissues that are sensitive to zinc depletion.

The effects of a zinc-deficient regimen for the maternal rat during gestation on zinc-containing enzyme activity were studied by Cox et al. (26). Female rats were individually housed in stainless steel cages and diets were fed ad libitum. The control group was given 9 ppm zinc and the experimental group was given 0.75 ppm zinc. All rats were killed at day 16 or 22 of fetal age for enzyme analysis (succinic dehydrogenase, lactic dehydrogenase, and ceruloplasmin). Compared with rats fed 9 ppm zinc, rats fed 0.75 ppm zinc showed the following effects. Activities of the enzymes were not altered in maternal and fetal tissues at day 16. Lactic dehydrogenase activity was not altered in maternal liver, heart, and brain, but was significantly increased in the serum at day 22 of fetal age. The following effects were also seen at day 22: Although succinic dehydrogenase activity was not changed in the maternal liver, activity was increased significantly in the heart. Maternal serum ceruloplasmin activity was reduced by zinc deficiency. Activities of lactic dehydrogenase and succinic dehydrogenase were not changed in the fetal liver. Activity of succinic dehydrogenase was higher in the fetal heart, but lactic dehydrogenase activity remained unaltered.

Nitrogen retention. Zinc is required for normal cell division and protein synthesis, therefore protein synthetic processes are depressed in response to dietary zinc deficiency (27,28). Zinc deficiency also results in a loss of protein from tissues, a process reflected by increased excretion of urinary nitrogen. Greely et al. (29) conducted a study to characterize nitrogen retention in response to marginal dietary zinc during gestation.

Long-Evans rats were assigned randomly to one of two dietary groups on day 1 of gestation. They were fed a basal diet supplemented with either restricted or control levels of zinc. Feces and urine were collected for 24 hours on day 20 of pregnancy and their nitrogen and zinc contents were determined. Urinary and fecal nitrogen excretions were similar for zinc-restricted and control dams, whereas fecal zinc excretions were depressed by feeding the zinc-restricted diet. Mean zinc and nitrogen retentions were negative for the zinc restricted and positive for the control groups. Multiple stepwise regression analysis showed that nitrogen retention on day 20 depended on both dietary nitrogen and zinc intakes. Zinc-restricted offspring weighed 12% less and the maternal plasma zinc concentrations were reduced by 66% when compared with the control group values on day 22. These results suggested that marginal dietary zinc limited fetal growth without causing excessive nitrogen excretion.

Naismith and Morgan (30) described the metabolism of nitrogen as falling into two distinct phases during the gestation of rats. During the anabolic state (days 1-14), the maternal organism stores nitrogen in peripheral tissue. In late gestation (after day 14), stored nitrogen is mobilized. The increasing requirements of the growing fetus are satisfied by the maternal mobilization process. Nitrogen withdrawal from stores apparently depends upon hormonal control rather than on the protein content of the diet (31). Rapid metabolic responses to decreased food intake ensure continued fetal growth. Like nitrogen, rapid fetal growth and the increased transport of zinc to the conceptus occurs during the final stage of gestation. However, unlike nitrogen, there is no maternal storage site of readily available zinc, and thus it must be supplied in the diet (32). Greely et al. (29) found that while the nitrogen excretion was not significantly increased during zinc deficiency, the nitrogen intake of zinc-restricted dams was decreased and retention values were negative as a consequence. A reduction in the exogenous nitrogen

intake, while maintaining comparable nitrogen excretions, caused some change in the deposition of endogenous nitrogen. As circulating levels of maternal zinc decreased, the amount of zinc available for placental transfer declined. Concurrent with depressed zinc transfer was the process of nitrogen mobilization. The maternal plasma amino acids would normally protect the fetus against low dietary protein intake and sustain fetal growth. Greely et al. concluded however, that limited zinc availability depresses fetal cell division and the requirement for amino acids in synthetic processes. The energy deficits induced by anorexia also play an important role in the disposition of maternal plasma amino acids. The decreased utilization of amino acids by zinc-restricted fetuses may then depress the normal rate of nitrogen mobilization.

Fetal pancreas. The pancreas is rich in zinc and hormone synthesis is extremely rapid in the fetal pancreas. Therefore glucagon and insulin levels might be vulnerable to the effects of maternal zinc deprivation (33). Insulin and glucagon in the fetus control fetal growth and development, but little is known regarding the nutritional regulation of their concentrations. Since pancreatic hormones are not transported across the placenta, the fetus must depend on an endogenous source of insulin and glucagon (34). The maternal diet may be one factor that could influence the concentration and secretion of these hormones in the fetus. For instance, the cultured fetal pancreas produced low insulin content in response to a medium with low amino acid and glucose levels (35). Robinson and Hurley (36) assessed the effect of maternal zinc deficiency or food restriction on the accumulation and release of glucagon and insulin in the fetal pancreas. Pregnant Sprague-Dawley rats were fed a zinc-deficient diet ( $0.4 \pm 0.1$  ppm zinc) ad libitum or a zinc-supplemented control diet (100 ppm zinc) either ad libitum or with restricted intake. The insulin content of the fetal pancreas was lower in fetuses from rats subjected to zinc deprivation or to food restriction than in controls. The



deficit of pancreas insulin was similar in the two treatment groups, which suggested that lower maternal food intake was the principal cause of the lower insulin level that was observed with zinc deficiency. However the treatment groups responded differently at the cellular level. The morphometric analysis of the fetal pancreas demonstrated that zinc deficiency resulted in a smaller than normal proportion of beta cells, while food restriction did not influence the beta cells proportion. The proportion of beta cells in the pancreas of zinc-deficient fetuses was 30% less than normal, but with food restriction, the proportion of beta cells remained normal, so the lower insulin content of fetal pancreas from rats subjected to food restriction was related to a less than normal amount of insulin per beta cell. These results suggested that zinc deprivation depressed the proliferation of the beta cells to a great extent. Glucagon concentration in the fetal pancreas was lower than normal in response to zinc deficiency but not to food restriction. The cause of the lower level of glucagon in the fetal pancreas with zinc deficiency is not known, but the depressed proliferation of the alpha cells is a possible explanation. Alpha cells develop in the fetal pancreas in close association with the beta cells. Since the zinc-deficient fetal pancreas contained a lower proportion of beta cells, there was a strong possibility that the proportion of alpha cells also was decreased. Robinson and Hurley (34) concluded that maternal zinc deficiency limited the fetal endocrine function and the development of the endocrine pancreas to a greater extent than food restriction.

DNA synthesis. Zinc deficiency is teratogenic to the developing rat embryo causing gross malformations in all organs, particularly in the central nervous system (1). Under these conditions, incorporation of thymidine into whole rat embryos was reduced greatly. These observations led to the hypothesis that the congenital malformations resulting from zinc deficiency were brought about by asynchronous differential growth rates stemming from de-



pressed synthesis of DNA.

Echkert and Hurley (37) studied the effect of zinc deficiency on regional DNA synthesis in the 12 day rat embryo. Zinc deficiency was achieved by feeding normal pregnant rats with a diet containing 100 ppm zinc, and dams were injected with  $^3\text{H}$ -thymidine on day 12 and embryos removed 1 hour later. In embryos from zinc deficient dams, there was a lower incorporation of  $^3\text{H}$ -thymidine into DNA in the head regions than in comparable regions from ad libitum and pair-fed controls. Total DNA and RNA contents in the head and body regions of embryos from zinc deficient dams were lower than respective regions of pair-fed controls, but the greatest deficit occurred in the head region. Replacement of zinc 28 hours prior to injection of label increased the low incorporation of  $^3\text{H}$ -thymidine/DNA in the head region of zinc-deficient embryos. Autoradiographs of the head region indicated that reduced uptake of  $^3\text{H}$ -thymidine and reversal by zinc replacement occurred mainly in the developing CNS. The difference in thymidine uptake between embryos of zinc-deficient and pair-fed controls was not brought about by decreased ability of the labeled nucleotide to cross the placenta, in fact, more label occurred in the amniotic fluid of the zinc deficient group. This was not observed when the zinc deficient rats were given zinc replacement as a result of a greater utilization of the thymidine by the embryo with less excretion of the label into the amniotic fluid. The incorporation of the label into the head region DNA of the pair-fed group was increased by prior injection of zinc. Increase in the incorporation of the label into DNA by zinc injection may be due to a mild zinc deficiency in the pair-fed rats resulting from a restriction of the protein intake. Total DNA and RNA were both lower in the head and body regions of embryos from zinc-deficient embryos than in those from pair fed controls although the head regions were more severely affected. After injecting dams with zinc only, the biochemical and autoradiographic differences between zinc-deficient and pair-fed controls were reduced

greatly. The lower uptake of  $^3\text{H}$ -thymidine into DNA extracted from embryonic heads in zinc deficient rats and the higher amount of  $^3\text{H}$ -thymidine in the amniotic fluid returned to control levels. The incorporation of  $^3\text{H}$ -thymidine into DNA was an indication of the effect of zinc deficiency over a short time interval, while the total DNA represented the effect of zinc deficiency on DNA synthesis over the 12 day period. Zinc supplementation increased the values for total head and body DNA in embryos from zinc-deficient dams. In this group, differences between head and body regions disappeared and the lower level of both DNA and RNA returned to control levels except for head DNA concentration which remained low. The pair-fed controls showed that the depressed DNA synthesis, regional differences in DNA synthesis, and low total DNA and RNA seen in zinc deficient rats were the result of a deficiency of zinc and not of food intake. The greater reduction in DNA synthesis in the head region may explain the increased vulnerability of the CNS to prenatal zinc deficiency.

In addition to its role in DNA synthesis, zinc is an integral structural component of nerve factor protein (38). So regulation by nerve growth factor of growth and differentiation of neural crest derivatives might be impaired in the absence of zinc, leading to increased vulnerability of the mammalian brain to zinc deficiency.

#### EFFECTS OF ZINC DEFICIENCY ON PREGNANT RATS

Anorexia and reduced weight. Zinc deficiency results in anorexia, usually characterized by a cyclical pattern of food intake. In a study by Fosmire et al. (39), pregnant rats fed a zinc deficient diet did not demonstrate a cyclical pattern of food intake, although there was severe anorexia. The effects of various degrees of zinc deprivation during pregnancy on dams and offspring were examined. After mating, the dams were fed a biotin enriched, 20% sprayed egg white diet which contained less than 1 ppm zinc. Zinc was provided in the drinking water at one of the following concentrations:

1, 2, 3, 5, 11 or 25 mg/liter. The level of zinc supplementation offered to the pregnant rats appeared to influence their food consumption, particularly during the last third of gestation. During the first 14 days, food consumption was not significantly different among groups, although there was a trend for the dams receiving the highest level of zinc in their drinking water to consume more diet. A significant decline in food consumption was seen on day 15 and anorexia was consistently present from day 17 in those rats receiving 1 ppm zinc in the drinking water. At higher levels of zinc supplementation, the anorexia occurred sequentially in response to the level of zinc (i.e., day 18 at 2 ppm, day 21 at 3 ppm, and day 22 at 5 ppm). Water consumption did not vary significantly among groups when considered on an average daily level throughout gestation.

Prior to day 18, weight gains were not significantly different among the various groups (39). The decrease in weight gain occurred 1 day after the onset of anorexia. Differences in weight gain between those dams receiving 11 ppm zinc and those receiving 25 ppm zinc were not significantly different, but dams receiving less zinc showed more depressed weight gain. No consistent cyclical pattern of food intake was observed. The energy needs of the pregnant dams were so great that any significant incidence of cyclical feeding may have been precluded. The differences in food consumption, both in the onset and severity of anorexia, in response to the different levels of dietary zinc indicated the sensitivity of the eating patterns of the animals to their zinc status. The anorexia experienced by the dams resulted in decreased weight gains and at the lowest level of zinc intake, in a straightforward loss of body weight.

Hurley and Cosens (40) studied a more severe zinc deficiency than did Foshmire et al. (39) and found that their dams experienced a greater degree of interference both in onset and extent of depressed weight gain. When the increased demands for zinc were imposed by fetal growth during the last

trimester, the dams entered the period of catabolism of protein stores and deposition of maternal fat stores, the dams became anorexic and failed to gain body weight in a normal fashion. There was little transfer of zinc to the fetus prior to day 18, but subsequent to this, there was a sharp increase in the permeability of the placenta to zinc. This corresponded well with the onset of loss of weight gain in those dams supplemented with the lowest level of zinc. Perhaps the fetal needs actually precipitated a more severe deficiency and thus induced the anorexia and weight loss which occurred.

Behavioral change. Zinc is essential for development of the brain. Fetuses of women who consume inadequate amounts of zinc during pregnancy may be at risk as far as intrauterine brain development and body growth are concerned. Damage caused to such fetuses may be persistent and may be manifested as mental retardation.

Halas and Sandstead (41) studied the effect of zinc deficiency during the latter third of gestation on avoidance conditioning of young adult male rats as compared with the effect of intrauterine starvation and normal pregnancy. From the 15th through the 20th day of gestation the rats were fed a sprayed egg white, biotin-enriched diet containing less than 1.0 ppm zinc. One control group (five dams) was pair-fed with the zinc-deficient group while a second control group (five dams) was fed the diet ad libitum. Both control groups were given drinking water containing 50 ppm zinc via an automatic watering device. The zinc-deficient group (five dams) was given trace mineral-free water before and after the "zinc-deficient interval." All dams were fed a standard laboratory rat chow ad libitum. Ten 60 day old male rats were selected randomly from the litters of each of the 3 groups of dams. All 30 rats were given behavioral training. The rats were given 25 trials per 24 hours of avoidance conditioning for 5 days. The number and latency of all jumps during the avoidance trial and intertrial period were recorded by a remote automatic device.

The results of Halas and Sandstead's study (41) suggested that both intrauterine zinc deficiency and intrauterine starvation during the latter one-third of the pregnancy have long term adverse effects on the behavior of the rat. The effects of zinc deficiency appear to be more severe than those of starvation alone. The rats which had been deprived of zinc during the latter one-third of pregnancy performed poorly and reached a peak of only 35% avoidance responses. In addition to the decreased avoidance response peak, there was a marked decrement in response due to an inability to tolerate the stress induced by the levels of shock used. The response latency of the zinc deficient group was significantly longer than that of either of the control groups. This finding supports the interpretation that the animals which had been exposed to intrauterine zinc deficiency were inferior in their ability to learn to avoid the shock. The level of activity of the intrauterine zinc-deprived group as measured by the intertrial responses was less than that for normal control groups but was equal to that of the rats born to the pair-fed (starved) dams, which suggested zinc deficiency in utero did not have adverse effects on activity which were different than the effects of intrauterine starvation alone. The rats whose dams were pair fed during the latter one-third of pregnancy did learn to avoid the shock 70% of the time. However, they readily discriminated the change from shock during conditioning to no shock during extinction and therefore stopped making avoidance responses. In contrast, the normal rats achieved an 80% level of avoidance and during the no shock situation (extinction) did not readily distinguish from the shock situation during conditioning. Thus the normal rats extinguished more slowly than the rats which had experienced intrauterine starvation. This finding supported the observation that the level of learning reached by the normal rats was greater than that of the offspring of the pair-fed dams. The response latency of the intrauterine starved rats was somewhat, but not significantly, longer than that of the normal rats. Once

rehabilitation was started, the food consumption of the zinc-deficient dams rapidly caught up and slightly exceeded the other groups. The weight gain of the zinc deficient dams also indicated that rehabilitation of the dams was rapid. Therefore the major injury occurred during intrauterine life when the brain was developing most rapidly and post natal effects were not a major factor in brain injury.

Compositional changes in fetal organs. McKenzie et al. (42) investigated the effects of zinc deficiency during the last third of gestation on the growth and development of the fetal rat at term. They chose to study this because the latter third of pregnancy is the time of the greatest zinc accumulation by the fetus and because in many studies behavioral abnormalities in nutritionally rehabilitated adult rats have been found. Previous studies had involved only zinc deficiency starting at or before mating. Virgin female Sprague Dawley rats were used. After being mated they were divided into three groups: zinc deficient, pair fed, and ad libitum fed. A biotin enriched 20% sprayed egg white diet, which contained less than 1 mg of zinc per kg, was fed. All rats were given drinking water containing 25 mg of zinc per liter of water. This gave them sufficient zinc, even in their pregnant state. On day 14 of gestation, the zinc deficient group of dams was changed to glass distilled and demineralized water. On day 21, they were anesthetized and the fetuses were delivered by cesarean section. Tissues for DNA, RNA, and protein analysis were taken from the eight fetuses nearest the median weight for each litter. The brains, livers, and placentas were pulled and weighed. DNA and lipid analysis were done, and protein, RNA, and hematocrit were measured. Zinc analysis by atomic absorption spectrophotometry was performed. The brain, liver, and placenta had very different susceptibilities to the zinc deficiency, but all were affected adversely. The reduced weights of the fetus and organs produced by the three groups are shown in table 5.

TABLE 5

Weight of fetuses, fetal brain, fetal liver, and placentas (42)

Group	Fetal Wt		Brain Wt		Liver Wt		Placental Wt	
	—	g	g	%fetal wt	g	%fetal wt	g	%fetal wt
Zinc deficient	X	3.98	0.166	4.2	0.227	5.7	0.455	11.4
	SEM	0.09	0.004		0.009		0.015	
	n	23	21		21		21	
Pair fed control	$\bar{X}$	4.25	0.178	4.2	0.260	6.1	0.473	11.1
	SEM	0.08	0.003		0.011		0.017	
	n	24	21		21		21	
Ad libitum fed control	$\bar{X}$	4.96	0.188	3.8	0.346	7.0	0.489	9.8
	SEM	0.06	0.002		0.008		0.017	
	n	27	27		27		27	

The most effected organ was the liver. In addition to decreased weight, the fetal livers of the zinc deficient group contained only 1/3 amount of zinc contained in the livers of either of the control groups (9.1 ug vs. 25.1 and 25.4). The placenta suffered a small decrease in zinc content (4.32 ug vs. 4.79 ug) and a correspondingly lower concentration of zinc (54.5 ug/g vs. 64.2 ug/g) from the ad libitum control group. The brain suffered a slight decrease in zinc content (1.86 vs. 1.92 ug), but showed a higher concentration due to decreased brain weight (89.4 ug/g vs. 83.7 ug/g).

Apparently the growing fetus sacrifices the less important liver in order to spare the brain of the full impact of the zinc deficiency. But the brain is still affected, primarily in total number of cells. Individual cells are larger when deprived of zinc, but it was hypothesized that the zinc deficiency impairs brain cell division. This mechanism is unknown, but may be the reason for the behavioral changes described in the previous section.

Parturition. A need for zinc for normal parturition in the rat has been demonstrated by Apgar (43) by use of a low zinc diet (1 ppm zinc). Two hundred gram female Sprague-Dawley rats were raised in stainless steel cages on chow (40-50 ppm zinc) and water until they weighed 250 g, and then mated.



On the 1st day of gestation, the females were put on a low zinc diet. Twelve females were assigned to each of three treatments, zinc deficient, pair fed controls, and ad libitum controls. On day 22, the females were transferred to 13 by 15 by 6.75 inch clear plastic cages with stainless steel lids. Observations were made at intervals until parturition began. Two hours after the birth of the last pup, a photograph was made of the cage and contents, after which the female and living pups were weighed. Two minutes after the female was replaced in the cage, five pups, age 1-5 days, were placed in the cage and the female observed for 10 minutes to determine the latency to retrieval of the first pup, time required to retrieve all pups, and time at which female took a position in which nursing would be possible.

Data on gathering and nursing for the zinc deficient females were not included, since nine of the females paid no attention to the pups, and the responses of the other two females were comparable to the ad libitum controls. After observations were completed, the females were given brown paper strips with which to make a nest.

Before parturition, some slight differences between the groups were observed. Both the zinc deficient and pair-fed groups experienced poor weight gain, although that of the pair fed group was slightly more than that of the zinc deficient. A few days before parturition, the zinc deficient dams became very inactive, often standing motionless except for occasional movements of just a few steps. There was a visible roughness in the coats of the zinc deficient rats compared with the two control groups.

Much greater differences were noticed once parturition began. The pair fed and ad libitum controls both showed very normal parturition, but the zinc deficient group had great difficulty. Total time for parturition was increased from .5 to 2 minutes for the two control groups to 6 to 75 minutes for the zinc deficient group. There was also severe bleeding in the zinc deficient group compared to the control groups.



After parturition was complete, abnormalities in the zinc deficient group continued, and the pair-fed group also began to show severe abnormal traits. The pair-fed rats ate their afterbirths (slightly more reliably than the control group, probably due to underfeeding) and cleaned up the cage as did the ad libitum group. They did not however remain active and clean and care for their pups, in fact they often ignored them. They did not lay down to nurse their pups, and often could not because they had failed to lactate.

The zinc deficient rats did not eat their afterbirths inspite of previous low food intake, possible due to continued anorexia. They did not clean their cages, and did not clean or care for their pups. Like the pair fed group, they could not nurse due to failure to lactate.

The abnormalities displayed by the pair fed group after parturition may have been caused by either the lack of energy resulting from malnutrition or due to failure to lactate. Lactation is related closely to maternal instincts so the cause of the abnormalities may have been an indirect hormonal change caused by malnutrition (44).

The great differences between the zinc deficient group and the two control groups indicated that zinc was very critical for parturition. The near normal parturition of the pair fed rats eliminated malnutrition as the cause of the abnormalities of the zinc deficient group.

#### EFFECTS OF ZINC DEFICIENCY ON HUMAN REPRODUCTION

Fetal and maternal complications. The serum zinc concentration decreases during human pregnancy (45). It is not known whether this decrease is a purely physiological effect or an expression of a deficiency state which implies a risk to mother or child (46). It is possible that changes in zinc metabolism during human pregnancy are much more important than was previously thought (6). Few pregnancies have occurred in women with acrodermatitis enteropathica (a zinc deficiency state) (47); severe fetal malformations have been recorded (one case of anencephaly, one of a chondroplastic dwarfism

and one infant with low birth weight.)

Sever and Emanuel (48) studied the possibility of a correlation between human maternal zinc deficiency and congenital malformations, especially anomalies of the central nervous system. Animal experimental zinc deficiency resulted in reduced fertility and the incidence of fetal malformations was demonstrated. These observations and the discovery of DNA-polymerase as a zinc metalloenzyme may imply that DNA-synthesis is impaired during zinc deficiency (49). When zinc deficiency occurs, it affects all systems in which there is a rapid cell-turnover.

Jameson (50) tried to determine whether serum zinc could prove useful in predicting complications affecting mother and/or child and whether serum zinc is correlated with other hematological factors. Eighty-four pregnant women in Sweden were investigated. All were healthy and none were receiving any medication. Hemoglobin estimations were made by the cyanmethemoglobin method and venous blood was taken. The duration of labor, blood loss during delivery, the weight and maturity grade of the infant, and any malformations were recorded. Women with complications such as abnormal labor or atonic bleeding had reduced ( $p < 0.001$ ) serum zinc concentration during early pregnancy. Women who gave birth to immature infants also had lower ( $p < 0.01$ ) serum zinc in early pregnancy. Women who delivered in the 37th week or earlier, or in the 43rd week or later showed lower ( $p < 0.005$ ) serum zinc during early pregnancy compared to women delivered in the 40th week. One infant had a congenital heart defect and her mother had the lowest serum zinc concentration recorded in the 13th week, but no other abnormal findings. Compared with women with abnormal labor and/or immature infants, mothers with normal deliveries and normal infants showed significantly higher serum zinc values ( $p < 0.001$ ) during early pregnancy. A notably high incidence of complication affecting mothers and infants was recorded among those with low serum zinc. Similarities to effects of experimental zinc deficiency in

animals were striking.

Congenital malformations, dysmaturity and abnormal parturition. It is known that serum zinc decreases during pregnancy. The fact that this phenomenon reflects altered zinc metabolism is confirmed by the observation that the zinc content of hair is lower in the 36th week of gestation than in early pregnancy (45).

Jameson (50) investigated a suspected connection between serum zinc and maternal or fetal complications and registered the variations in serum zinc during pregnancy. Pregnant women attending Regional Hospital in Sweden were investigated throughout pregnancy. Two hundred forty-five women were examined (mean age 25.4 yrs, range 16 - 41 years). Spontaneous abortion occurred in 11 cases so these were excluded. Zinc estimation was carried out by atomic absorption spectrophotometry, and 24 hour excretion of estriol in urine was measured. Data concerning blood loss during delivery, duration of labor, and birth weight were obtained from case records, along with clinical obstetric and pediatric observations.

The serum zinc fell slowly as pregnancy progressed, but the mean value leveled out after the 25th week. Some women showed a low serum zinc in early pregnancy. The serum zinc concentration varied little in the individual women with uncomplicated pregnancy. The pregnant women gave birth to 22 immature infants. Labor was induced before the expected date in three cases with normal serum zinc. Four infants were classed as small for date. Eight infants had congenital malformations of different types and severity; five mothers of those eight infants had the lowest serum zinc concentrations registered in the respective week of gestation. Fourteen mothers who gave birth to abnormal infants also delivered abnormally. Altogether 50 abnormal deliveries occurred, and in six women, labor was induced owing to a prolonged pregnancy. One hundred forty-five of 234 women had deliveries at the expected time, and the infants were normal; these 145 women were called group 1.

Eighty-nine women had abnormal deliveries and/or abnormally developed infants; these formed group 2. Each individual patient was represented by the lowest serum zinc concentration recorded during pregnancy.

Compared to the normal group 1 women, 50 patients with abnormal deliveries showed lower ( $p < .001$ ) serum zinc during pregnancy (Table 6). The 22 women with immature infants had lower serum zinc than group 1, but the difference was not significant ( $p < 0.3$ ). The 13 mothers of dysmature (postterm) infants had serum zinc concentrations less than group 1 ( $p < 0.02$ ). Mothers of malformed infants also had lower serum zinc than group 1 ( $p < 0.001$ ). The groups were comparable with regard to the time when the lowest zinc concentrations were recorded, mean values concerning the week when the lowest zinc concentration was recorded did not differ significantly (group 1, 20.3; group 2, 21.9). This very small difference in time cannot explain the different zinc concentrations. In one case, a diabetic woman with placental insufficiency, there was a sudden fall in estriol excretion which failed to evoke a change in serum zinc. This hormone appeared to be of minor importance with regard to low serum zinc concentrations. Eight infants had congenital malformations. Five of the eight mothers had the lowest serum zinc concentrations recorded during pregnancy.

TABLE 6

Differences between serum zinc means<sup>1</sup> (50)

S-zinc $\mu\text{mol/l}$	n <sup>2</sup>	mean	SD	t	p
Group I	145	14.5	2.0	-	-
Group II	89	13.3	2.1	4.14	<0.001
abnormal deliveries	50	13.2	2.0	3.82	<0.001
dysmature infants	13	13.0	2.1	2.53	<0.02
malformations	8	12.3	1.7	3.06	<0.005
immature infants	22	13.9	2.1	1.27	<0.3

<sup>1</sup> When testing Group I against other groups the probability of difference was considered to be significant when  $p$  was <0.05.

<sup>2</sup> n = number of women per group.

A diabetic woman gave birth to an immature infant with multiple skeletal malformations. She had the lowest serum zinc in the 21st week, and at the same time a very low alkaline phosphatase activity.

Women with dysmature infants had lower zinc values during pregnancy ( $p < .02$ ) than women with mature infants born by normal delivery. Schraer and Calloway (51) studied zinc balance in four teenagers during the last trimester of pregnancy. A zinc retention of 3.6 mg per day was observed. This means that for the normal weight gain of pregnancy at least 375 mg of zinc would have to be retained. To this requirement of 375 mg must be added an extra supplement to compensate for daily losses.

#### Methods of investigation and treatment.

Jameson (50) investigated the reason for the low serum zinc concentrations in 20 pregnant women and in 7 of them examined the effects of oral zinc sulphate supplementation. The subjects were women whose zinc concentration was below 11.5  $\mu\text{g/l}$ . They were referred to the hospital because they had unsatisfactory hemoglobin concentrations in spite of routine treatment with oral iron and vitamin supplementation, they were investigated as outpatients.

The standard investigation included hemoglobin estimation, determinations of serum zinc, serum vitamin B<sub>12</sub>, folate, estimations of serum iron and total iron binding capacity, bone marrow aspiration, and analysis of urine and feces. Thirteen had hemoglobin concentrations below 110 g/l and in 7 of them the cause of this anemia was not found. Histological investigations indicated increased intramedullary cell destruction. Findings from the 8 pregnant women referred for further investigations made it hardly probable that the low serum zinc concentrations were secondary to somewhat low plasma proteins or liver damage. None of them showed conventional signs of increased hemolysis. But they and altogether 17 women investigated showed a moderate or marked increase in intracellular cell fragments in bone marrow macrophages.

Changes of this type are in keeping with increased intramedullary hemolysis. Dash et al. (52) suggested that a low serum zinc concentration may impair the deformability of red blood cells. Zinc seems to be necessary to the stability of cell membranes (53).

Jameson (50) also found 7 cases of anemia that were not fully explained. Their marrow smears showed moderate or strong increase in cell fragments in the macrophages which may indicate intramedullary hemolysis and may explain the anemia. Bone marrow macrophages phagocyte denaturated red blood cells in experimental animals and act as an erythroclastic organ (54). The low serum zinc concentrations received no probable explanation other than zinc deficiency. Seven pregnant women were treated with 90 mg zinc daily as zinc sulphate by mouth during the latter part of pregnancy. Zinc excretion in urine was increased ( $p < 0.005$ ) after one week's therapy. The serum zinc also increased ( $p < 0.05$ ). Zinc therapy gave no reticulocytosis within 8-12 days. Three women reported spontaneously an improvement in sense of taste. Seven women who received zinc therapy had all normal deliveries, but labor was prolonged in one. No side effects of zinc therapy were noted except for nausea in one case. Five of 20 pregnant women without zinc therapy had dysmature infants. Heavy bleeding occurred at delivery in 6 cases, secondary to impaired uterine contractility. Jameson suggested that zinc supplementation in women with low serum zinc concentrations during pregnancy seems to be justified.

Zinc is a very important trace element of the diet, and one which can easily become deficient. The body stores very little zinc. Most of the body zinc is in the skeleton and is not readily available for use by the body. Zinc is an element of many bodily functions. Body zinc turnover rate is rather high so there must be a fairly constant source of dietary zinc. The availability of zinc from the diet, however, is often questionable. Vegetarian diets and diets of low income people may not contain zinc rich animal protein sources, furthermore the phytate and fiber in these diets can bind zinc and make it unavailable. Alcoholism affects zinc status by increasing urinary zinc excretion.

Pregnancy, which increases the need for zinc due to its role in growth of the fetus, makes zinc status even more critical in the pregnant women. First, with limited stores of zinc, the increased demands of pregnancy can quickly cause a deficiency. Secondly, the key role of zinc makes the effects of the deficiency serious. The embryo in mammalian species has no endogenous nutrient source so it must depend on the oviductal and uterine fluids for all nutrients. If the mother's zinc status is low, the results can range from slight congenital physical abnormalities to compositional changes in fetal organs, to severe birth defects or even spontaneous abortion. Also affected is the brain, with changes in composition and number of cells.

Behavioral and learning ability changes also result from zinc deficiency and they can be permanent. Effects of zinc deficiency also are seen in the mother during and after pregnancy. During pregnancy a lack of zinc accentuated by anorexia and the stress of the pregnancy can have a large toll. The growing fetus takes priority on limited nutrients. Effects of zinc deficiency also are seen during parturition, and afterwards in difficulty of labor and improper care for offspring.

Although most studies of zinc deficiency have been done with animals,

some data from human studies are also available. Zinc deficiency in humans also correlates with maternal complications, congenital malformations, and difficulties in parturition.



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ZINC DEFICIENCY DURING PREGNANCY

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Zinc is a very important trace element of the diet, and one which can easily become deficient. The body stores very little zinc. Most of the body zinc is in the skeleton and is not readily available for use by the body. Zinc is an element of many bodily functions. Body zinc turnover rate is rather high so there must be a fairly constant source of dietary zinc. The availability of zinc from the diet, however, is often questionable. Vegetarian diets and diets of low income people may not contain zinc rich animal protein sources, furthermore the phytate and fiber in these diets can bind zinc and make it unavailable. Alcoholism affects zinc status by increasing urinary zinc excretion.

Pregnancy, which increases the need for zinc due to its role in growth of the fetus, makes zinc status even more critical in the pregnant women. First, with limited stores of zinc, the increased demands of pregnancy can quickly cause a deficiency. Secondly, the key role of zinc makes the effects of the deficiency serious. The embryo in mammalian species has no endogenous nutrient source so it must depend on the oviductal and uterine fluids for all nutrients. If the mother's zinc status is low, the results can range from slight congenital physical abnormalities to compositional changes in fetal organs, to severe birth defects or even spontaneous abortion. Also affected is the brain, with changes in composition and number of cells.

Behavioral and learning ability changes also result from zinc deficiency and they can be permanent. Effects of zinc deficiency also are seen in the mother during and after pregnancy. During pregnancy a lack of zinc accentuated by anorexia and the stress of the pregnancy can have a large toll. The growing fetus takes priority on limited nutrients. Effects of zinc deficiency also are seen during parturition, and afterwards in difficulty of labor and improper care for offspring.

Although most studies of zinc deficiency have been done with animals,

some data from human studies are also available. Zinc deficiency in humans also correlates with maternal complications, congenital malformations, and difficulties in parturition.