

PREGNANCY AND PARTURITION IN RATS ON A ZINC DEFICIENT
DIET WITH VARYING LEVELS OF TRYPTOPHAN

by

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Chapter I
INTRODUCTION

The essentiality of zinc for normal growth of the laboratory rat has been known since 1934 (49). Halsted and Prasad (28) observed a zinc deficiency syndrome in humans characterized by retarded growth, delayed sexual development, hepatomegaly, splenomegaly, and anemia. Acrodermatitis enteropathica is a genetic malabsorption of zinc characterized by a number of symptoms (29). Recently, zinc has been shown to be necessary for normal parturition (1,2) and normal offspring (34) in the laboratory rat. Zinc deficiency has been shown to be teratogenic in man (29,37).

Apgar (1) described zinc deficiency in the pregnant rats. Food intake and weight gain were decreased in zinc deficient dams. Severe stress due to zinc deficiency occurred prior to parturition. Lethargy, a reddish exudate on the vibrissae, and a rough coat were followed by lengthy and difficult parturition, and often the death of the dam and her pups. Hypertrophied adrenal glands, hyperkalemia, diuresis, and kidney nephrosis in zinc deficient dams indicate that the clinical signs of zinc deficiency may be attributed to a biochemical lesion at the kidney and/or adrenal gland (12,31,42). The biochem-

ical lesion which results in the clinical pathology Apgar observed has not been defined.

Pregnancy and lactation are times of increased nutrient needs in most mammals. Not only is there an increased requirement for protein, carbohydrates, and lipids but vitamin and mineral requirements are also increased. The increased nutrient needs during pregnancy increase the likelihood that marginal nutrient deficiencies in the diet will result in clinical symptoms of nutrient deficiency. In using purified research diets with free amino acids as the protein source, the need for great care in balancing the amino acid mixture and in choosing vitamin and mineral mixes is particularly important when the diet is fed to pregnant animals. Poor reproductive success in research has been attributed to poorly balanced amino acids, zinc deficiency, and other nutrient deficiencies (27).

Hill (31) observed difficult parturition, maternal mortality, and fetal mortality in Sprague-Dawley rats fed a diet with L-amino acids as the protein source. A diet low in tryptophan was protective against the pathology. Zinc was present in the diet as part of the Rogers-Harper (R-H) mineral mix. The R-H salt mix was 4 % of the diet by weight, adding 4 ppm of zinc to the diet. This is less zinc than is required by pregnant female

rats. Zinc deficiency caused the observed diuresis, eye lesions, poor appetite, and mortality (12,31). Low dietary tryptophan levels exercised a sparing effect on zinc deficiency symptoms.

The objectives of this study were to determine whether low dietary tryptophan levels protect against zinc deficiency by decreasing the transfer of zinc from the maternal organism to the fetuses and to determine whether zinc deficiency induces diuresis and renal pathology. A further objective was to confirm Hill's observations of high urine volume on a zinc deficient diet and of the protective influence of a diet low in tryptophan against zinc deficiency symptoms.

Chapter II
LITERATURE REVIEW

Zinc Deficient Pregnancy and Parturition

The high concentration of zinc in male sexual glands and seminal fluid prompted research into the amount of zinc required for reproduction in the male rat (33,40, 50). Although it was known that female rats are in continual anestrus when fed a diet low in zinc, the effect of zinc on the reproductive function of female rats was not studied until 1968. Apgar (1) reported that zinc was necessary for normal parturition in the rat even when the diet was sufficient in zinc prior to pregnancy.

Zinc affects the ability of the female rat to maintain pregnancy. Hurley and Swenerton (34) found that only 50 % of rats maintained on a severely zinc deficient diet from the first day of pregnancy to term were pregnant at term. Those pregnant had a lower number of fetuses than controls. Apgar (3) found that only 50 % of rats on a diet containing 1 ppm zinc were pregnant at day 8 and none were pregnant at day 21. Three ppm zinc was enough to insure that all animals carried their young to term. Females reacted to the zinc deficient diet by having a decreased number of implantation sites (fetuses) and an increased rate of reabsorption of fetuses.

Apgar (1) fed Sprague-Dawley rats a commercial rat chow containing 40 to 50 ppm zinc until the first day of pregnancy at which time they were placed on a diet containing 1 ppm Zn. The control animals were given 2 ml of a 5 mg/ml zinc acetate solution by stomach tube daily. The zinc deficient dams were given 2 ml of deionized water. Food intake of the zinc deficient dams dropped on the eleventh day of pregnancy, indicating the onset of zinc deficiency. The weight gain and food consumption of the zinc deficient females were less than that of the controls. Two or three days before parturition the zinc deficient dams became lethargic, sitting quietly in one place unless disturbed. By the twenty-first day of pregnancy there was a reddish material on the vibrissae and the coat looked rough.

Parturition was extremely difficult in the zinc deficient rats. Parturition was preceded in six of the eight females in the zinc-deficient group by the secretion of a vile smelling yellow fluid from the vaginal area. Parturition was long and difficult in the zinc deficient dams. The controls moved around the cage during parturition cleaning their pups, themselves, and the cage while the deficient females remained motionless (1,2). Average length of parturition was 240 min for the zinc deficient dams (2). Length of parturition was within the normal 60 to 120 min range for the controls

(26). In Apgar's studies (1,2) some females died during parturition. The cages of the zinc deficient females after parturition were characterized by scattered pups (often dead), uneaten placentas, and excessive blood. Most of the zinc deficient pups died shortly after birth and the rest within a few days (1).

O'Dell et al (42) observed that rats on a zinc deficient diet lose more blood during parturition than zinc supplemented females. Non-pregnant females were fed a diet based on EDTA treated soybean protein with extra calcium in the diet to accentuate the effects of phytate, a component of soybeans which binds zinc making it unavailable. The control diet had 100 ppm of zinc added in the form of zinc carbonate. The animals were mated and maintained on the experimental or control diet until parturition was completed. Three hours after parturition bleeding time and bleeding rate were quantitated by measuring blood loss from a tail wound. Total blood loss was determined gravimetrically by placing the severed tail in a test tube of known weight containing physiological saline solution at 37^o. Zinc deficient females bled more profusely (282 ± 25 mg at 36 mg/min) than the controls (86 ± 11 mg at 14 mg/min) although total bleeding time was not significantly different (7.8 min \pm 1.2 min and 6.0 ± 1.4 min respectively). The time from the first incision to the first stop of bleeding was

longer in the zinc deficient animals (5.4 ± 1.2 min) than in the controls (2.4 ± 0.4 min). Zinc deficiency increased the amount of blood lost during parturition.

O'Dell et al (42) reported that female rats on a zinc deficient diet exhibited signs of physiological shock during parturition. Rectal temperature was $36.9 \pm 0.17^{\circ}$ C in zinc deficient dams three hours after parturition as opposed to $38 \pm 0.17^{\circ}$ C in controls. The zinc deficient females were cold to the touch and appeared to be dazed. Blood pressure was lower (90 ± 4 mm Hg) in the zinc deficient females than in the controls (104 ± 3 mm Hg). Zinc deficiency caused physiological stress in female rats during parturition.

Apgar (4) looked at the effects of zinc deprivation (1 ppm Zn in the diet) instituted on day 1, 12, 15, or 18 of gestation. Although dams in the group fed a low zinc diet from day 18 were alive at parturition they gained less weight than the zinc-supplemented controls. The animals on the zinc deficient diet from day 1 actually showed fewer signs of stress than dams placed on the zinc deficient diet at days 12, 15, or 18. The females deprived from day 1 consumed more of the afterbirths, took less time to deliver the young, and showed less hypertrophy of the adrenal glands. Apgar (4) concluded that zinc is required through day 18 for the female to

produce a normal litter without a stressful parturition. There is a flux of zinc from the mother to the fetus between day 18 and parturition which puts a great demand on the maternal zinc pool. The females fed a zinc deficient diet from day 1 of pregnancy had adapted to the zinc deficiency. Intestinal absorption of zinc could have been enhanced, zinc stores in the body could have been mobilized, or possibly zinc was not transported into the fetus in the same amounts as in the other groups. In an earlier study Apgar (3) found that females reacted to zinc deficiency by having a decreased number of implantation sites and an increased number of reabsorbed fetuses.

Apgar (5) looked at the effect of repleting zinc deficient females during pregnancy. Fifteen or 30 mg of zinc acetate was administered on day 15, 18, 19, 20, 21, or 22 of pregnancy. Parturition was normal in females repleted on day 15, 18, or 19. Some, but not all, females who were given zinc on day 20 or 21 completed parturition successfully. There was no difference in the difficulty of parturition exhibited by animals given zinc on day 22 and by the zinc deficient females. These studies indicate that dietary zinc is highly critical between day 19 and 21 for parturition in rats.

In Apgar's study (5), pair-fed controls behaved

similarly to the ad libitum controls during parturition. Hence, it appeared to be zinc deficiency and not general low nutrient intake which caused difficult parturition. Pair feeding with 50 ppm zinc added to the water decreased the number of animals pregnant at day 8 to 60 % of those pregnant at day 1. All the pair-fed, zinc-supplemented animals pregnant at day 8 maintained the pregnancy to term. It is the zinc deficiency and not the low food consumption which was responsible for the termination of pregnancy after day 8 (3). Berg (10) reported that restriction of food intake to 75 % of ad libitum intake does not interfere with pregnancy and restriction to 50 % of ad libitum intake interrupted only 23 % of pregnancies. The limited food intake caused by zinc deficiency was not the cause of difficult parturition.

Absorption of Zinc During Pregnancy

Changes in the pH of the intestine due to the hormonal changes of pregnancy may cause an increase in the absorption of zinc (30). The observation was made that zinc is more rapidly absorbed during the last trimester of pregnancy when there is a general decrease in the pH of the intestinal tract. It is not certain whether these changes are the specific effect of female hormones.

The ^{65}Zn uptake by the ovaries, fallopian tubes,

uterus, cervix, and vagina of rats during different stages of pregnancy was similar in all tissues (17). $^{65}\text{ZnCl}_2$ was injected intraperitoneally in a dose of 1 $\mu\text{Ci/g}$ of body weight on day 1 to 7, 9, 10, 12, or 20 of pregnancy. The rats were autopsied 24 hours later and ^{65}Zn content of the maternal tissues was measured. ^{65}Zn uptake occurred during the first week of pregnancy, the only decrease being on day four. On day seven ^{65}Zn uptake reached its peak and remained constant through day 10. From day 10 through parturition there was a gradual decrease in the uptake of ^{65}Zn .

Davies and Williams (15,16) studied ^{65}Zn absorption, in situ by isolating 150 mm segments of duodenum loops of at different stages of pregnancy. The duodenum of non-pregnant controls absorbed ^{65}Zn at a rate of 0.40 $\mu\text{g Zn/loop}$ per 15 min. The rate decreased to 0.37 $\mu\text{g Zn absorbed/loop}$ per 15 min on day 12 of pregnancy and increased continually during the last half of pregnancy up to an absorption rate of 0.72 $\mu\text{g Zn/loop}$ per 15 min on the twenty-first day of pregnancy. Thus, the physiological changes of pregnancy may include an increased absorption of zinc. Davies and Williams (16) injected L - [4,5 - ^3H] lysine monohydrochloride along with the ^{65}Zn and found L - [4,5 - ^3H] lysine monohydrochloride absorption was increased when zinc was present.

These studies pointed out that the absorption of

nutrients is influenced by the presence of other nutrients. Increased absorption of zinc in situ in a physiological saline solution during pregnancy does not necessarily indicate a similar occurrence when zinc is consumed in the diet.

Serum and Tissue Zinc Content During Pregnancy

Plasma zinc levels fall dramatically soon after a zinc deficient diet is begun (18,50). Dreosti et al (18) placed pregnant rats on a soybean diet containing 0.36 ppm of zinc. The control animals were fed the same diet with 100 ppm of zinc as $ZnCO_3$ added. The plasma zinc levels of the control animals remained around 95 $\mu g \%$ through the end of the second week of the pregnancy. Plasma zinc levels fell during the last week of pregnancy to 70 $\mu g \%$. The plasma zinc levels of the zinc deficient animals fell 38 % (to 60 $\mu g \%$) the first day of pregnancy. Dreosti (18) saw a similar decrease in plasma zinc levels in male weanling rats on the same diet. O'Dell et al (42) found lower plasma zinc levels (73 ppm vs 131 ppm) at the time of parturition in rats fed a diet containing 1 ppm zinc than in controls given 100 ppm zinc. Zinc deficiency in rats causes a decrease in the serum zinc content. There is also a decrease in serum zinc levels during pregnancy.

Other mammals have been reported to have decreased serum zinc levels during pregnancy. Dufty et al (19) reported that plasma zinc levels remained constant in

Hereford cattle until late in pregnancy when a decline occurred. A further decline occurred during parturition. Labor was longer with a greater decrease in plasma zinc levels in cattle which experienced dystocia than in animals calving normally. Plasma zinc levels in sheep fall to 0.75 mg/ml during parturition (McSporran, 1977). There is also a decrease in plasma zinc levels of pregnant women (50,25).

There are two major ligands of zinc in serum, α_2 -macroglobin and serum albumin. Giroux et al (25) looked at the effect of pregnancy in women on serum levels of these ligands and the affinity of zinc for these ligands. Serum albumin levels were lower in pregnant women than in non-pregnant women. Alpha- $_2$ macroglobulin concentrations were not significantly different. The decrease in total serum zinc levels of pregnant women was of similar magnitude as the decrease in serum albumin. The serum albumin of pregnant women had less affinity for zinc than the serum albumin of non-pregnant women. Both hypoalbuminemia and low serum albumin affinity for zinc contribute to the hypozincemia observed in pregnant women.

Changes occurred in the zinc content of maternal tissues, fetal tissues, and placentas during pregnancy (54). The maternal body increased in zinc content during the first 15 days of pregnancy in rats. There was no further

significant increases from day 15 on. Non-pregnant rats, however, showed three times the rate of increase in zinc over the same time period. On the diet used, which was sufficient in zinc, there was no depletion of liver stores of zinc in pregnant rats. There was a slight increase in the zinc content of the femur of pregnant rats during the first 15 days of gestation. Non-pregnant females had a much greater increase in zinc content of femurs (30 μg as opposed to 10 μg). The uterus gained 8.8 μg zinc from day 15 to 21 of the pregnancy. The placenta gained 3.6 μg zinc per day during the same time period. Uterine and placental weight correlated with fetal number but their zinc content was not related to fetal number. The zinc content of fetuses was 0.48 μg on day 18, and 77.3 μg on day 21. Fetal weight increased from 1.54 g on day 18 to 5.32 g on day 21. This tremendous increase in the weight and zinc content of fetuses during the last three days of pregnancy which puts a great demand on the maternal organism for zinc and other nutrients. The uterus, mammary glands, and pups were lighter in weight in zinc deficient females due to lack of sufficient zinc for growth of these tissues (4).

Because of increased need for zinc during pregnancy and the rapid growth of these tissues during pregnancy, the zinc turnover during pregnancy is greater than in

nonpregnant females (23). Evans used ^{65}Zn to measure the turnover of zinc in mice during pregnancy. A single dose of 0.1 μCi of ^{65}Zn was injected intraperitoneally on the first day of pregnancy. Controls and pregnant mice were monitored daily for radioactivity throughout pregnancy and for 20 days afterwards. The pregnant females lost 1.65 ± 0.06 % of the ^{65}Zn during the first two weeks of pregnancy and retained zinc during the third trimester. Non-pregnant females lost 0.85 ± 0.01 % of the ^{65}Zn per day. 100 % of the ^{65}Zn lost around parturition could be accounted for in the pups. During lactation 50 % of the ^{65}Zn lost by the females was accounted for by ^{65}Zn accumulation in the pups.

Although other studies found that zinc is not well mobilized from stores during pregnancy (35), Evans and Reis (23) concluded that zinc losses are increased during pregnancy. This supports Sansteads' (46) recommendation that zinc intake be increased during pregnancy and lactation. Food intakes, however, were not recorded for Evans' and Reis' study (23). The accelerated loss of ^{65}Zn could have been the result of consumption of additional zinc in the diet since food consumption increases during pregnancy.

Hormonal levels during pregnancy and Zn deficiency

Several hormones may be influenced by zinc defi-

ciency. Some examples are the female steroids estrogen and progesterone, the adrenal steroid aldosterone, and the prostaglandins (6,7,12,31,42,47). Changes in the levels of these hormones may explain the difficult parturition experienced by zinc deficient dams.

Serum progesterone concentration was higher on day 18 (156 ± 16 mg/ml) in zinc deficient animals than in ad libitum control animals (110 ± 10 mg/ml) (6,7). Control values were close to those previously reported (40). The higher progesterone value may be the result of lower plasma volume. Hematocrit values for zinc deficient dams were elevated beginning on the day 14 of pregnancy (6,7). O'Dell et al (42) also observed high hematocrits, indicating hemoconcentration. High progesterone levels during pregnancy may compound zinc deficiency. Sata and Henkin (47) found that estrogens do not alter serum zinc levels and progesterones cause a decrease in the zinc concentration of serum.

Progesterone-estrone therapy increased the number of pregnancies in zinc deficient dams (3). Female rats were fed a diet containing 1 ppm of zinc from day 1 through day 21 of gestation. Subcutaneous injections of 1 μ g estrone and 4 mg progesterone were given daily from day 4 to 21. Only 50 % of the females on a zinc deficient diet without hormone therapy were pregnant 8 days

after copulation. All zinc deficient females receiving hormone therapy were pregnant on day 8 of gestation. Both groups had a 50 % incidence of pregnancy on day 21. Progesterone or estrone or both may be a limiting factor early in pregnancy. Zinc or some other factor is limiting in later stages of pregnancy. Progesterone/estrone imbalances may cause zinc deficient animals to reabsorb or abort their young.

Aspirin was used to imitate the effects of prostaglandin insufficiency in rats (42). The resulting pathology found in rats fed 200 mg aspirin by stomach tube twice a day was similar to the pathology of zinc deficient animals. Gestation period was increased, a number of the pups were born dead, rectal temperature was low, and blood pressure was low in both the zinc deficient and aspirin fed animals. Blood loss was greater in the females given aspirin. Zinc may be involved in prostaglandin biosynthesis or function since the prostaglandin inhibitor, aspirin, causes the same stress signs in periparturient animals as zinc deficiency. Prostaglandin levels rise dramatically during labor and the potential for prostaglandin F_2 increases during the last week of pregnancy in rats (13). If zinc deficiency affects prostaglandin biosynthesis, parturition might be difficult.

O'Dell (42) found that zinc deficient rats exhibited

signs of adrenal insufficiency during parturition. Low aldosterone levels may have accounted for the decrease in blood pressure and hyperkalemia. Ten mg of aldosterone was administered twice a day beginning on day 15 of pregnancy to rats on a diet containing 1 ppm zinc. Aldosterone did not decrease the number of dams that exhibited signs of stressful parturition on a zinc deficient diet. Adrenal glands, however, were significantly heavier ($P < 0.05$) in the zinc deficient animals. O'Dell hypothesized that adrenal steroids are not released or are unable to perform their normal function in zinc deficiency. This is supported by observations that electrolyte balance is disturbed (42) and kidney nephrosis (12) is present in pregnant, zinc deficient rats.

The electrolyte balance may be disturbed in zinc deficient pregnant rats (42). Plasma zinc concentration fell from 1.31 ± 0.13 ppm in controls to 0.73 ± 0.5 ppm in zinc deficient animals. Potassium levels were 6.46 ± 0.48 mg/liter in zinc deficient animals and 5.22 ± 0.24 mg/liter in control animals. Sodium and calcium levels remained unchanged. The electrocardiograms of zinc deficient females were typical of those observed in hyperkalemia.

Kidney nephrosis has been observed in zinc deficient female rats sacrificed on the twentieth day of pregnancy

(12). An increase in non-urea urinary nitrogen along with diuresis was reported in animals fed a low zinc (6-8 ppm) and high L-amino acid (24 %) diet (12,31). Reduction in the amount of tryptophan in the diet from 250 mg % to 75 mg % was significantly protective against the pathological changes. The kidney pathology resulting from zinc deficiency during pregnancy probably is a major factor in the stressful parturition observed in zinc deficient dams.

Serum hormone levels are altered in zinc deficiency and this may explain the difficult parturition by zinc deficient animals. Progesterone which is necessary for maintenance of pregnancy is present in lower levels in the serum of zinc deficient animals. Aspirin, a prostaglandin inhibitor, results in stressful parturition similar to that of zinc deficient females indicating that prostaglandin synthesis or function is depressed by zinc deficiency. Hypertrophied adrenal glands, hyperkalemia, diuresis, and kidney nephrosis in zinc deficient dams are indicators of possible aldosterone insufficiency.

Inadvertent Zinc Deficiency

Many commercially available salt mixes for nutritional studies with laboratory rats are deficient in zinc (27). Greenfield and Briggs (27) found that out of 100 papers using rat diets published in Volume 97-99

of the Journal of Nutrition, 23 used diets lacking zinc and 16 used diets containing less than one half of the NRC requirement for zinc. The salt mixes considered best by Greenfield and Briggs were Bernhart-Tomarelli (11), G2 (37), Jones-Foster (36), Rogers-Harper (45), USP 17 (14), and Williams Briggs Modified (UCB-1R6) (53). Still, the Jones-Foster, USP 17, and Rogers-Harper salt mixes are low in zinc. Greenfield and Briggs (27) conclude that mineral deficiencies, quite frequently zinc, were the "deficiency" that researchers described when another mineral, vitamin, or even fat, carbohydrate, or protein was restricted from the diet.

Zinc requirements are highest in animals undergoing rapid growth (41). Therefore, special care should be taken in designing diets for pregnant, lactating, and immature rats. Zinc deficiency causes the toxic pregnancy and parturition described earlier. Hirt et al (32) related the protein and fat content of a diet the used to litter survival and lactation defects. The diet used Wesson 86 salt mixture which lacks zinc. Zinc is a special problem in diets based on soybean protein concentrate, rapeseed protein content, and other vegetable protein sources containing phytate. A number of papers have described serious complications during pregnancy and parturition which could be attributed to zinc deficiency

(20,21,22,38,48). Zinc deficiency was probably the cause of the problems Hill (31) had in breeding rats.

While studying the effect of maternal diet on cataract formation, Hill (31) made the observation that female Sprague Dawley rats were becoming ill a couple days prior to parturition. Those who survived parturition did not properly care for their young. The eyes of the females who exhibited the pathology became encircled with blood-like secretions, and the coat became sparse and rough, between the nineteenth day of pregnancy and the second day after parturition. The eye lesions were unresponsive to medicate salve. The mineral mix used (45) was lower in zinc than recommended (27) and zinc deficiency may have accounted for the pathology observed.

Hill (31) used a L-amino acid diet which contained 7.5 g of tryptophan per 10 kg of diet and no tocopherols (-E, -Trp). The other dietary treatments had 4.0 g tocopherol in an ether solution added per 10 kg of diet (+E, -Trp); 42.50 g of tryptophan per 10 kg of diet added at the expense of alanine (-E, +Trp); and both tocopherol and tryptophan added (+E, +Trp) in the same amounts as in the other +E and +Trp diets. Diet (+E, -Trp) was protective against the pathology which occurred around parturition. The pathological effects were more pronounced with a higher (24 %) protein diet than at a

24 % amino acid diet. Diuresis also occurred with the 12 % protein diets which were tested. Mean urine (ml/48 hr) volumes (\pm S.D.) were (-E, -Trp)₂₄, 115 ± 29 ; (+E, -Trp)₂₄, 33 ± 2 ; (-E, +Trp)₂₄, 110 ± 20 ; (+E, +Trp)₂₄, 112 ± 20 ; (-E, -Trp)₁₂, 60 ± 6 ; (+E, -Trp)₁₂, 87 ± 6 . It should be noted that water consumption was not measured. The diet and/or pregnancy might have induced a higher water consumption and therefore a large urine volume.

About the nineteenth day of gestation the female rats began to show the pathological signs mentioned previously. Appetite also decreased, possibly due to zinc deficiency. Not only were many of the females lost during parturition but many of the young were lost within a few days after birth. Twenty percent of the females died at parturition and only 25 % of the pups survived to weaning. The pathology correlates well with Apgar's (1-7) observations on the effects of zinc deficiency on parturition. On about the nineteenth day of gestation, Apgar also noticed a rough coat and "reddish material on the vibrissae." Apgar also reported fetal and maternal deaths.

Zinc deficiency could have occurred on the diet Hill used. The mineral mix used was Rogers and Harpers mix (45), which at 4 % provides 0.4 mg Zn/100 g diet (4 ppm),

30 % of the NRC requirements for growth (27,41). The cages used were stainless steel and the water used was double distilled. The only source of zinc was the diet. Subsequent experiments were conducted to ascertain whether the pathology was derived from a zinc deficiency. Supplementation of the test diets with zinc did allow the females to survive parturition and care for their young (12).

Experimental Rationale

The biochemical lesions which result in the overt pathology observed in zinc deficient dams and their pups have not been fully identified. Two interesting observations made on zinc deficient dams are that low dietary levels of tryptophan protect against the symptoms of maternal zinc deficiency and that water and electrolyte balance are disturbed in zinc deficient dams.

Low levels of tryptophan may protect against zinc deficiency by increasing the absorption of zinc, decreasing the requirement for zinc, or decreasing the excretion of zinc. A decreased requirement for zinc could be the result of several changes in metabolism: tryptophan metabolism may require a large amount of zinc, low levels of dietary tryptophan may decrease the growth of the fetus, or mobilization of zinc stores may be increased by low dietary tryptophan levels. Hormones,

notably serotonin, and enzymes containing a large amount of tryptophan may be biosynthesized in low amounts when dietary levels of tryptophan are low. If these same hormones and enzymes are zinc dependent, low dietary levels of tryptophan would have a sparing effect on zinc deficiency. How low dietary tryptophan levels spare zinc is unknown.

The kidney and adrenal gland may be a major site of the biochemical lesions seen in zinc deficiency. Hypertrophied adrenal glands, hyperkalemia, diuresis and kidney nephrosis occurs in zinc deficient dams and are possible indicators of aldosterone insufficiency. Disturbed water and electrolyte balance may result in the pathological symptoms observed in zinc deficient dams. Pregnancy is normally accompanied by shifts in water and electrolyte balance. The influence of zinc deficiency upon water and electrolyte balance has just begun to be addressed.

This study was designed to test the hypothesis that zinc deficiency induces diuresis and renal pathology in pregnant female rats. A second hypothesis tested was that a low dietary tryptophan level during pregnancy exerts a protective influence against the symptoms of maternal zinc deficiency by limiting the transfer of zinc from the maternal organism to the fetus. If low

dietary tryptophan levels are protective against maternal zinc deficiency, low dietary tryptophan levels would be protective against renal pathology which is caused by zinc deficiency.

A number of measures were taken in order to test the hypothesis. Water consumption and urine volume during pregnancy were measured and renal tissue was examined to determine the effect of zinc deficiency upon water balance and the kidney. Fetal zinc content was measured to determine the influence of maternal dietary tryptophan levels on zinc transfer from the maternal organism to the fetus. Measures of food consumption, weight gain, zinc balance, and peri-parturient illness were used as indicators of maternal zinc status.

Chapter III

EXPERIMENTAL PROCEDURES AND METHODS

Three experiments were conducted using Sprague-Dawley rats obtained from Flow Laboratories in Dublin, Virginia. Eight to 10 week old females weighing 160 to 200 g and males of the same age were housed in an animal room with an automatic 12 hour light-dark cycle. Heating and air conditioning systems kept the room between 19° and 23°, although failure of the heating-cooling system caused the temperature in the room to go as high as 26° and as low as 16° for short periods of time. The animals were housed in stainless steel cages and provided with deionized water for drinking, in order to limit zinc intake. At the beginning of each experiment, the animals were adjusted for one week to one of four experimental diets.

The experimental design was a 2 x 2 factorial with nesting. Two different levels of tryptophan (.75 g Trp/Kg diet and 5.0 g Trp/Kg diet) and two different levels of zinc (6 ppm of diet and 50 ppm of diet) were fed in 4 different combinations (see Table 1). A group, in each experiment, consisted of 6 females and 6 males. Measurements were made on each female and on her progeny. The dams on the diet containing the higher level of zinc and the higher level of tryptophan and their

Table 1

Experimental Design

Diet	-Tryptophan (.75 g/kg diet)			+Tryptophan (5.0 g/kg diet)		
	dam 1	dam ... 2	dam 6	dam 1	dam ... 2	dam 6
-Zinc (6 ppm)	fetus 1	fetus 1	fetus 1	fetus 1	fetus 1	fetus 1
	fetus 2	fetus 2	fetus 2	fetus 2	fetus 2	fetus 2
	fetus n	fetus n	fetus n	fetus n	fetus n	fetus n
+Zinc (50 ppm)	dam 1	dam ... 2	dam 6	dam 1	dam ... 2	dam 6
	fetus 1	fetus 1	fetus 1	fetus 1	fetus 1	fetus 1
	fetus 2	fetus 2	fetus 2	fetus 2	fetus 2	fetus 2
	fetus n	fetus n	fetus n	fetus n	fetus n	fetus n

pups were considered the positive controls. The dams on the diet containing the lower level of zinc and the lower level of tryptophan were considered the negative controls.

The constituents of the diets are listed in Table 2 and the components of the amino acid and mineral mixes are listed in Tables 3 and 4-5 respectively. The tryptophan and zinc content of the diets varied. Rogers-Harper Salt Mix from ICN Pharmaceuticals, Inc., Cleveland, Ohio was used as the source of minerals in the diets for experiment 1. A mineral mix patterned after the R-H salt mix was prepared by the experimenter and used in the diets for experiments 2 and 3. The diets were stored in a freezer maintaining a temperature around -10° C until used.

After the one week adjustment period, the males and females were caged in a one-to-one pairing with the experimental diet and distilled water provided ad libitum. The papers beneath the cages were examined for copulation plugs and changed daily during the mating period. The presence of a copulation plug was considered evidence of mating and the day on which it was found was considered day 1 of gestation. The females were weighed and placed in a separate cage from that of the males. The males were returned to a rat chow diet

Table 2

Composition of BASAL DIET

g/kg diet

Glucose	450.00
Cornstarch	80.60
Alphacel	50.00
Corn Oil (tocopherol stripped)	100.00
Vitamin Mix ^a	25.00
Mineral Mix ^b	40.00
Amino Acids ^c	253.05

^aObtained from ICN Pharmaceuticals, Inc., Cleveland, Ohio. A complete Vitamin Mix except for tocopherol was used in the first experiment. Tocopherol was added to each diet as tocopherol acetate suspended in ether (1 g tocopherol acetate/10 ml ether) at levels of .4 g tocopherol/kg diet. The vitamin mix used in the second and third experiment was the ICN complete vitamin mix.

^bMineral mix for the first experiment R-H salt mix from ICN Pharmaceuticals, Inc., Cleveland, Ohio. Mineral mix for the second and third experiment was similar to the R-H salt mix and made at V.P.I. & S.U. See Table 4 for the mineral composition of mixes.

^cObtained from Ajinomota, New York, NY. See Table 3 for the amino acid composition of the diets.

Table 3
 Amino Acid Composition of Diets
 g/kg diet

^a amino acid	Diet 1 and 2	Diet 3 and 4
Histidine	10.80	10.80
Lysine	31.00	31.00
Leucine	16.00	16.00
Isoleucine	10.00	10.00
Phenylalanine	12.00	12.00
Methionine	6.00	6.00
Cysteine	6.00	6.00
Tyrosine	6.00	6.00
Threonine	10.00	10.00
Valine	14.00	14.00
Arginine	15.00	15.00
Glutamine	18.00	18.00
Asparagine	18.00	18.00
Proline	18.00	18.00
Alanine	26.00	21.75
Glycine	18.00	18.00
Serine	18.00	18.00
Tryptophan	.75	5.00
Total	253.05	253.05

^aAmino acids were obtained from Ajinomoto, N.Y.

Table 4

Composition of Mineral Mixes used in Diet

g / kg diet

Mineral	Mineral mix for Experiment 1	Mineral mix for Experiments 2 and 3
Ca	4.732	4.743
Cl	6.084	6.134
Cu	0.025	0.035
I	0.00015	0.00015
Fe	0.039	0.039
Mg	0.394	0.394
Mn	0.016	0.016
Mo	0.0005	0.0006
P	3.155	2.479
K	3.943	6.161
Se	0.00018	0.00026
Na	3.954	3.944
S	0.541	0.546
Zn	0.004	0.003

Table 5

Components of Mineral Mix Patterned after Rogers
and Harpers and used for Experiments 2 and 3
g / kg diet

Mineral	g / kg
Ammonium Molybdate ^a	0.0012
Calcium Carbonate ^a	11.716
Calcium Phosphate (2H ₂ O) ^b	0.172
Cupric Sulfate ^a	0.062
Ferric Citrate (6 H ₂ O) ^b	0.2492
Magnesium Sulfate (7 H ₂ O) ^b	3.992
Manganese Sulfate (1 H ₂ O) ^b	0.0484
Potassium Iodide ^a	0.00002
Potassium Phosphate, Monobasic ^b	13.724
Sodium Chloride ^b	10.026
Sodium Selenite (5 H ₂ O) ^c	0.00008
Zinc Chloride ^a	0.00624

In all experiments 0.84 g/10 kg of zinc acetate was added to diets 2 and 4.

^aFrom S.T. Baker Chemical Co. Phillipsburg, N.J. 08865.

^bFrom Fisher Scientific Co., Chemical Manufacturing Division, Fairlawn, N.J. 07410.

^cPrepared at VPI & SU, Dept. of Biochemistry and Nutrition, Dr. G. E. Bunce's lab. 1972.

and the females were fed the experimental diet for the remainder of the gestation period.

The females were weighed and placed in metabolic cages on day 7 of gestation. Forty-eight hour urine and fecal collections were made from day 7 to day 20 of gestation. In experiment 1, one ml of toluene was placed in the acid-washed collection bottles to prevent microbial growth. Urine pH was measured in experiment 1. One ml of 2 N HCl · 2 N H₂SO₄ was used as the antimicrobial agent in experiments 2 and 3. After 48 hours, urine volume was measured and both urine and feces were frozen until later analysis. In order to prevent contamination by zinc or bacteria, the bottoms of the metabolic cages were washed and rinsed with distilled water every other day.

Food consumption was recorded daily from day 1 of pregnancy through parturition in all experiments. The small size of the feed cups in the metabolic cages necessitated feeding the females two or three times a day. Water consumption was recorded daily during experiments 2 and 3 and the data gathered were used to compare water consumption with urine volume. The females were weighed every seven days and just before sacrificed. Food consumption was related to weight gain during the pregnancy. Food consumption was also used as an indi-

cation of zinc deficiency.

In experiment 1, those animals which survived parturition were allowed to raise their young. Data were collected on maternal and fetal morbidity and mortality. The same females and males were used for experiment 2. In experiment 2, the females which did not deliver prematurely were sacrificed on day 21 by decapitation under CO₂ anaesthesia. Serum was collected and analyzed for zinc content.

In experiment 3, the pregnant females were anaesthetized with CO₂ and sacrificed by decapitation on day 21 of pregnancy. The fetuses were removed by caesarean section, examined for defects, counted, and individually weighed. The fetal carcasses were freeze dried and stored in a dessicator until further analysis. The maternal carcasses were sent to the Veterinary Science Department at V.P.I. & S.U. for histological examination.

In all experiments, zinc balance was determined by analysis of diet, urine, and feces for zinc content. Duplicate samples of diet were wet ashed in acid washed 400 ml beakers covered with watch glasses. Concentrated nitric acid was added in 3 serial additions to one gram of diet sample. After each dilution the samples were digested for 45 min on a 250° F hotplate. The samples were then heated for 30 min with one ml 30 % hydrogen

peroxide, 45 min with 10 ml concentrated nitric acid, 30 min with 3 ml of hydrogen peroxide, and then 45 min with 10 ml of nitric acid. The samples were brought to dryness after the last treatment with nitric acid, allowed to cool to ambient temperature and diluted to 10 ml with 10 % hydrochloric acid.

Fecal samples for 6 days were combined by placing them in a flask of a tissue homogenizer, bringing the volume up to 20 ml with deionized water, and then homogenizing the samples thoroughly. Duplicate 2 ml aliquots were then analyzed for zinc content in the same manner as the diet samples.

Urine samples for each animal were composited for two six day periods - day 7 to 13 of gestation and day 14 to 20 of gestation. Ten ml of concentrated nitric acid was added to the sample, an acid washed watch glass placed over the beaker, and the sample heated at 250° F for 45 min. After the samples were brought close to dryness, 1 ml of 30 % hydrogen peroxide was added. After cooling to ambient temperature, the sample was brought to 10 ml with 10 % hydrochloric acid.

The dry weight of the fetuses from experiment 3 was taken. Half of the fetuses from each dam were randomly chosen for zinc determination and the other half were analyzed for nitrogen content. Since there was an

average of 64 fetuses per dietary group, 32 were analyzed for zinc content and 32 were analyzed for nitrogen content. Actual numbers ranged from 36 fetuses in the low trp, low Zn group to 52 fetuses in the high Zn, high trp group. The fetuses used for zinc determination were wet ashed individually in acid washed 400 ml beakers with watch glasses. The samples were heated at 250° F in 10 ml of concentrated nitric acid for one hour. One ml of 30 % hydrogen peroxide was added and the samples heated for 30 min. The samples were heated three more times with 10 ml of nitric, 1 ml of hydrogen peroxide, and 10 ml of nitric. After cooling to ambient temperature, the samples were brought up to 75 ml with 10 % hydrochloric acid.

The zinc content of the prepared samples was determined on a Perkin-Elmer 306 atomic absorption spectrophotometer. Acetylene fuel with air as the oxidant was used for the flame source. Absorption of the samples in 10 % hydrochloric acid was recorded at a wavelength of 213.9 nm. Sample zinc concentration was determined by comparing absorption values with a standard curve formed from zinc chloride standards. The standards contained 0.1, 0.25, 0.5, 1.0, and 2.0 ppm zinc and were prepared and run with each set of samples.

Nitrogen content of fetuses and diets was analyzed

by the AOAC modified Kjeldahl procedure (8). Diet samples weighing 0.5 g were analyzed in triplicate. Half the freeze-dried fetuses from each dam in experiment 3 were weighed, one fetus was placed whole into each Kjeldahl flask, and analyzed. The fetuses weighed between 0.25 g and 0.6 g after drying. Samples were digested in 20 ml of concentrated sulfuric acid with 0.2 g cupric sulfate acid and 10 g sodium sulfate as catalysts. The ammonia was released from the digestion mixture with 110 ml of 50 % sodium hydroxide. A small amount of granulated zinc was added to the sodium hydroxide mixture to produce even boiling. The ammonia was trapped in 25 ml of 4 % boric acid and titrated with 1.036 N hydrochloric acid to the methyl-red-methylene-blue endpoint.

Urine creatinine and nitrogen content were analyzed simultaneously on a Technicon Autoanalyzer II. Urine was frozen previous to analysis. Urine creatinine and nitrogen values were calculated using standard curves from solutions containing known concentrations of creatinine and ammonium sulfate, respectively.

Data obtained from each experiment was analyzed separately using SAS 76 (9). Means were compared and analysis of variance tests performed. When a significant difference was found, Duncan's Multiple Range Test was used to determine the location of the difference.

Chapter IV

RESULTS AND DISCUSSIONS

The different intents of the three different experiments necessitated measuring slightly different parameters in the three experiments. In all experiments, maternal health, water intake, urine volume, maternal zinc intake, and maternal zinc excretion were measured. Maternal health was observed for symptoms of zinc deficiency. Water intake and urine volume were measured in order to determine whether water balance was affected by zinc deficiency. Zinc balance was calculated from maternal zinc intake and excretion. In the experiment 1 the survival rate of the pups was used as an indicator of maternal zinc deficiency. Pup survival was observed in all but 9 litters in experiment 2. Locating the site of the primary lesion causing the pathology in the zinc deficient dams was an objective of experiment 2. Nine of the dams were sacrificed on day 20 of gestation and histopathology of lungs, heart, spleen, liver, and kidney tissues were observed for signs of lesions. Blood was collected from these animals and serum zinc concentration was measured to determine whether it was affected by dietary zinc level. In experiment 3, the movement of zinc from the dam to the fetuses was observed. The number of fetuses and implantation sites were counted in

order to determine the number of reabsorptions. The water content, wet weight, dry weight, zinc content, and nitrogen content of the fetuses were measured.

A. Physical Appearance and General Health of Females During Gestation and Parturition

The females in experiments 1 and 2 exhibited symptoms of a respiratory infection which began 2 to 3 days after the animals were received from Flow Laboratories. Males received at the same time had similar symptoms, noticeably sneezing and bleeding at the nostrils. The symptoms subsided by the end of the one week adjustment period. The females used in all experiments were free of clinical symptoms until day 18 of gestation.

Between day 18 and day 21 of gestation, 20 to 50 %, depending upon the experiment, of the females on the low zinc dietary treatments exhibited signs of ill health (Tables 6-8). The animals in experiment 1 on a low zinc diet did not exhibit the pathological symptoms to the degree the animals in the other experiments did. The vibrissae of animals in experiment 1 which were affected were coated with a red exudate. In the affected females from experiments 2 and 3, during day 18 to 20 a red exudate encircled the eyes. A reddish material also coated parts of the vibrissae by day 21 of pregnancy. The coat became sparse and rough. The zinc deficient

Table 6

Experiment 1

Reproductive Success: Fertility, Maternal Survival
at Parturition, and Pup survival 24 hours after
Parturition

	Experimental Treatment			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
<u>Females Pregnant</u> No.	5/6	4/6	6/6	5/6
<u>Females Mated</u> %	83	67	100	83
<u>Females dying at Parturition</u> No.	1/5	2/4	0/6	0/5
<u>Females Pregnant</u> %	20	50	0	0
<u>Pups surviving 24 hrs</u> No.	21/37	8/21	61/65	57/63
<u>Pups delivered</u> %	57	40	94	90

Table 7

Experiment 2

Reproductive Success: Fertility, Maternal Moribundity
at Parturition, and Pup Survival 24 hours after
Parturition

		Experimental Treatment			
		-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
<u>Females Pregnant</u>	No.	3/6	6/6	6/6	4/6
<u>Females Mated</u>	%	50	100	100	67
Females moribound close to parturition					
<u>Females Pregnant</u>	No.	1/3	2/6	0/6	0/4
	%	33	33	0	0
<u>Pups surviving 24 hrs</u>	No.	-	5/17	21/23	7/7
<u>Pups delivered</u>	%	-	30	91	100

¹Data on pups surviving 24 hours does not include data
from animals which were sacrificed.

Table 8

Experiment 3

Reproductive Success: Fertility, Maternal Morbidity at parturition, Number of implantation sites, Number of fetuses, and number of reabsorptions.

		Experimental Treatment			
		-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
<u>Females Pregnant</u>	No.	4/6	6/6	6/6	6/6
<u>Females mated</u>	%	67	100	100	100
Females moribound close to parturition					
<u>Females Pregnant</u>	No.	1/4	2/6	0/6	0/6
	%	25	33	0	0
<u>Implantation Sites</u>	No.	15	13	14	14
<u>Pregnant Female</u>					
<u>Fetuses</u>	No.	9	12	12	13
<u>Pregnant Female</u>					
<u>Reabsorptions</u>	No.	6	1	2	1
<u>Pregnant Female</u>	%	40	8	14	7

dams became lethargic 2 to 3 days before parturition. By the time of parturition, the affected females stood in one place and moved only if disturbed. The most severely affected dams died soon during parturition or within 24 hours after parturition. The clinical symptoms were most severe in dams on the low zinc diets in the second experiment.

Dams on zinc deficient diets exhibited signs of zinc deficiency. The pathology observed around the time of parturition was identical with Apgar's (1,2,3) description of zinc deficient dams except for the red exudate around the eyes. Maternal mortality was not quite as high as in Apgar's studies, but higher levels of dietary zinc were used in this study than were used by Apgar in low zinc groups.

B. Food Consumption During Gestation

The four dietary treatments differed in zinc and tryptophan content (Tables 1-5). The tryptophan content of the diets was 7.5 g/10 kg in the low tryptophan diets and 50 g/10 kg in the high tryptophan diet. The analyzed zinc content of the diets was 8.8 ppm, 7.5 ppm, and 6.8 ppm in the low zinc diets for experiments 1, 2, and 3 respectively, or 47.6 ppm, 51 ppm, and 46 ppm in the high zinc diets for experiments 1, 2, and 3 respectively.

In all experiments, there was a significant increase

($p < .01$) in the mean daily food consumption of the females during gestation as the dietary zinc level increased (Tables 9-12 and Figures 1-3).

Females on a diet low in zinc consumed a mean of 22 ± 7 g diet/day. Females on the diets with a higher zinc content consumed a mean of 29 ± 8 g diet/day. Food consumption of pregnant females on a zinc deficient diet dropped during the last week of gestation and food consumption of females obtaining adequate dietary zinc increased slightly or remained constant during the last week of gestation.

The lower food intake of females in the zinc deficient diets is the result of zinc deficiency (Tables 9-12). Although, statistically there was no influence of tryptophan level of the diet upon food consumption, the trend in experiments 1 and 3 was for an increase in food consumption when dietary tryptophan was increased in diets sufficient in zinc. Suppression of appetite in zinc deficient animals has been observed by other researchers (1,2,42).

Appetite suppression by low levels of tryptophan when dietary zinc is adequate can not be adequately explained. An hypothesis is that the low levels of dietary tryptophan led to an amino acid imbalance which caused the decreased appetite or aversion to the diet.

Table 9

Experiment 1

Food Consumption Records for Females Through Gestation

(g of diet consumed /24 hours)

Days of Gestation	Experimental Procedure			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(5)	(4)	(6)	(5)
1 - 7	18±4	22±4	19±4	28±4
7 - 14	19±1	23±2	21±2	30±8
14 - 21	21±3	26±2	22±5	30±3
Mean daily food consumption ^{1,2}	19±2 ^a	24±2 ^b	21±3 ^{a,b}	29±13 ^b
Mean Total food consumption	410	508	448	623

¹Means ± SD²Means with different letters are significantly different, p < 0.05

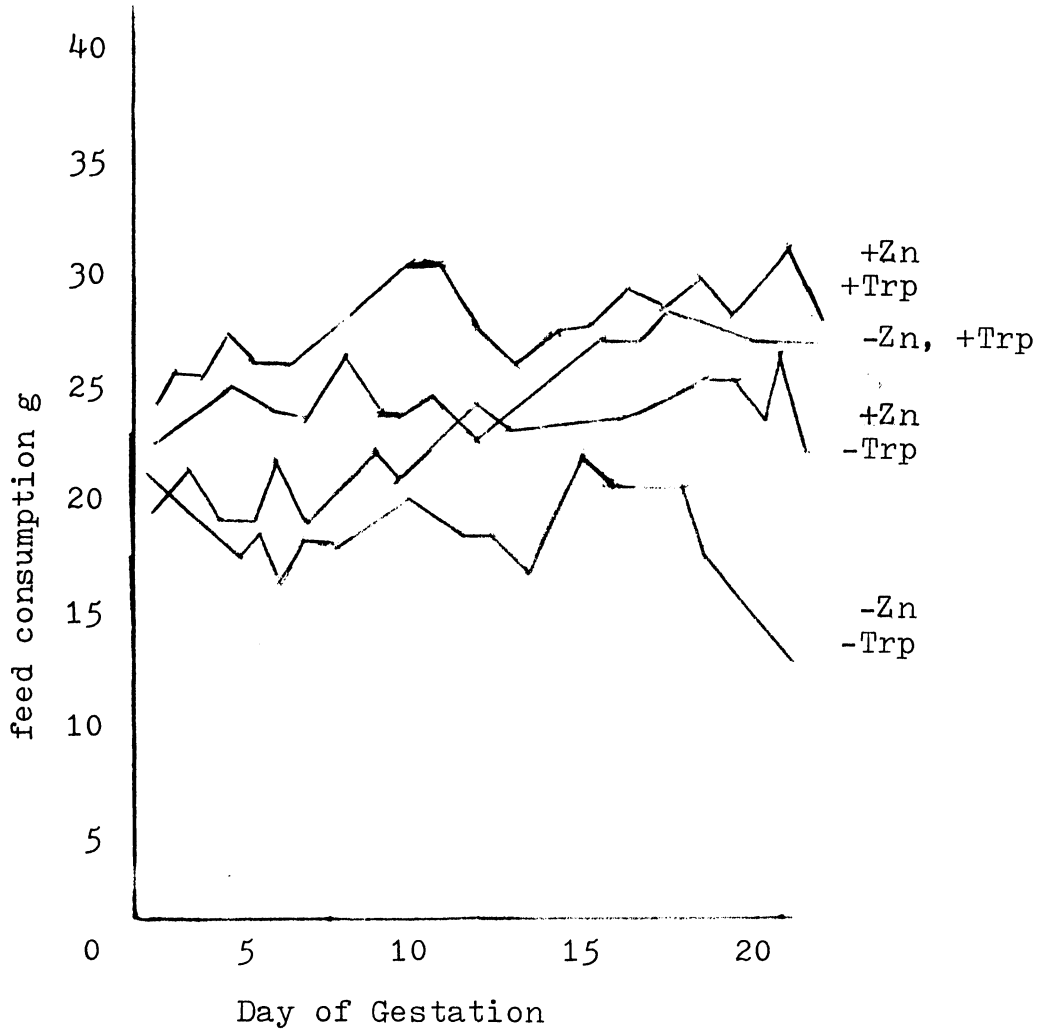


Figure 1: Food Consumption of Females in Experiment 1:
g/24 hours

Table 10
Experiment 2

Food Consumption Records for Females Through Gestation
(g of diet consumed /24 hours)

Days of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(3)	(6)	(6)	(4)
1 - 7	26±2	24±5	31±5	29±2
7 - 14	25±9	22±4	32±3	34±7
14 - 21	23±6	19±4	33±6	35±6
Mean daily food consumption ^{1,2}	25±5 ^a	22±3 ^a	32±3 ^b	33±3 ^b
Mean Total food consumption	525	462	672	693

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

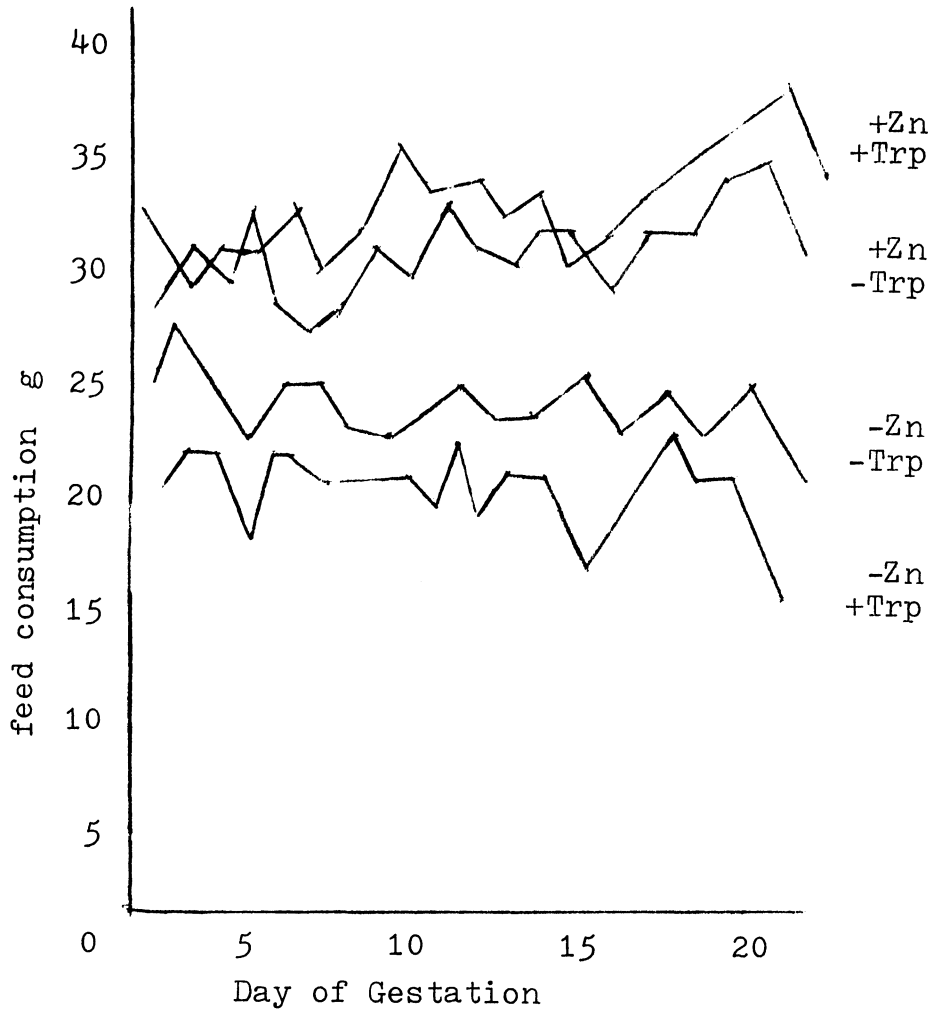


Figure 2: Food Consumption of Females in Experiment 2:
g/24 hours

Table 11

Experiment 3

Food Consumption Records for Females Through Gestation
(g of diet consumed /24 hours)

Days of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(4)	(6)	(6)	(6)
1 - 7	21±3	23±4	25±6	31±6
7 - 14	20±1	22±4	26±4	33±4
14 - 21	17±2	20±2	26±7	32±5
Mean daily food consumption ^{1,2}	19±2 ^a	22±3 ^{a,b}	26±5 ^{b,c}	32±4
Mean Total Food Consumption	399	462	546	672

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

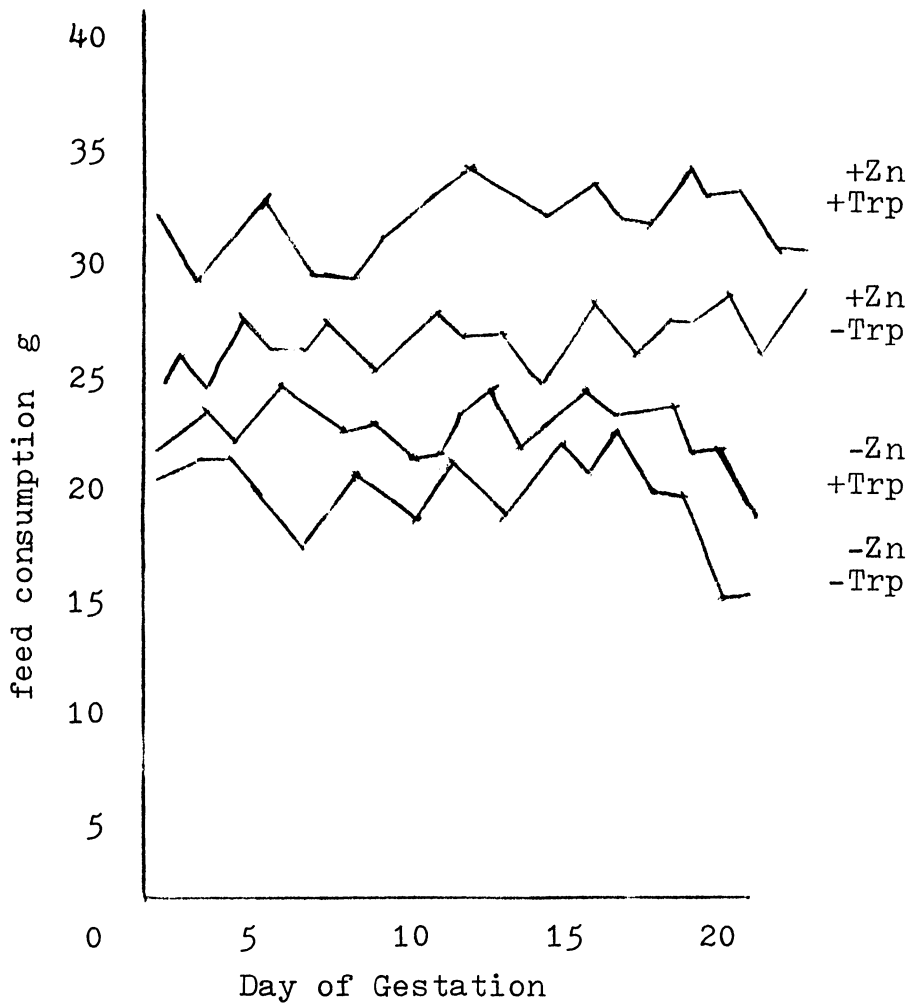


Figure 3: Food Consumption of Females in Experiment 3:
g/24 hours

Table 12

Data from Experiments 1, 2 and 3
Pooled using a Block Design

Food Consumption Records for Females Through Gestation
(g of diet consumed /24 hours)

Day of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(12)	(16)	(18)	(15)
1 - 7	22±7	23±3	25±14	29±4
7 - 14	21±6	22±2	26±11	32±4
14 - 21	20±6	22±6	27±11	32±6
Mean daily food consumption ^{1,2}	21±6 ^a	23±3 ^a	25±10 ^{a,b}	32±9 ^b
Mean Total food consumption	441	483	525	672

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

C. Weight gain during Gestation

The animals in all dietary groups gained weight during each 7 day period of parturition (Tables 13-16). In experiments 2 and 3, there was a significant difference ($p < 0.005$) in weight gain dependent upon dietary zinc level. Animals receiving the larger quantity of zinc in the diet had significantly larger weight gains during parturition. This was not true in experiment 1. Tryptophan level of the diet did not affect weight gain significantly.

Decreased weight gain during pregnancy in zinc deficient animals has been observed by other researchers (1,2,42). The decreased weight gain observed was the result of zinc deficiency caused appetite suppression.

D. Reproductive Success

In none of the experiments were all the females fertile (Tables 6-8). The greatest difficulty with fertility was experienced in experiment 1. In experiment 1, three females on zinc deficient diets died during parturient period (Table 6). Since animals were sacrificed on day 21, no observations on peri-parturient mortality were taken from experiments 2 and 3. In experiment 2, 3 of the 9 females on zinc deficient diets exhibited the pathology described in Section A of this chap-

Table 13

Experiment 1

Mean Body Weight Change (g) for Females
During Gestation

Day of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(5)	(4)	(6)	(5)
1	199±11	208±12	212±13	210±9
7	218±12	217±15	225±15	233±14
14	243±9	239±14	246±16	267±17
21	265±13	264±10	264±15	304±19

¹Means ± SD

Table 14

Experiment 2

Mean Body Weight Change (g) for Females
During Gestation

Day of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(3)	(6)	(6)	(4)
1	276±16	272±14	281±31	274±28
7	288±23	287±19	293±24	296±13
14	301±29	298±23	315±20	329±15
21	322±34	326±17	347±35	372±21

¹Means ± SD

Table 15

Experiment 3

Mean Body Weight Change (g) for Females
During Gestation

Day of Gestation	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(4)	(6)	(6)	(6)
1	215±13	221±12	211±9	218±13
7	228±14	236±17	233±10	229±16
14	244±11	251±23	254±13	261±14
21	272±12	282±19	285±11	307±13

¹Means ± SD

Table 16

Experiments 1, 2 and 3

Mean Total Body Weight Gain During Gestation

(in g)

Experiment	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(12)	(16)	(18)	(15)
1	46±12	54±14	66±11	98±20
2	66±23	46±28	52±12	94±14
3	57±12	61±17	74±13	89±21
Weight gain (g) experiments 1, 2, 3 pooled using a Block Design ^{1,2}	56±18 ^a	57±20 ^{a,b}	64±15 ^b	94±18 ^c

¹Means ± SD²Means with different letters are significantly different, P < 0.05.

ter (Table 7). In experiment 3, 1 of the 4 animals on the -Zn, -Trp diet and 2 of the 6 animals on -Zn, +Trp diet exhibited pathological symptoms prior to being sacrificed (Table 8). Maternal health was influenced by the dietary zinc content.

Pup survival for 24 hours after birth was decreased in the zinc deficient dams. In experiment 1, 21 out of 37 pups in the low Zn, low trp group, 8 of 21 pups in the low Zn, high trp group, 61 of 65 pups in the high Zn, low trp group, and 57 of 63 of the pups in the high Zn, high trp group were alive 24 hours after parturition (Table 6). Some of the females were sacrificed prior to parturition in experiment 2. Animals which were allowed to deliver their young were used to gather data on pup survival. In experiment 2, 5 of 17 pups in the low Zn, high trp group, 21 of 23 pups in the high Zn, low trp group, and 7 of 7 pups in the high Zn, high trp group survived for more than 24 hours after parturition (table 7).

Pup survival was determined by maternal health. Dams which were ill failed to clean the cage after delivery, and did not clean, retrieve, or nurse their pups. Pups were scattered around the cage and died of maternal neglect.

In experiment 3, the number of fetuses was less

than the number of implantation sites on the uterine horns of the dams. The number of reabsorptions was 6 per female in the low Zn, low trp group, 1 per female in the low Zn, high trp group, 2 per female in the high Zn, low trp group, and 1 per female in the high Zn, high trp group (Table 8). Zinc deficiency increased the number of reabsorption sites.

Reproductive failure, including the failure of fetuses to be implanted, the failure of fetuses to be carried to term, the birth of dead pups, and the death of pups soon after birth have all been attributed to zinc deficiency (3,34). The occurrence of these problems during the experiments in this study is further evidence of zinc deficiency (Tables 6-8).

E. Water Intake and Urine Volume

There was no significant difference ($p < 0.05$) in water intake or in urine volume between dietary groups (Tables 17 and 18). This was true in each experiment.

Hill's (31) research led to the hypothesis that zinc deficiency causes an increased urine volume. This study does not support that conclusion. However, animals from this study which were zinc deficient did exhibit renal pathology (12). Although diuresis was not observed in this study it is possible that zinc deficiency did result in altered electrolyte balance and/or water bal-

Table 17

Experiments 1, 2 and 3

Mean Water Intake from day 7 to day 21 of Gestation
(ml /24 hrs)

Experiment	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(12)	(16)	(18)	(15)
1	17±10	19±6	19±3	18±4
2	17±3	18±7	17±4	16±4
3	19±4	19±8	16±6	18±4
Data from experiments 1, 2, 3 pooled using a Block design ^{1,2}	18±4 ^a	19±7 ^a	17±4 ^a	17±4 ^a

¹Means ± SD²Means with different letters are significantly different, P < 0.05.

Table 18

Experiments 1, 2, and 3

Mean Urine Volume from day 7 to day 21 of Gestation
(ml /24 hrs)

Experiment	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group	(12)	(16)	(18)	(15)
1	15±4	16±3	17±3	14±3
2	14±7	14±3	16±3	16±3
3	18±3	17±6	16±5	17±5
Data from experiments 1, 2, 3 pooled using a block design ^{1,2}	15±4 ^a	16±4 ^a	17±4 ^a	16±4 ^a

¹Means ± SD²Means with different letters are significantly different, P < 0.05.

ance secondary to the renal pathology which was observed.

F. Renal Histopathology

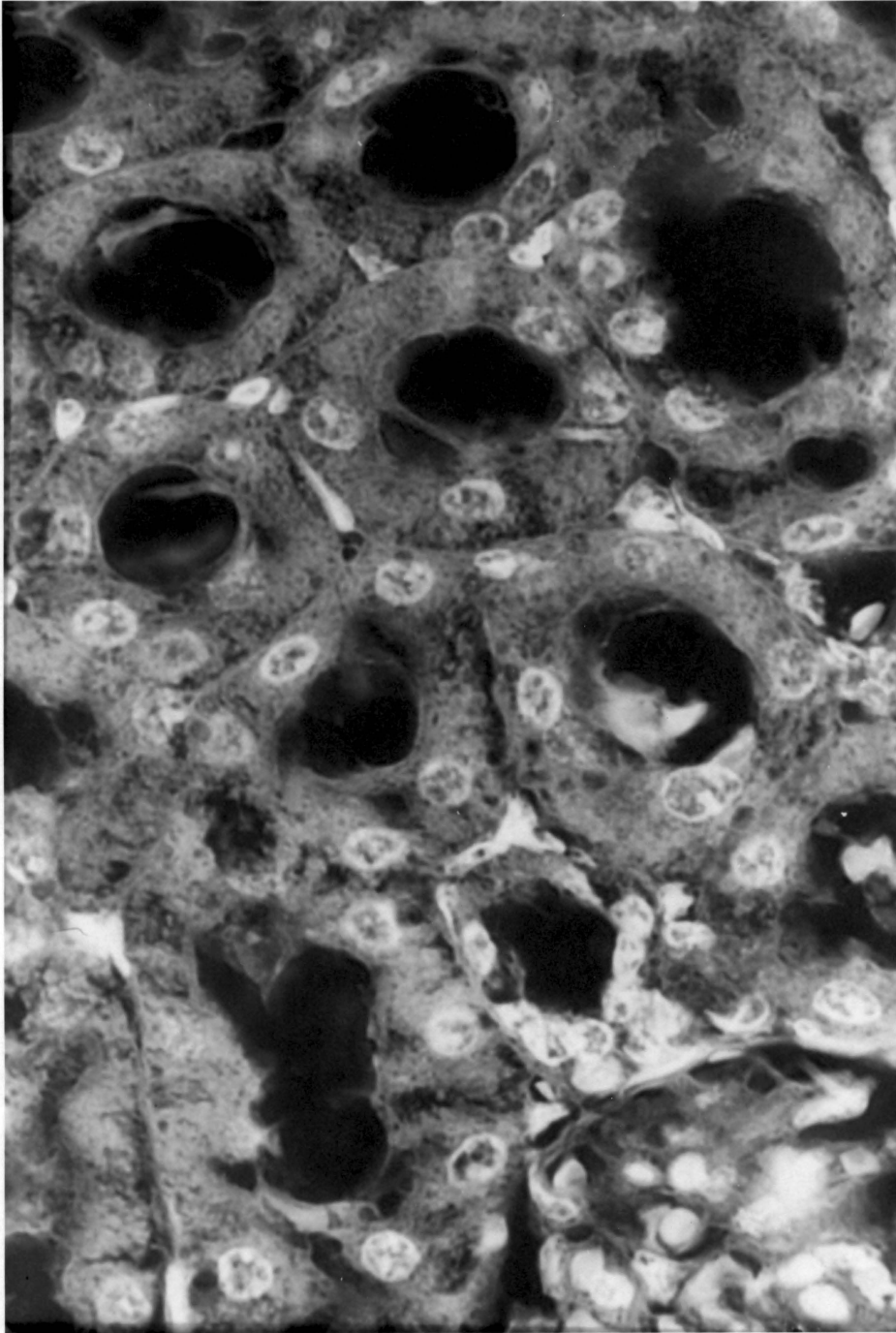
Histological examination of 9 of the animals sacrificed on day 20 of pregnancy in experiment 2 revealed acute nephrosis in animals which had been on a low zinc diet. The low zinc group fed high tryptophan levels had a slightly higher degree of histological changes than the low zinc group fed low tryptophan levels.

The 2 animals fed a high zinc, high tryptophan diet exhibited no remarkable histological lesions in the kidneys, pancreas, heart, spleen, small intestine, stomach, liver or lungs. The 3 animals in the low zinc, low tryptophan group had kidney lesions and 2 animals from this group had lung pathology indicative of chronic respiratory illness which was not at an acute stage at the time the animals were sacrificed. All 4 of the animals in the low zinc, high tryptophan group had kidney lesions. The lung pathology observed in 2 animals in the low zinc, low tryptophan group was present in 2 of the animals in the low zinc, high tryptophan group. In addition, 2 of the animals in the low zinc, high tryptophan group exhibited a small amount of centrilobular fatty changes in the liver.

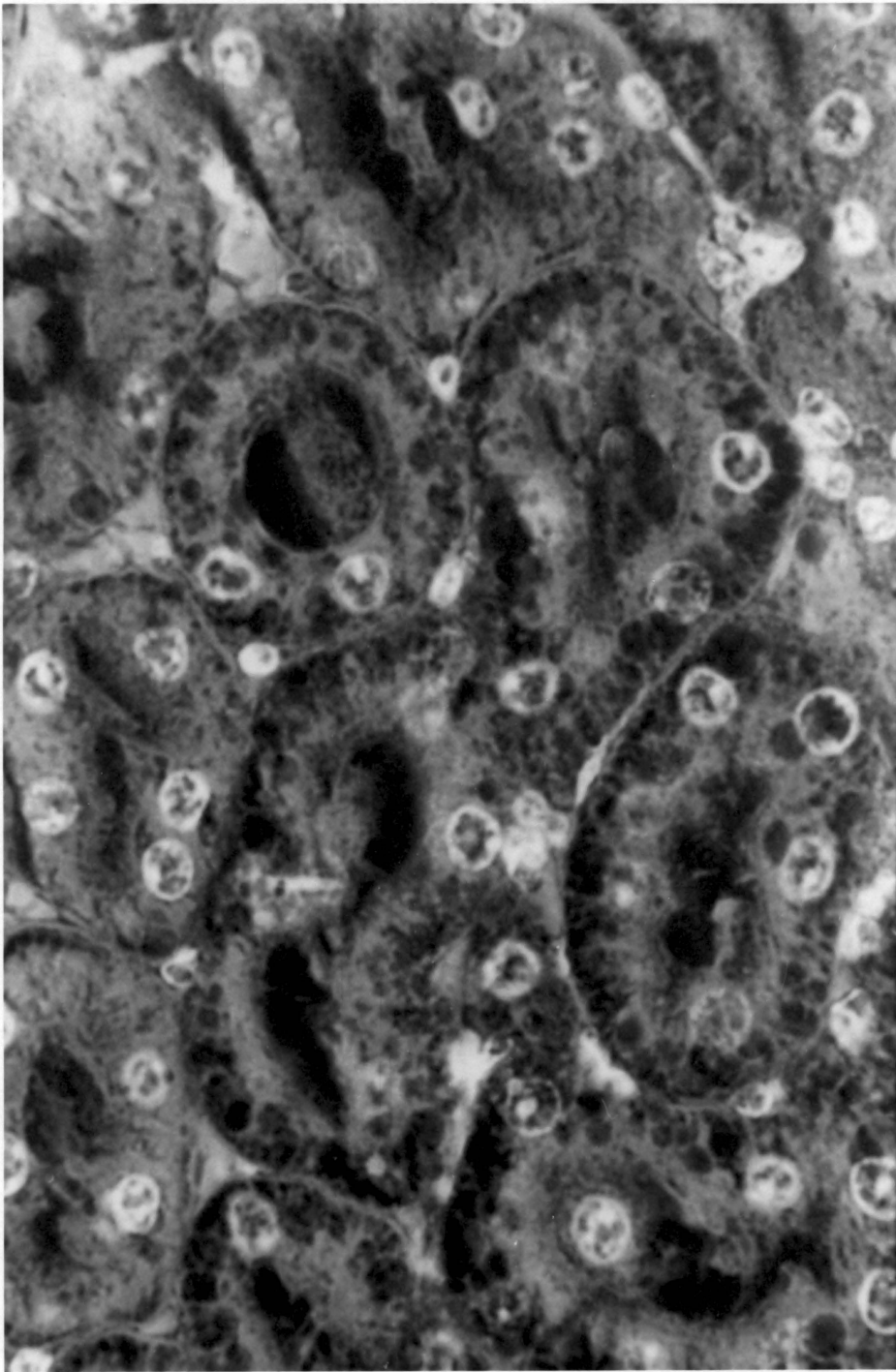
The primary kidney lesions noted were cellular

swelling, vacuolization, eosinophilia, intracytoplasmic granulation, and disruption of the brush border (pictures 1-2). The most frequent change was the hydrophic (cellular) swelling. Swelling occurred most often in the proximal convoluted tubules and less often in the collecting tubules or loops of Henle. Vacuolization occurred in some tubules to a greater degree than in others and most often in the proximal convoluted tubules. Eosinophilia was intermittent and usually related to a few cells within a tubule. Intracytoplasmic granulation which was periodic-acid-shift-reaction-stain positive was found in all rat kidneys so examined. In the low tryptophan groups, the size and concentration of these granules varied in relation to the degree of other changes. The cells which were swollen and contained vacuoles often had more granules than normal looking cells. Some cells on thin section appeared to have either sloughed or shortened brush borders.

The second common lesion noted in rats which had been fed a low zinc diet was centrilobular fatty change. This change is often seen in anorectic animals and represents mobilization of fat by the animal. The decreased food consumption of the zinc deficient animals was the cause of the centrilobular fatty change. Pair fed controls were not used in the experiment to deter-



Photograph 1: Tissue of normal rat kidney from an animal fed the high zinc, high tryptophan diet. H and E stain of 2μ section at 2800 x magnification.



Photograph 2: Tissue of rat kidney from an animal fed the low zinc, high tryptophan diet. H and E stain of 2 μ section at 2800 x magnification.

mine the effect of zinc deficiency on the centrilobular fatty change.

The insult to the kidney tissue was such that while there was distinctive degeneration occurring particularly in the proximal convoluted tubules, the process did not generally lead to necrosis. Other degenerative lesions of the kidney were not noted. Cellular (hydropic) changes are related to the dilatation of one or more cellular organelles. The vacuolization was non-lipid in nature and may be a more advanced manifestation of the disturbance causing cellular swelling. The positive periodic-acid-shift-reaction-stain (PAS) indicate the aggregation of PAS + substances. Many substances including mucins, polysaccharides, and collagen are PAS + so further work is needed to determine the composition of the PAS + granules. The eosinophilia noted is due to the binding of eosin by cytoplasmic proteins.

The primary lesion noted in the zinc deficient dams was acute nephrosis in the proximal convoluted tubules. These lesions do not appear in ad libitum fed control animals. It is assumed that the kidney lesions are the result of zinc deficiency but this data has not been confirmed.

G. Zinc Balance

Dietary zinc intake was between 120 and 190 $\mu\text{g}/24$

hours for animals on the low zinc diets and between 1000 and 1700 $\mu\text{g}/24$ hours for animals on the high zinc diets (Tables 19-21). Urinary losses of zinc were between 40 and 90 $\mu\text{g}/24$ hours and were not related to diet. Fecal zinc content varied with the dietary zinc content. Fecal loss of zinc was greatly increased on the high zinc diet (900-1600 $\mu\text{g Zn}/24$ hours) over losses on the low zinc diet (30-125 $\mu\text{g Zn}/24$ hours). There was no significant difference in zinc balance due to dietary treatment.

Zinc content ranged from 0.7 $\mu\text{g}/\text{ml}$ to 1.6 $\mu\text{g}/\text{ml}$ in the serum taken from the 9 animals from experiment 2 used for histopathological examination. The 2 animals fed a high zinc, high tryptophan diet had serum zinc levels of 0.9 $\mu\text{g}/\text{ml}$ and 1.2 $\mu\text{g}/\text{ml}$. The three animals on the low zinc, low tryptophan diet had a mean serum zinc content of 1.16 $\mu\text{g}/\text{ml}$. The four animals from the low zinc, high tryptophan group had a mean serum zinc content of 1.25 $\mu\text{g}/\text{ml}$. There was no significant difference ($p < 0.05$) in serum zinc content between groups.

Animals which are pregnant, because of the growth of new tissue, should show zinc retention when balance data is calculated. It is suspected that in this experiment the fecal collections were contaminated with zinc. A possible source of contamination was the food.

Table 19

Experiment 1

Zinc Balance Data

No. females/ group	Days of gesta- tion	Experimental Treatments ¹			
		-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
		(5)	(4)	(6)	(5)
Mean Dietary Intake, Zinc ($\mu\text{g}/24$ hr)	7-14	134 \pm 9	166 \pm 14	1001 \pm 23	1375 \pm 19
	14-20	124 \pm 24	161 \pm 13	998 \pm 16	1385 \pm 24
Mean Urinary Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	46 \pm 25	72 \pm 14	79 \pm 27	59 \pm 21
	14-20	51 \pm 18	74 \pm 9	83 \pm 25	57 \pm 16
Mean Fecal Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	75 \pm 53	98 \pm 73	925 \pm 76	1324 \pm 53
	14-20	86 \pm 64	85 \pm 77	909 \pm 87	1318 \pm 38
Mean Zn Balance ($\mu\text{g}/24$ hr) ^{1,2}		-0.9 \pm 1.7 ^a	-0.8 \pm 2 ^a	0.6 \pm 1.7 ^a	0.5 \pm 1.7 ^a

¹Means \pm SD

²Means with different letters are significantly different, $p < 0.05$.

Table 20
 Experiment 2
 Zinc Balance Data

	Days of gestation	Experimental Treatments ¹			
		-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/group		(3)	(6)	(6)	(4)
Mean Dietary Intake, Zinc ($\mu\text{g}/24$ hr)	7-14	185 \pm 23	162 \pm 12	1609 \pm 14	1689 \pm 9
	14-20	188 \pm 29	168 \pm 9	1653 \pm 17	1678 \pm 8
Mean Urinary Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	72 \pm 33	39 \pm 19	72 \pm 19	83 \pm 19
	14-20	75 \pm 29	44 \pm 17	67 \pm 14	89 \pm 27
Mean Fecal Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	119 \pm 59	133 \pm 53	1579 \pm 93	1583 \pm 54
	14-20	108 \pm 48	120 \pm 51	1548 \pm 101	1608 \pm 49
Mean Zn Balance ($\mu\text{g}/24$ hr)		-0.4 \pm 1.5 ^a	-0.9 \pm 1.9 ^a	0.4 \pm 1.9 ^a	0.7 \pm 1.7 ^a

¹Means \pm SD

²Means with different letters are significantly different, $P < 0.05$.

Table 21
 Experiment 3
 Zinc Balance Data

	Days of gesta- tion	Experimental Treatments ¹			
		-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. females/ group		(4)	(6)	(6)	(6)
Mean Dietary Intake, Zinc ($\mu\text{g}/24$ hr)	7-14	124 \pm 8	142 \pm 16	1191 \pm 9	1465 \pm 9
	14-21	116 \pm 6	136 \pm 17	1201 \pm 13	1479 \pm 6
Mean Urinary Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	89 \pm 39	52 \pm 27	77 \pm 22	74 \pm 21
	14-21	86 \pm 41	56 \pm 24	74 \pm 31	71 \pm 18
Mean Fecal Losses, Zinc ($\mu\text{g}/24$ hr)	7-14	32 \pm 29	88 \pm 58	1098 \pm 83	1415 \pm 49
	14-21	35 \pm 33	84 \pm 54	1140 \pm 67	1381 \pm 61
Mean Zn Balance ($\mu\text{g}/24$ hr)		-1.1 \pm 1.7 ^a	-0.6 \pm 1.6 ^a	0.5 \pm 1.8 ^a	0.5 \pm 1.4 ^a

¹Means \pm SD

²Means with different letters are significantly different, $P < 0.05$.

The high zinc diet if spilled and mixed with the feces would have added a large amount of zinc to the feces. Maternal stores would not have been adequate to supply sufficient zinc for the fetus (52). Therefore the failure to have zinc retention indicates poor collection technique and/or contamination.

Weigand and Kirchgessner (51) collected zinc balance data on weanling rats. They found that fecal losses of zinc were one fourth of the dietary intake when dietary zinc levels were between 39 and 70 ppm of the diet. The proportion of the zinc in the diet which was absorbed increased from 34 to 100 % as the dietary zinc levels decreased from 141 ppm to 5.6 ppm of the diet. There was no difference in urinary zinc losses. These results are typical of other findings (44,50). The maternal organism during pregnancy, in order to produce viable new tissue, should have been in positive zinc balance (44,50).

H. Fetal Weights and Moisture Contents

There were no significant differences ($p < 0.05$) between the wet weight, dry weight, or moisture content of fetuses between maternal dietary treatment groups (Table 22). The mean wet weight was 3.4 g. Mean dry weight was 0.42 g. Mean moisture content was 88 %.

Table 22

Experiment 3

Fetal Weights and Moisture Content

	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. fetuses analyzed	18	72	72	78
Mean Net Weight g ^{1,2}	3.26 ±0.04 ^a	3.45 ^a ±0.02 ^a	3.28 ±0.03 ^a	3.57 ^a ±0.02 ^a
Mean Dry Weight g ^{1,2}	0.37 ^b ±0.03 ^b	0.42 ^b ±0.01 ^b	0.43 ^b ±0.02 ^b	0.45 ^b ±0.03 ^b
Mean Moisture Content %	89±2 ^c	88±1 ^c	87±2 ^c	87±1 ^c

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

There was a significant difference ($p < 0.05$) in the wet weight and dry weight of fetuses which was dependent upon the dam. Moisture content did not vary significantly with the dam.

In this study there was no significant difference in fetal weights between groups (Table 22). Apgar (6) reported a significant difference in fetal weights with a decreased fetal weight when dams were fed a diet containing 1 $\mu\text{g Zn/g}$ diet. The low zinc diets used in this study contained more zinc than the diets Apgar used and zinc levels were enough that fetal weight was not affected.

Fosmire et al (24) found a sparing of fetal growth at the expense of the dam at very low levels of zinc intake. At higher levels of zinc, still suboptimal, fetal growth was retarded and maternal weight gain was normal.

I. Fetal Zinc Content

The fetal zinc content was dependent on maternal dietary zinc content ($p < 0.001$). Zinc content of fetuses ranged from a mean of $45.5 \pm 2 \mu\text{g Zn/fetus}$ in the low zinc, low tryptophan group to $65.3 \pm 5 \mu\text{g Zn/fetus}$ in the high zinc, high tryptophan group (Table 23). Statistically, dietary tryptophan did not affect fetal zinc content. In the low zinc diets, however, fetal

Table 23
 Experiment 3
 Fetal Zinc Content

	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. fetuses analyzed	(18)	(36)	(36)	(39)
$\frac{\text{mean no. fetuses}}{\text{dam}}$	8.75	12	11.67	10.66
No. dams	4	6	6	6
$\frac{\text{mean } \mu\text{g zinc}}{\text{fetus}}$ ^{1,2}	45.2±2 ^a	49.6±3 ^a	59.3±4 ^b	65.3±5 ^b
$\frac{\text{mean } \mu\text{g Zn}}{\text{g dry weight}}$ ^{1,2}	123±3 ^c	118±1 ^c	138±4 ^d	145±6 ^d
mean total zinc (μg) in fetuses from one dam ^{1,2}	398±15 ^e	595±13 ^f	692±18 ^g	696±12 ^g

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

zinc content was lowest when tryptophan levels were low.

The total amount of zinc in the fetuses from one dam was dependent upon dietary treatment. The fetal compartment of dams on the low zinc, low tryptophan diet contained 394 ± 15 μ g of zinc. The fetal compartment of dams on the low zinc, high tryptophan diet contained 595 ± 13 μ g of zinc. The fetal compartment of dams on the high zinc, low tryptophan and high zinc, high tryptophan diets contained 692 ± 18 μ g zinc and 696 ± 17 μ g zinc respectively.

Fetal zinc content was a function of maternal zinc intake. Dams on a diet containing higher levels of zinc, did transfer more zinc to the fetuses. A lower level of dietary tryptophan resulted in lower zinc content of the fetuses only when maternal dietary zinc levels were low. The protective influence of tryptophan against symptoms of zinc deficiency could be exercised in part by decreasing zinc transfer into the fetuses. The average weight of fetuses from the third experiment in this study was lower at a lower level of dietary tryptophan. Lower fetal accretion of zinc may have been partially induced by lower tissue weight of fetuses.

Fetal zinc content was similar to that reported by other studies (4,24), although the zinc content of fetuses from high zinc dams was not as high as expected.

Fosmire (24) reported fetal zinc contents of 95.9 μ g per fetus when the maternal diet contained 25 ppm of zinc.

J. Nitrogen Content of Fetuses

The nitrogen content of fetuses was not dependent upon dietary zinc or upon dietary tryptophan content (Table 24). The mean nitrogen content of fetuses was between 0.095 g N/g dried weight and 0.0103 g N/g dried weight. When a factor of 6.25 was used to determine fetal protein content, the fetuses were found to contain between 0.24 μ g and 0.28 μ g of protein.

Fetal nitrogen content was not affected by maternal dietary zinc or tryptophan level. Further studies are needed to determine the specific amino acid and mineral composition of fetuses from dams on low zinc and low tryptophan diets before the affect of maternal diet on fetal nitrogen content with these diets is understood.

Table 24
 Experiment 3
 Nitrogen Content of Fetus

	Experimental Treatments ¹			
	-Zn -Trp	-Zn +Trp	+Zn -Trp	+Zn +Trp
No. fetuses analyzed	18	36	36	39
Mean <u>g Nitrogen</u> fetus	0.038	0.041	0.041	0.044
Mean <u>g Nitrogen</u> ^{1,2} g dried weight	0.013 ± .02 ^a	0.098 ± .03 ^a	0.095 ± .01 ^a	0.098 ± .02 ^a
Mean <u>g Nitrogen</u> g wet weight	0.012	0.012	0.013	0.012
Mean <u>g Protein</u> fetus	0.24	0.24	0.26	0.28

¹Means ± SD

²Means with different letters are significantly different, $P < 0.05$.

Chapter V

SUMMARY AND CONCLUSIONS

In the three experiments of this study, rats were fed diets throughout gestation containing 7.5 mg Trp/100 g diet or 500 mg Trp/100 g diet and 7.5 ppm zinc or 50 ppm zinc in a 2 X 2 factorial design. The physical appearance and general health of the females during gestation and parturition and the rate of reproductive success were observed. Food consumption and weight gain of the females were measured throughout gestation and water intake and urine volume of the same dams were measured the last 2 weeks of gestation. Urine and fecal zinc content were determined from collections made the last 2 weeks of gestation and subtracted from zinc intake in order to determine zinc balance of the females during gestation. Nine females from experiment 2 were sacrificed on day 20 of pregnancy and examined for pathological lesions. Serum zinc content was analyzed in blood from the same females.

The females from experiment 3 were sacrificed on day 20 of pregnancy and the fetuses were removed by caesarian section. The number of implantation sites on the uterine horn were counted and compared to the number of fetuses from the same dam. Fetal wet weight was taken and fetal moisture content calculated. From each dam

half of the fetuses were analyzed for zinc content and half were analyzed for nitrogen content.

The females on the low zinc diets exhibited the symptoms of zinc deficiency reported by Apgar (1-4). In some cases, the pathology was severe enough that maternal and fetal death occurred. The affected females gained less weight and consumed less food than females on a zinc sufficient diet. Tryptophan level did not affect weight gain or food consumption. Urine volume and water consumption did not differ significantly with dietary treatment. Zinc balance data did not differ significantly but contamination of the urine and fecal samples with zinc was suspected.

In experiment 2, the pathological examination revealed 2 abnormalities in the zinc deficient animals: acute renal nephrosis primarily in the proximal convoluted tubules and centrilobular fatty change of the liver. There was a higher degree of histological changes in the group fed the low zinc, high tryptophan diet than in the group fed a low zinc, low tryptophan diet. Serum zinc content was not significantly different between groups.

The females from the third experiment fed a low zinc diet had a higher number of reabsorptions than the females fed a high zinc diet. Dietary tryptophan level

did not affect reproductive success.

Fetal wet weight and dry weight were not affected by the maternal diet, but were affected by the individual dams. Fetal zinc content was dependent upon maternal dietary zinc content and not significantly related to maternal dietary tryptophan content. Dietary zinc content and tryptophan content did not affect fetal nitrogen content.

The biochemical lesions which result in the pathology observed in pregnant rats on zinc deficient diets are not well understood. However, some conclusions were drawn from this study.

1) Female rats fed diets containing 6-9 ppm of zinc throughout gestation do become zinc deficient by the time of parturition.

2) Zinc deficiency causes decreased appetite and decreased weight gain during pregnancy.

3) Zinc deficiency causes an increased incidence of reproductive failure.

4) Zinc deficiency does not cause diuresis. Renal pathology, however, does occur in zinc deficiency.

5) Low dietary tryptophan levels (75 mg %) were protective against the renal pathology observed in zinc deficiency.

6) Zinc deficiency results in a lower zinc content

of the fetal compartment. Zinc content of individual fetuses was lower when maternal dietary zinc levels were lower.

7) Fetal weight and moisture content were not influenced by maternal dietary zinc or tryptophan at the levels fed in this study.

Further research is necessary to define more clearly the biochemical lesions of zinc deficiency and to elucidate the influence of dietary tryptophan upon these lesions.

Literature Cited

1. Apgar, J. (1968) Effect of zinc deficiency on parturition in the rat. *Am. J. Physiol.* 215:160.
2. Apgar, J. (1968) Comparison of the effect of copper, manganese, and zinc deficiencies on parturition in the rat. *Am. J. Physiol.* 215:1478.
3. Apgar, J. (1970) Effect of zinc deficiency on maintenance of pregnancy in the rat. *J. Nutr.* 100:470.
4. Apgar, J. (1972) Effect of zinc deprivation from day 12, 15, or 18 of gestation on parturition in the rat. *J. Nutr.* 102:343.
5. Apgar, J. (1973) Effect of zinc repletion late in gestation on parturition in the zinc deficient rat. *J. Nutr.* 103:973.
6. Apgar, J. (1975) Effects of some nutritional deficiencies on parturition in rats. *J. Nutr.* 105:1553.
7. Apgar, J. (1977) Use of EDTA to produce zinc deficiency in the pregnant rat. *J. Nutr.* 107:539.
8. Association of Official Analytical Chemists (1976) Official Methods of Analysis. 12th ed. Washington, D.C.
9. Barr, A.J. and Goodnight, J.H. (1976) Statistical Analysis System. North Carolina University, Raleigh, N.C.
10. Berg, B.N. (1965) Dietary restriction and reproduction in the rat. *J. Nutr.* 87:344.
11. Bernhart, F.W. and Tomarelli, R.M. (1966) A salt mixture supplying the National Research Council estimates of the mineral requirements of the rat. *J. Nutr.* 89:495.
12. Bunce, G.E., Hess, J.L., Veit, H., Hill, J.L. and McLellan, M. (1978) Nephrosis in female rats fed a low zinc diet during gestation. *Fed. Proc. Soc. Exp. Biol. Med.* March 1, 1978 #2391 p. 668

13. Carminati, P., Luzzani, F., Soffientini, A. and Lerner, L.J. (1975) Influence of day of pregnancy on rat placental, uterine and ovarian prostaglandin synthesis and metabolism. *Endocrinology* 97:1071.
14. Committee of Revision (1955, 1960, 1965) *Pharmacopeia of the United States* 15th, 16th, 17th ed. Easton, Pa. Mack Publishing Co.
15. Davies, N.T., Williams, R.B. (1976) Zinc absorption in pregnancy and lactation. *Proc. Nutr. Soc.* 35:5A.
16. Davies, N.T., Williams, R.B. (1977) Zinc balance during pregnancy and lactation. *Am. J. Clin. Nutr.* 30:300.
17. Dhar, J.O., Roy, S.K. and Kar, A.B. (1976) In vivo uptake of ⁶⁵zinc with female genital tract of rat during pregnancy. *Indian J. Exp. Biology* 14:319.
18. Dreosti, I.E., Tao, S.H. and Hurley, L.S. (1968) Plasma zinc and leukocyte changes in weanling and pregnant rats during zinc deficiency. *Proc. Soc. Exp. Bio. Med.* 128:169.
19. Dufty, J.H., Bingley, J.B., and Cove. L.Y. (1977) The Plasma zinc concentration of non-pregnant, pregnant, and parturient hereford cattle. *Aust. Vet. J.* 53:519.
20. Ekland, A. (1973) Influence of a detoxified rapeseed protein concentrate on reproduction in the female rat. *Nut. Rep. Int.* 7:647.
21. Ekland, A. (1975) Outcome of pregnancy from day 0 to 19 and serum tocopherol levels in mother rats fed on a rapeseed protein concentrate essentially free from glucosinolates. *Nutr. and Metabolism* 19:173.
22. Ekland, A. And Agren, G. (1978) Effect of dietary rapeseed protein concentrate on the contents of α -tocopherol and zinc in serum, liver and tibia of rats. *Nutr. Metab.* 22:218.
23. Evans, G.W. and Reis, B.L. (1976) Zinc turnover in mice during pregnancy, lactation, and growth. *Am. J. Clin. Nutr.* 29:814.

24. Fosimire, G.T., Greeley, S. and Sanstead, H.H. (1977) Maternal and fetal response to various sub-optimal levels of zinc intake during gestation in the rat. *J. Nutr.* 107:1543.
25. Giroux, E., Schechter, P.J., and Schown, J. (1976) Diminished albumin binding of zinc in serum of pregnant women. *Clin. Sci. Mol. Med.* 51:545.
26. Griffith, J.Q. and Farris, E.J. (ed.) (1942) The Rat in Laboratory Investigations. Philadelphia, Pa. Lippincott.
27. Greenfield, H. and Briggs, G.M. (1971) Nutritional Methodology in metabolic research with rats. *Annual Review of Biochemistry* 40:449.
28. Halstead, J.A. and Prasad, A.S. (1960) Syndrome of iron deficiency, anemia, hepatosplenomegaly, hypogonadism, dwarfism, and geophagia. *Tran. Amer. Clin. Climat. Assn.* 72:139.
29. Hambidge, K.M. (1978) Zinc and chromium in human nutrition. *J. Human Nutr.* 32:99.
30. Henkin, R.I. (1976) Trace metals in endocrinology. *Med. Clin. of North Amer.* 60:770.
31. Hill, J.E. (1977) Cataract formation in young rats as a consequence of maternal diets containing excess phenylalanine and low in tryptophan and or vitamin E. Thesis done at V.P.I. & S.U. Dept. of Nutr.
32. Hirt, J., Gier, H.T. and Marion, G.B. (1968) Effects of dietary protein - fat deficiencies on lactation and survival of young in laboratory mice. *J. Reprod. Fert.* 17:59.
33. Hoekstra, R.L. (1978) In: Trace Element Metabolism in Man and Animals. Kirchgessner, M. ed. Technisch Universitat Munchen, G.D.R.
34. Hurley, L.S. and Swererton, H. (1966) Congenital Malformations resulting from zinc deficiency in rats. *Proc. Soc. Exp. Biol. Med.* 123:692.

35. Hurley, L.S. and Swererton, H. (1971) Lack of mobilization of bone and liver zinc under teratogenic conditions of zinc deficiency in rats. *J. Nutr.* 101:597.
36. Jones, J.H. and Foster, C. (1942) A salt mixture for use with basal diets either low or high in phosphorous. *J. Nutr.* 24:245.
37. Jones, K.L., Smith, D.W., Ulleland, C.N., and Streissguth, S. (1973) Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* i,1297.
38. McLaughlan, J.M., Jones, J.D., Shah, B.G., and Beare-Rogen, J.L. (1975) Reproduction of rats fed protein concentrate from mustard or rapeseed. *Nutr. Rep. Int.* 18:245.
39. McSporran, K.D. and Lortentz, P.P. (1977) Plasma zinc levels in sheep in the peri-parturient period. *Res. Vet. Sci.* 22:393.
40. Morishige, W.K., Pepe, G.J., and Rothschild, I. (1973) Serum luteinizing hormone, prolactin, and progesterone levels during pregnancy in the rat. *Endocrinology* 92:1527.
41. Nutrient Requirements of Laboratory Animals. (1972) National Research Council, Washington, D.C.
42. O'Dell, B.L., Reynolds, G., and Reeves, P.G. (1977) Analogous effects of zinc deficiency and aspirin toxicity on the pregnant rat. *J. Nutr.* 107:122.
43. Prasad, A.S., Miale, A., Farid, Z., Sansted, H.H., and Schulert, A.R. (1963) Zinc metabolism in patients with the syndrome of iron deficiency anemia, heptosplenomegaly, dwarfism, and hypogonodism. *J. Lab. Clin. Med.* 61:537.
44. Prasad, A.S. and Oberleas, D. ed. (1976) Trace Elements in Human Health and Disease. Volume 1: Zinc and Copper. Academic Press, New York.

45. Rogers, Q.R. and Harper, A.E. (1965) Amino acid diets and maternal growth in the rat. *J. Nutr.* 87:267.
46. Sanstead, H.H. (1973) Zinc nutrition in the United States. *Am. J. Clin. Nutr.* 26:1251.
47. Sato, N. and Henkin, R.I. (1973) Pituitary-gonadal regulation of copper and zinc metabolism in the female rat. *Am. J. Physiol.* 225:508.
48. Sharpe, G.L., Larsson, K.S., and Lieden, S.A. (1975) Toxicological and teratological studies of a rapeseed protein diet in rats and mice. *Nutr. Metab.* 18:245.
49. Todd, W.R., Elvehjem, C.A., and Hart, E.B. (1934) Zinc in the nutrition of the rat. *Am. J. Physiol.* 107:146.
50. Underwood, E.J. (1971) Trace Elements in Human and Animal Nutrition. Ed. 2 Academic Press, New York.
51. Weigand, E. and Kirchgessner, M. (1978) Homeostatic adjustments in zinc digestion to widely varying dietary zinc intake. *Nutr. Metab.* 22:101.
52. Widdowson, E.M. and Dickerson, J.W.T. (1964) Chemical composition of the body. In: Mineral Metabolism Vol. 2. ed. Comar, C.L. and Browner, F. Academic Press, N.Y.
53. Williams, M.A., Chu, L.C., McIntosh, D.J., and Hincenbergs, I. (1968) Effects of dietary fat level on pantothenate depletion and liver fatty acid composition in the rat. *J. Nutr.* 94:377.
54. Williams, R.B., Davies, N.T. and McDonald, I. (1977) The effects of pregnancy and lactation on copper and zinc retention in the rat. *British J. Nutr.* 38:407.

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PREGNANCY AND PARTURITION IN RATS ON ZINC
DEFICIENT DIETS WITH VARYING LEVELS OF TRYPTOPHAN

by

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(ABSTRACT)

Three experiments, each with 24 pregnant female rats as subjects, were conducted. Four experimental diets contained either 7.5 mg tryptophan or 500 mg tryptophan/100 g and either 7.5 ppm or 50 ppm zinc. Dams on the low zinc diets throughout gestation consumed less food and gained less weight than those on the high zinc diets. Reproductive success was less on the low zinc diet. The incidences of maternal mortality, fetal mortality, and reabsorptions were higher in the dams fed a low zinc diet. There were no significant differences in water intake, urine volume, fetal weight, or fetal nitrogen content which were dependent upon dietary treatment. Dams on the low zinc diets had acute renal nephrosis which was most severe when dietary tryptophan levels were high. Fetal zinc content was higher in fetuses from dams fed the high zinc diets. Although the dams on the low zinc diet did exhibit signs of zinc deficiency, the deficiency was not severe enough to affect fetal weight. Diets containing between 6 and 9 ppm were not sufficient for normal parturition.