ZINC AND TAURINE INTERACTIONS IN MATERNOFETAL METABOLISM

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ABSTRACT

It was proposed that zinc and taurine interact *in vivo* to influence reproductive outcome in the rat. The effect of taurine deficiency induced by guanidinoethyl sulfonate (GES), a taurine transport antagonist, on fetal development was examined. Female Sprague-Dawley rats were mated overnight and assigned to 1 of 4 treatments through gestation. Animals received taurine-restricted diet and 0.5% 1.0% or 2.0% GES in drinking water. Control animals received taurine-adequate diet and no GES. Taurine deficiency produced a sharp decline in maternal and fetal liver taurine ($p<0.05$) but did not result in intrauterine growth retardation or significant external, visceral or skeletal malformations. It was evident that reproductive outcome might be an inadequate endpoint to explore this interaction. Instead, development of eye structure and function was examined. Virgin female Sprague-Dawley rats were bred overnight and assigned to 1 of 4 treatments in a 2 x 2 factorial design with two levels of zinc (50 or 15 µg/g through gestation; 50 or 7.5 µg/g after parturition) and two levels of taurine (2 or 0 µmol/g). GES (1% w/v) was provided in drinking water to the animals receiving 0 µmol/g taurine. A fifth group, fed control diet, was pairfed to the marginally zinc-deficient group. At postnatal day 23, male pups were weaned onto their respective diets. Analysis of plasma zinc, tibial zinc and liver taurine concentrations at 7½-8½ weeks showed an interaction in these tissues. Dark-adapted oscillatory potentials

(OPs) and electroretinograms (ERG) were recorded. Two-way ANOVA revealed a significant interaction between zinc and taurine for OP_2 and OP_3 amplitudes. ERG b-wave amplitude as a function of log stimulus intensity was plotted and an iterative curve fitting procedure used to determine the maximum response (Vmax), slope (n) and half-saturation constant (σ). No interaction was noted. Zinc or taurine deficiency each independently depressed Vmax. Zinc deficiency depressed the amplitude of OP_1; taurine depletion similarly affected the amplitude of OP_1, OP_4 and OP_5. Histological examination of the retinas from animals deficient in both zinc and taurine revealed photoreceptor degeneration and retinal dysplasia. These data provide evidence that zinc and taurine interact physiologically and influence retinal function.

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LIST OF ABBREVIATIONS

AMD	Age-related Macular Degeneration
CV	Coefficient of Variation
ERG	Electroretinogram
GES	Guanidinoethyl Sulfonate
HPLC	High Performance Liquid Chromatography
OP	Oscillatory Potential
OPA	Ortho-phthalaldehyde
RPE	Retinal Pigment Epithelium
SAD	Sulfinoalanine Decarboxylase

Chapter 1

INTRODUCTION

1.1 BACKGROUND

Although the importance of nutrition in pregnancy outcome is increasingly recognized, there is widespread belief that nutrient intake among women of childbearing age is inadequate. A review of data from the Second National Health and Nutrition Examination Survey (NHANES II), the USDA Continuing Survey of Food Intakes of Individuals (CSFII) and the National Health Interview Survey (NHSIS) revealed that large segments of this population consume levels of major nutrients that are well below recommended levels (Block and Abrams 1993). For example, only 10% achieve the recommended daily allowance (Commission on Life Sciences, National Research Council 1989) for iron or zinc placing them at risk for poor status of these nutrients.

Developmental defects occur in approximately 3% of all infant births (Centres for Disease Control 1989). Despite intensive research efforts over the

past two decades, causative factors can be identified in only 35-60% of these cases (Schaffer 1993). It has been suggested that environmental factors such as maternal nutritional status may be involved in the expression of these unknown teratogens (Bendich 1993).

It is unlikely that poor maternal intake of a single nutrient is responsible for reproductive defects in the human population. Instead, it is more likely that the compromised status of an individual nutrient increases the risk to other potential teratogens or that there is a synergistic interaction between nutrients that results in poor pregnancy outcome (Keen 1992). The bases of the research reported herein suggests that zinc and taurine are nutrients that interact in this way.

1.2 RATIONALE

Reports that the nutrients zinc and taurine interact have prompted interest in the physiological significance and mechanisms responsible for this phenomenon (Pasantes-Morales et al. 1987a). The strongest evidence for an interaction comes from *in vitro* research on isolated frog rod outer segments. When these cells are exposed to ferrous sulfate they display extensive disruption of their structure, characterized by acute swelling and disc membrane disorganization. The addition of zinc or taurine individually to the culture

medium is ineffective in protecting against this outer segment damage; however, zinc and taurine, added together, provide protection in a synergistic manner (Pasantes-Morales and Cruz 1984). Whether zinc and taurine interact *in vivo* is not known.

The mechanism by which these nutrients may interact is unclear. Zinc has been proposed to play a physiological role in plasma membranes by its effects on membrane protein conformation and protein-protein interactions (Bettger and O'Dell 1993). While the precise biochemical mechanisms have not been fully elucidated, it has been suggested that the loss of zinc from specific proteins in the plasma membrane alters water and ion channels. Ultimately intracellular ion and water concentration is changed (Bettger and O'Dell 1993). It is proposed that taurine, as an intracellular osmoregulator (Huxtable, 1992), can respond to these changes by regulating water and ion flow across cell membranes.

1.3 HYPOTHESIS

It is hypothesized that zinc and taurine will interact in their physiological functions *in vivo*. Marginal zinc deficiency imposed throughout gestation and postnatal life will result in membrane damage and subsequent osmotic stress in cells. These alterations will, in turn, influence pre- and postnatal development

as examined by reproductive outcome, incidence of congenital malformations, and retinal electrophysiology and morphology. Taurine deficiency imposed upon zinc deficiency will worsen these outcomes in a synergistic manner by further increasing the osmotic stress.

1.4 OBJECTIVES

1. To determine whether zinc and taurine interact *in vivo* by examining reproductive outcome, incidence of congenital malformation and retinal electrophysiology and morphology in response to varying zinc and taurine status in the rat.

2. To establish the effect of this interaction on zinc and taurine status in this model.

Chapter 2

REVIEW OF LITERATURE

2.1 ZINC AND TAURINE IN HUMAN HEALTH

2.1.1 Zinc

Although zinc has been recognized as an essential nutrient in animals since the early 1930's (Todd et al. 1934), a role for this metal in human nutrition was not established until 30 years later. Prasad et al. (1963) reported growth retardation, delayed secondary sexual maturation, poor appetite, mental lethargy and skin changes in immature males from Iran and Egypt. These clinical features were corrected by zinc supplementation. Geophagia, parasitic infestation and diets high in phytates with negligible animal protein were thought to have contributed to the severe zinc deficiency (Prasad 1991).

In North America severe zinc deficiency was first recognized in hospitalized patients receiving parenteral nutrition (Arakawa et al. 1976).

Prior to the 1970's, the protein source for these solutions contained zinc as a contaminant. When the protein was changed from hydrolysates to crystalline amino acids, zinc deficiency symptoms developed. Secondary zinc deficiency has also been documented in patients with cystic fibrosis, liver disease, burns and alcoholism. In these states, the deficiency is thought to be the result of increased loss of zinc in urine and gastrointestinal secretions (Aggett and Harries 1979). Some patients with sickle cell anemia have also been reported to suffer from zinc deficiency (Prasad and Cossock 1982). Acrodermatitis enteropathica, a genetic defect resulting in impaired intestinal uptake and transfer of zinc (Barnes and Moynahan 1973) results in severely compromised zinc status.

The effects of a severe deficiency of zinc are dramatic; however, this occurrence in human populations under normal conditions is relatively rare. Marginal zinc deficiency can occur in large groups of people; it is estimated that four million people in the United States fall into this category (Walsh et al. 1994). The most vulnerable groups are those who are experiencing rapid growth, reproduction or new tissue synthesis (Aggett 1995) such as women in late pregnancy, infants and adolescents.

Marginal zinc deficiency is characterized by neurosensory changes, slowing of physical growth, poor appetite and diminished taste acuity (Aggett and Comerford 1995). Behaviour is also affected (Golub et al. 1995a). This condition has been identified in apparently healthy

infants and children in the United States (Hambidge et al. 1972; Walravens and Hambidge 1976; Walravens et al. 1983) and Canada (Gibson et al. 1989; Smit-Vanderkooy and Gibson 1987). Other groups susceptible to marginal zinc deficiency include pregnant women (Solomons et al. 1986) and the elderly (Sandstead et al. 1982). Preterm infants may be particularly sensitive to zinc deficiency due to limited stores of this mineral. Estimates of zinc accretion during human pregnancy indicate that about two-thirds of the zinc in the fetus at term is transferred during the last 10-12 weeks of gestation (Widdowson et al. 1974).

Marginal zinc deficiency is thought to be the result of inadequate intakes and/or poor availability of dietary zinc associated with excessive intakes of dietary fibre, polyphosphates, iron, copper, phytate and calcium (Solomons 1982). Zinc deficiency often accompanies protein/calorie malnutrition (Golub et al. 1995b). Breast milk zinc content is not particularly sensitive to maternal zinc intake (Lönnerdal 1986) but it can be deficient in generally malnourished populations (Lehti 1990). In the elderly, the presence of disease and the use of medications may also be contributing factors (Aggett and Harries 1979).

It is important to note that the diagnosis of zinc deficiency is limited by the lack of a specific and sensitive biochemical index of zinc status. The most reliable method for diagnosing marginal zinc deficiency is a

positive response to zinc supplementation (Gibson 1990).

2.1.2 Taurine

Cats have a restricted ability to synthesize taurine from endogenous precursors and rely on diet to obtain this nutrient (Knopf et al. 1978). A reduction in taurine below 50% of the normal tissue concentration in this species results in retinal degeneration characterized first by a decrease in amplitude of the electroretinogram (ERG) (Berson et al 1976) followed by severe morphological alteration of the photoreceptor cells (Hayes et al. 1975b). Consequently, taurine is considered an essential nutrient for the cat. Whether taurine is essential for human health is less clear.

Adult patients maintained parenterally on protein hydrolysates lacking taurine have reduced plasma (Kopple et al. 1990; Vinton et al. 1986) and urine (Vinton et al. 1990) taurine concentrations. Though significant, these assessments of taurine status do not correlate with abnormal visual function as measured by ophthalmoscopic examination and electroretinography (Vinton et al. 1990). Conversely, children receiving long-term total parenteral nutrition have reduced plasma taurine concentrations accompanied by depressed ERG (Arment et al. 1986; Geggel et al. 1985). These abnormalities were corrected in some of the children when taurine was added to the infusate. Parenterally fed

children may be more susceptible to the effects of taurine deficiency on retinal function due to limited taurine pools (Järvenpää et al. 1982); many of the children studied began total parenteral nutrition shortly after birth and were never fed human milk. Human milk contains substantial amounts of taurine, especially during the first few days after birth (Sturman 1993). It is also possible that the developing retina has a greater requirement for taurine.

The human neonate is dependent on exogenous taurine (Rigo and Senterre 1977). Infants fed taurine-free formula have decreased plasma and urine taurine levels. The addition of taurine to synthetic formula results in tissue taurine concentrations similar to those of infants fed human milk (Rassin et al. 1983). These low taurine levels, however, do not correlate with poorer growth rates (Järvenpää et al. 1982) or impaired bile acid kinetics (Watkins et al. 1983). Preterm infants may be more vulnerable to the effects of low taurine intake due to limited taurine stores (Gaull et al. 1977; Ghisolfi 1987) and poor renal taurine conservation (Zelikovic et al. 1990). For example, preterm infants fed taurine-free diets demonstrate similar growth rates but less mature auditory-evoked responses at 37 weeks postmenstrual age when compared with infants receiving taurine-supplemented formula (Tyson et al. 1989).

Though taurine-free diets do not depress growth or elicit overt pathological changes in the human population, can taurine still be

considered essential? Although somewhat controversial, taurine has been labelled a conditionally essential nutrient for human infants and children, especially those born prematurely (Gaull 1986; Sturman 1988). A conditionally essential nutrient refers to a normally nonessential nutrient which, in some clinical circumstances must be supplied exogenously due to inadequate endogenous synthesis. The changes in ERG and auditory evoked potentials in children on total parenteral nutrition and preterm infants fed taurine-free formula emphasize the need to evaluate nutritional needs by methods other than the presence of clinical symptoms and depressed growth.

2.2 ZINC AND TAURINE IN PRENATAL AND POSTNATAL DEVELOPMENT

2.2.1 Zinc

Adequate maternal zinc nutrition during pregnancy is essential for normal pre- and postnatal development in experimental animals. Severe maternal zinc deficiency in rats results in an increased number of fetal resorptions, malformations, intrauterine growth retardation and poor survival of offspring (Hurley 1981). It has been estimated that up to 90% of the implantation sites can be adversely affected (Hurley 1981; Rogers

et al. 1985). Pups that do survive generally weigh less than controls and have a variety of congenital defects including skeletal abnormalities (Da Cunha Ferreira et al. 1989) and internal soft tissue malformations affecting the brain, heart, lungs and urogenital systems (Hurley 1981; Hurley and Swenerton 1966; Keen and Hurley 1987). Virtually every developing organ system can be affected (Hurley and Mutch 1973). In addition to morphological abnormalities, biochemical and functional abnormalities in the young can also occur as a result of maternal zinc deficiency. These include defects in pancreatic function, lung metabolism and immune competence (Keen and Gershwin 1990; Keen and Hurley 1989)

Marginal zinc deficiency has been found to cause intrauterine growth retardation, as well as delayed skeletal maturation, immune dysfunction and behaviour deficits in rat pups (Peters et al. 1986). Behavioural abnormalities (Golub et al. 1994; Sandstead et al. 1978), reduced birth weights and decreased muscle tone (Golub et al. 1984; Haynes et al. 1985) in the offspring of marginally zinc-deficient rhesus monkeys have also been reported. Additionally, adult rats from marginally zinc-deficient dams suffer from significant learning deficits and inferior working memory (Halas et al. 1986; Hughes and Horsburgh 1982).

In contrast, there is little definitive information pertaining to zinc deficiency in human pregnancy. There is, however, some evidence that

suggests the human is at risk for similar problems. Seven pregnancies in three women with acrodermatitis enteropathica who were not receiving zinc therapy have been reported. Two mothers gave birth to babies with congenital malformations similar to those observed in pups of zinc-deficient rats; one had an anencephalic fetus, the other an achondroplastic dwarf. Two other babies were low birth weight (Hambidge et al. 1975; Verburg et al. 1974). After the introduction of zinc therapy for acrodermatitis enteropathica patients, Brenton et al. (1981) reported that pregnant women with this condition who are able to maintain normal plasma zinc levels via supplementation have normal deliveries and births.

Overt zinc deficiency, as seen in acrodermatitis enteropathica patients, is rare in human populations, but marginal zinc deficiency can occur. A number of studies have suggested a link between congenital malformations, intrauterine growth retardation and maternal zinc status (Swanson et al. 1987). Women with low serum zinc levels in the first trimester have an increased risk of pregnancy complications, including intrauterine growth retardation and increased incidence of congenital malformations (Jameson 1976; Neggers et al. 1990; Soltan and Jenkins 1982). Leucocyte zinc content has been shown to be significantly lower in a group of 44 women delivering small-for-gestational-age infants than in women whose infants were of normal birth weight (Meadows et al.

1981). Previously, Jameson (1976) had reported that in a study of 316 pregnancies, a high proportion (60%) of the women who gave birth to infants with congenital defects had low serum zinc concentrations in the first trimester. Scholl et al. (1993) examined the dietary intake of 818 pregnant girls and women. They found low zinc intake was associated with a two-fold increase in the risk of low birth weight (<2,500 g). The risk of very preterm delivery (<33 weeks gestation) was increased threefold.

In alcoholic mothers, plasma zinc concentrations are inversely related to the incidence of congenital malformations in their babies (Flynn et al. 1981). Similarly, term serum zinc concentrations tend to be lower in mothers who deliver infants with neural tube defects (anencephaly and spina bifida) compared with controls (Buamah et al. 1984; Cavdar et al. 1988). The research described above must be interpreted with caution. Serum zinc concentration is not a specific measure of zinc status (Gibson 1990). The use of this indicator is further complicated by uncertainty regarding normal plasma zinc concentrations at different stages of gestation (Hambidge et al. 1983).

If poor zinc status is associated with complications in human pregnancy, then zinc supplementation should be beneficial to women at risk. In a double-blind randomized trial of oral zinc supplementation in women at risk for delivering small-for-gestational-age babies, 100 mg of zinc citrate reduced the incidence of intrauterine growth retardation and

decreased the number of inductions of labour and Caesarean section deliveries (Simmer et al. 1991). More recently, in a randomized double-blind placebo-controlled trial of 580 African-American pregnant women with low plasma zinc levels, a 25 mg supplement of zinc, resulted in increased birth weights and increased head circumference (Goldenberg et al. 1995).

Maternal zinc deficiency in experimental animals has severe consequences for the developing fetus. Assessing the importance of maternal zinc status as an etiological factor in intrauterine growth retardation or the development of congenital anomalies in the human fetus, however, is much less certain. Further research is required to establish a definitive link between marginal zinc status and poor reproductive outcome in the human population.

2.2.2 Taurine

Taurine or 2-aminoethane sulfonic acid (Figure 2.1) is present in mammalian tissues in high concentrations (Huxtable 1992). Taurine is an amino acid, but because the amino group is on the ß-carbon and the acid group is sulfonic rather than carboxylic, it is not used as a structural unit in protein (Chapman and Greenwood 1988). Instead, it is found primarily as an intracellular free amino acid (Huxtable 1992). For decades, the only known role for taurine was in bile acid conjugation (Schersten 1970).

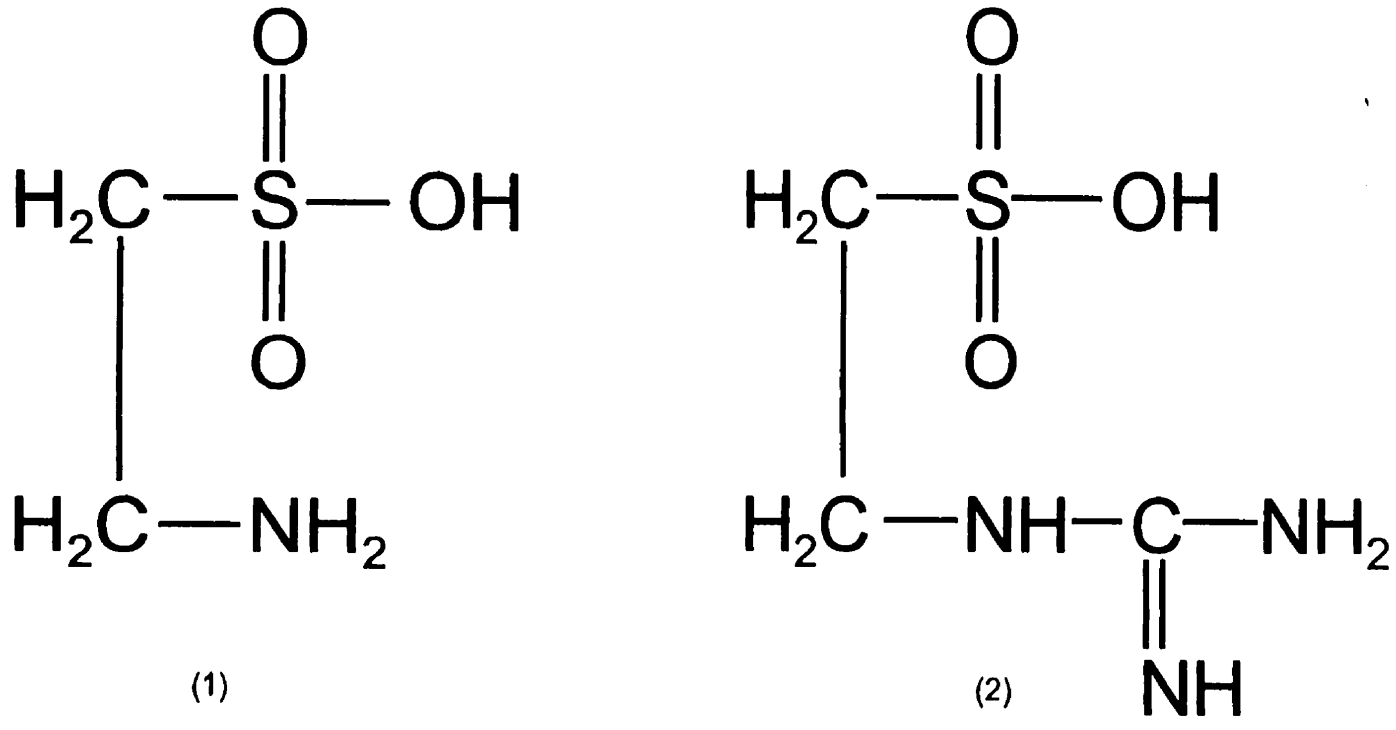

Figure 2.1

Structure of Taurine and Guanidinoethyl Sulphonate

Taurine or 2-aminoethane sulphonic acid (1) and guanidinoethyl sulphonate (GES), a structural analogue of taurine (2).

There is now evidence for its importance in the function of the heart, brain, retina and in reproduction (Huxtable 1992).

Taurine in mammalian tissues is obtained from both exogenous sources and endogenous synthesis (Huxtable 1986). Although several pathways exist, the preferred pathway for taurine biosynthesis is believed to involve oxidation of cysteine to 3-sulfinoalanine with subsequent oxidation and decarboxylation to taurine (Figure 2.2) (Sturman and Hayes 1980). The rate limiting enzyme in the pathway is the vitamin B_6 dependent 3-sulfinoalanine decarboxylase (SAD), also known as cysteine sulfinic acid decarboxylase (Sturman and Hayes 1980). Taurine status is a balance between the amount of taurine added to the body pool (endogenous synthesis and exogenous sources) and the amount removed primarily via urinary excretion.

There is considerable variation among species in their ability to synthesize and conserve taurine (Huxtable and Lippincott 1981). Cats have low SAD activity and limited renal adaptation; therefore, their tissues are readily depleted when fed diets free of taurine (Knopf et al. 1978). Conversely, both diet and endogenous synthesis contribute to the taurine requirements of humans and rats (Huxtable and Lippincott 1981; Huxtable and Lippincott 1982). As described previously, studies of term and preterm infants indicate that the human infant is also dependent on a supply of exogenous taurine (Gaull et al. 1977; Järvenpää et al. 1982;

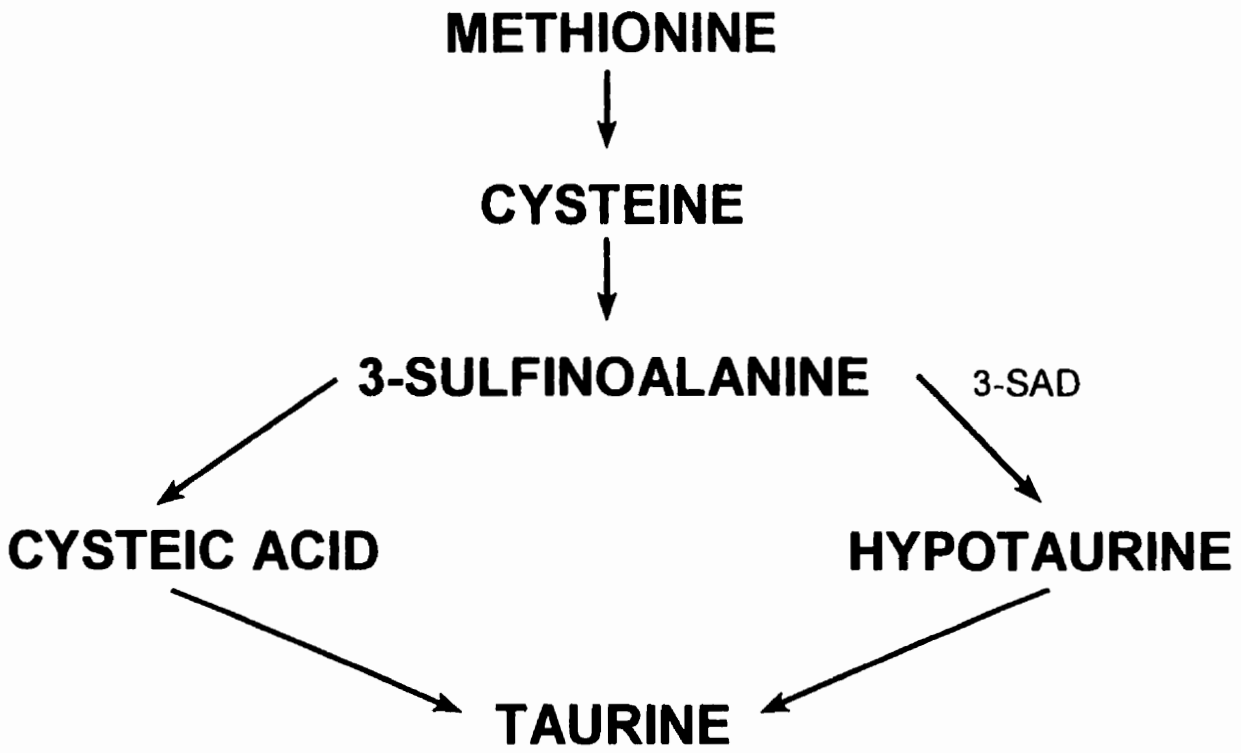

Figure 2.2

Taurine Biosynthesis Pathway

The preferred pathway for taurine biosynthesis is believed to involve oxidation of cysteine to 3-sulfinoalanine with subsequent oxidation and decarboxylation to taurine. The rate limiting enzyme in the pathway is the Vitamin B_6 dependent 3-sulfinoalanine decarboxylase (3-SAD).

Rassin et al. 1983; Rigo and Senterre 1977).

Because the rat has relatively high SAD activity and efficient renal adaptation, their pools are not easily depleted by dietary manipulation (Chesney et al. 1986; Huxtable and Lippincott 1981). Instead, guanidinoethyl sulfonate (GES), a taurine transport inhibitor, can be used to induce taurine deficiency in this species (Figure 2.1) (Huxtable et al. 1979; Ejiri et al. 1987; Pasantes-Morales et al. 1983).

Fetal and neonatal animals are at increased risk for taurine deficiency. They generally have lower SAD activity and less efficient renal adaptation to limited dietary taurine than adult animals (Kuo and Stipanuk 1984). These factors, along with the high concentrations of taurine in fetal and neonatal tissues (Jacobsen and Smith 1968; Sturman and Gaull 1975), suggest that the fetus is largely dependent on the mother for a source of taurine. In fact, 60-70% of the total taurine present in rats pups at birth is derived from the mother's taurine pool (Huxtable and Lippincott 1982; Sturman 1982). By weaning, 3 weeks after birth, the amount of taurine derived from the mother during gestation has dropped to about 5% of the total taurine in the pup. Approximately 10% has been derived from the mother's milk and the remainder has been biosynthesized by the pup (Huxtable 1981; Huxtable and Lippincott 1983).

Fetal and neonatal outcome is compromised by decreased taurine supply. Taurine-deficient queens frequently abort or resorb their fetuses

and have stillborn or live low-birth-weight kittens with decreased tissue taurine concentrations (Sturman and Messing 1991). A number of neurological and morphological abnormalities occur; kittens born to taurine-deficient queens display abnormal hind leg development, thoracic kyphosis (Sturman et al 1985), hydrocephalus (Sturman and Messing 1991) and failure of cells in the cerebellar external granule cell layer to migrate properly (Sturman et al 1985). These kittens have also been reported to exhibit retinal degeneration (Sturman et al. 1986; Sturman and Messing 1991) and abnormal visual cortex development (Palackal et al 1986). The growth of kittens suckling taurine-deficient mothers is also impaired (Sturman 1993).

When GES is administered to rats during pregnancy, the taurine concentrations of fetal tissues (Bonhaus et al. 1985; Ejiri et al. 1987) and placentas (Ejiri et al. 1987) are depressed. This model has been used to study the effects of taurine deficiency on fetal (Ejiri et al. 1987) and neonatal growth (Bonhaus et al. 1985; De la Rosa and Stipanuk 1984); however, it has not been carefully explored as a model to study the other developmental effects reported in the taurine-deficient cat.

When GES was administered to female rats prior to mating and throughout pregnancy, no effect on litter size, birth weight or mortality of the pups during lactation was reported (Bonhaus et al. 1985; De la Rosa and Stipanuk 1984); incidence of congenital malformations was not

assessed. Pre- and postnatal exposure to GES has been used to study the effect of taurine depletion on retinal structure and function. This research demonstrated that morphological changes to the photoreceptors of the GES-induced taurine-deficient rat are similar to those reported in taurine-deficient kittens (Bonhaus et al. 1985; Pasantes-Morales et al. 1983).

2.3 ZINC AND TAURINE IN RETINAL MORPHOLOGY AND PHYSIOLOGY

2.3.1 Retinal morphology and physiology

The retina is a complex photosensory structure consisting of 10 layers (Figure 2.3): (1) retinal pigment epithelium, (2) photoreceptor layer, (3) external limiting membrane, (4) outer nuclear layer (photoreceptor nuclei), (5) outer plexiform layer, (6) inner nuclear layer (nuclei of Müller, amacrine, horizontal and bipolar cells), (7) inner plexiform layer, (8) ganglion cell layer, (9) nerve fibre layer (axons of ganglion cells) and (10) internal limiting membrane (Müller cell processes). The primary function of this layered structure is to transform light stimuli into a form of neural information that can be interpreted by the brain (Pycock 1985).

Eye development begins as an outgrowth of the neural plate

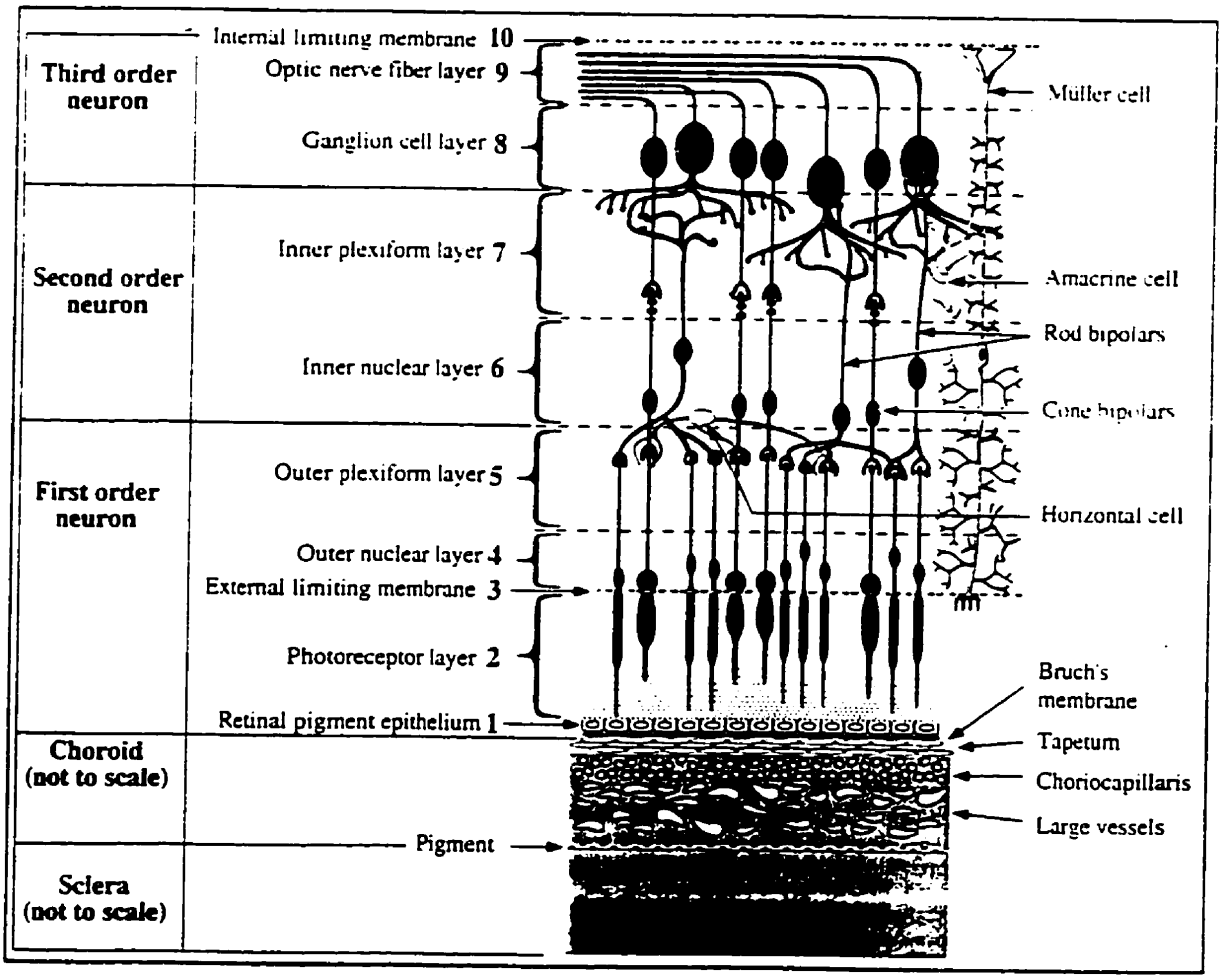

Figure 2.3

Layers of the Retina and Adjacent Choroid

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(Forrester et al. 1996). By the end of the embryonic period the retina can be clearly differentiated into a thin outer layer which will form the retinal pigment epithelium and a much thicker inner neural retina. These two layers are separated by a narrow subretinal space (Forrester et al. 1996). In the albino rat, the retinal layers form in sequence, moving from the inner retinal border outward, always beginning posteriorly and then spreading peripherally. All adult layers are present by eight days after birth and each layer thins after reaching its maximal thickness (Braekevelt and Hollenberg 1970).

The vertebrate retina is inverted, meaning the photoreceptive rod and cone cells lie near the choroid and the optic nerve fibres near the vitreous (Pycock 1985). This places the photoreceptors in close proximity to the choroidal blood supply (Caldwell et al. 1982) but means that light must pass through all retinal layers, except the retinal pigment epithelium (RPE), before reaching the photoreceptors (Pycock 1985).

The rod photoreceptor consists of two segments: a slender rod-like outer segment that contains rhodopsin, linked to a highly metabolic inner segment via a connecting cilium. The cone photoreceptor is similar in structure, one major difference being a conical outer segment that contains no rhodopsin (Pycock 1985; Stryer 1987). This difference allows rod photoreceptors to operate under more scotopic and cone photoreceptors under more photopic conditions. Consequently, the rat

eye, which contains primarily rod photoreceptors, is well adapted for nocturnal vision (Belhorn 1991).

Rod and cone photoreceptors are highly polarized cells that continually renew themselves throughout life. The outer segments contain a stack of light-sensitive double membranes, called discs, that are derived through constant growth of the plasma membrane, from components synthesized in the inner segment (Bok 1985). When older discs are shed, they are taken up by phagocytosis, internalized by the RPE and degraded by lysosomes. Products of this process, in particular retinoids, are shuttled back to the photoreceptors for the regeneration of rhodopsin (Bok 1985).

The most important molecule associated with the disc membrane is rhodopsin (Bok 1985). Rhodopsin is made up of 11-cis retinal, an organic molecule derived from vitamin A, and opsin, a protein with enzymatic capabilities (Stryer 1987). When a single photon of light is absorbed by the 11-cis retinal of rhodopsin, it triggers the enzymatic activity of opsin and sets into motion a biochemical cascade that results in a nerve impulse being transmitted to the central nervous system (Stryer 1987).

Activation of rhodopsin is achieved by isomerization of 11-cis retinal to all-trans retinol (Stryer 1987). When all-trans retinol is subsequently converted to all-trans retinal it no longer fits within the rhodopsin molecule and detaches. In the dark, the detached all-trans

retinal undergoes re-isomerization within the RPE cell to 11-cis retinal, which can reattach to the opsin molecule to regenerate rhodopsin (Forrester et al 1996). The conversion of the energy stored in a single photon of light to an electrical response is possible because of amplification of the cascade involved in closing the sodium channels of the photoreceptor (Forrester et al. 1996).

In the dark state, a continuous electrical current maintains the photoreceptors with a relatively positive charge. When light is absorbed the sodium channels of the receptor membrane are blocked. Rhodopsin activation causes hyperpolarization of the rod membrane, closing of sodium channels and an increased efflux of sodium ions from the photoreceptor (Forrester et al. 1996). The hyperpolarizing response is transmitted along the length of the photoreceptor to the bipolar and horizontal cells, and eventually to the optic nerve. Neurotransmitters are responsible for the relay of this response across the synapse between retinal cells (Forrester et al. 1996).

Retinal neurons conduct their visual signals by electronic conduction which is a direct flow of electrical current not action potentials (Guyton and Hall 1996). The process allows graded conduction which means the hyperpolarizing output signal of the retina is directly related to the intensity of illumination (Guyton and Hall 1996). This electrical current can be measured by electroretinography. The resulting ERG

waveform is comprised of an a-wave, produced when light strikes rhodopsin in the photoreceptor outer segments, and a b-wave generated at a more proximal location, possibly in the Müller cells of the inner nuclear layer (Berson 1992). The a-wave is the first ERG component that can be detected in the neonatal rat; its amplitude plateaus by postnatal day 30. The b-wave appears later and reaches maximal amplitude by six weeks postnatal age (Grün 1982).

Oscillatory potentials (OP) are an additional electrophysiological parameter that can be measured in the retina. These waveforms represent a series of rhythmic consecutive discharges of retinal neurons found superimposed on the b-wave of the ERG (Speros and Price 1981). It is thought that these oscillations are generated independently from the mechanism producing the primary components of the ERG, the a- and b-waves, at a more proximal location, possibly the amacrine cells of the inner nuclear layer (el Azazi and Wachtmeister 1990). In the neonatal rat, OP are detected after the a- and b-waves appear but achieve maximal amplitude at approximately the same postnatal age as the b-wave. The retina is not considered mature until approximately 6 weeks postnatal age (Braekevelt and Hollenberg 1970).

2.3.2 Zinc and retinal function

Ocular tissues contain substantial amounts of zinc (Eckhert 1983;

Karcioglu 1982). In particular, zinc has been found concentrated in the retina associated with photoreceptors (Hirayama 1990; Wu et al. 1993), the tapetum lucidum (Kohler 1981; Sturman et al. 1981) and retinal pigment epithelium (Ulshafer 1989; Ulshafer et al. 1990). In photoreceptors of Wistar rats fed zinc-adequate diets, histochemical localization by dithizone revealed the presence of zinc in outer segments. The photoreceptors from animals fed zinc-deficient diets displayed a weaker reaction suggesting a decreased zinc content (Hirayama 1990). No reaction was observed in other ocular tissues. Neo-Timm staining of zinc in the retinas of larval tiger salamanders revealed the heaviest concentrations in the outer nuclear layer (Wu et al. 1993). Histochemical localization of zinc in the tapetum lucidum of the cat, shows it to be present on the periphery of the tapetal rods (Kohler 1981; Sturman et al. 1981) situated between the tapetal rod and tapetal rod membrane (Wen et al. 1982).

Pigmented ocular tissues such as the iris and RPE have been reported to contain especially high concentrations of zinc (Eckhert 1983). Isolated human and intact rat RPE cells have also been reported to contain metallothionein (Nishimura et al. 1991; Oliver et al. 1992; Tate et al. 1993), a protein thought to play a major role in intracellular zinc metabolism (Bremner 1993). Energy dispersive x-ray microanalysis of human (Ulshafer 1989; Ulshafer 1990) and porcine (Samuelson et al.

1993) RPE cells localize much of this zinc in melanin granules. The zinc content of RPE cells can be affected by exogenous zinc intake. Pigs maintained on suboptimal zinc diets exhibited a trend of decreasing levels of zinc in the RPE when compared with zinc-adequate controls. These changes are most pronounced in the melanosomes (Samuelson et al. 1993). Though melanin can not account for all zinc present in isolated RPE cells (Newsome and Rothman 1987), suggesting other possible sites of accumulation, it is thought to influence general zinc uptake and accumulation in this cell layer (Newsome et al. 1992).

Feeding zinc-deficient diets has been shown to affect the amount of zinc localized in rat photoreceptors (Hirayama 1990) and porcine RPE cells (Samuelson et al. 1993). What effect does this apparent depression have on the structure and function of the human retina? Patients with alcohol-induced cirrhosis frequently have elevated dark adaptation thresholds that can be corrected by zinc supplementation (Morrison et al. 1978; Russell et al., 1978). Karcioglu et al. (1984) described depressed plasma zinc concentrations in patients with retinitis pigmentosa, a disease characterized by RPE changes and a reduced (ERG) response (Berson 1992). Plasma zinc concentrations in patients receiving total parenteral nutrition have been shown to correlate positively with visual function (Vinton al. 1990). Photoreceptor loss and degeneration of the RPE have been reported in patients with AE (Cameron et al. 1986).

Recent research has focused on the possible involvement of zinc in age-related macular degeneration (AMD), a condition known to affect the sensory retina and RPE (Newsome et al. 1988). Patients with this degenerative eye disease have significantly lower plasma zinc concentrations when compared with age-matched controls (Wu et al. 1994). In a heterogeneous group of patients with AMD, an oral supplement of 200 mg zinc sulphate provided daily for a two-year period was found to increase serum zinc and significantly reduce visual loss (Newsome et al. 1988). In contrast, a follow-up study in a homogeneous group of patients with AMD showed no improvement in functional eye tests in response to the same dose of zinc (Stur et al. 1996). Conclusions from these studies are limited based on the short-term nature of these trials. The Eye Diseases Case-Control Study Group (1988) was also unable to demonstrate a positive relationship between serum zinc levels and incidence of AMD; however, this finding may be of limited significance as serum zinc concentration is not a sensitive or specific indicator of zinc status (Gibson 1990). Interestingly, the concentration of zinc in retina but not plasma in Cynomolgus Monkeys, a primate with early onset AMD, was found to be significantly depressed when compared with unaffected controls (Nicolas et al. 1996).

DeVirgiliis et al. (1988) reported retinal abnormalities characterized by decreased ERG amplitudes and defective dark adaptation in

thalassaemia patients treated with desferrioxamine (DeVirgiliis et al. 1988). Depressed granulocyte zinc and alkaline phosphatase activity accompanied by increased faecal zinc excretion was also observed. Dithizone administration produced densely staining inclusion bodies in the RPE of Sprague-Dawley rats (Leure-duPree 1981), possibly through the sequestration of zinc. Whether these observations are due solely to the poor availability of zinc is questionable as both compounds can chelate other minerals.

It has been hypothesized that zinc is involved in the maintenance of normal plasma transport of vitamin A. Severe zinc deficiency in rats results in a decreased concentration of vitamin A in plasma with a parallel reduction in retinol-binding protein, despite adequate liver vitamin A stores (Smith et al. 1974). When zinc concentrations in ocular tissues are reduced by feeding a zinc-deficient diet, retinal alcohol dehydrogenase is also significantly lowered (Huber et al. 1975). The authors speculated that this change in alcohol dehydrogenase activity could significantly reduce the conversion of retinal to retinol.

Leure-DuPree and Bridges (1982) fed Sprague-Dawley rats diets containing 0.7 ppm zinc. They reported decreased growth rate, dermatological changes and depressed tissue zinc concentrations when compared to zinc-adequate controls. As well, irregularly shaped osmiophilic inclusion bodies similar to those seen in the RPE of rats

administered zinc chelators (Leure-duPree 1981) and photoreceptor degeneration, were apparent. Based on the proportions of retinol and retinyl esters found in the zinc-deficient and control eyes, they speculated that the zinc-deficient rats were able to form retinol from the retinal that is released when rhodopsin is bleached. Rhodopsin regeneration in zinc-deficient and pair-fed rats has been reported to be almost half that found in *ad libitum* fed controls (Dorea et al. 1986), suggesting that depressed feed intake and not zinc deficiency, per se, depresses the synthesis of rhodopsin.

The studies described above suggest that zinc may be important in retinal health. Unfortunately, these observations can not be ascribed solely to zinc status. Therefore, it is important to determine whether zinc deficiency, in the absence of possible secondary effects, can alter the structure and function of the retina. Feeding 0.5 µg Zn/g diet (severe zinc deficiency) to Long Evans rats throughout gestation produced microphthalmia in 38% of the fetuses examined at day 21 (Rogers et al. 1984). Examination of fetuses from zinc-deficient dams, at day 12 and 14 of gestation revealed that invagination of the optic cup was often deficient and that closure of the choroid fissure did not occur, resulting in retinal folding visible at term (Rogers et al. 1987). No incidence of microphthalmia was observed in rats fed 4.5 (marginal zinc deficiency) or 100 (control) µg Zn/g diet (Rogers et al. 1984). When zinc deficiency was

instituted at parturition. eye opening was delayed but no morphological differences were noted among photoreceptor cells at weaning (Sinning et al. 1984). In this study, zinc deficiency appeared to have a minimal effect on the topography of developing photoreceptor cells; however, internal morphological alterations may have been present.

When weanling male Sprague-Dawley rats were maintained for 7 weeks on a diet containing 0.7 µg Zn/g diet (severe zinc deficiency), they showed markedly retarded growth, dermatologic signs characteristic of zinc deficiency and depressed serum and femur zinc concentrations. Electron microscopic examination of the retina revealed vesiculation and degeneration of the photoreceptor outer segments and accumulation of osmiophilic inclusion bodies in the RPE. The osmiophilic inclusions are morphologically identical to those seen in the RPE of rats administered dithizone and 1,10-phenanthroline, known zinc chelators (Leure-duPree 1981). Retinal tissue was normal in pair-fed and weight-paired control rats (Leure-duPree and McClain 1982).

The severe deficiency of zinc described above appears to have dramatic effects on the eye. This occurrence in human populations under normal conditions is relatively rare. Marginal zinc deficiency has been reported in large numbers of the population (Walsh et al. 1994) and has not been extensively explored in relation to eye structure and function. Cats fed diets containing less then 7 ppm zinc for 4 months, gained less

weight and experienced depressed muscle zinc concentrations. These observations were accompanied by changes in retinal function measured by a reduction in the electroretinogram b-wave amplitude that was reversible upon zinc repletion (Jacobson et al. 1986). These results were not consistent in all animals studied. Samuelson et al. (1991), in an attempt to model human zinc deficiency, fed pigs diets marginally deficient in zinc for a 12 month period. Histological examination of their retinas revealed a decrease in cone photoreceptors and an increase in phagosomes and residual bodies in the retinal pigment epithelium that appeared to progress over time. No measurements of tissue zinc levels were provided.

These studies provide additional evidence that zinc plays an important role in retinal health. It appears that feeding diets deficient in zinc can affect the structure and function of the retina; however in most studies reported, zinc concentration was never measured in ocular tissues. That zinc plays an important role in the morphology and physiology of the retina is certain; what this exact role is has yet to be confirmed.

2.3.3 Taurine and retinal function

The retina contains a high concentration of taurine (24 μmol/g wet weight). In fact in some animal species the taurine concentration in the

retina is the highest of any other tissue (Cohen et al. 1973; Gupta et al. 1981; Heinämäki 1986). In the rat retina, taurine is the most abundant free amino acid comprising more than 50% of the total amino acid pool (Heinämäki 1986; Macaione et al. 1974). Within the retina taurine appears to be unevenly distributed and is concentrated in the photoreceptor layer (Orr et al. 1976; Voaden et al. 1977; Fletcher and Kalloniatis 1996).

A high degree of immunostaining, reflecting the presence of taurine, was observed in photoreceptor inner segments and synaptic terminals. Amacrine and bipolar cell bodies, including their synaptic processes in the inner plexiform layer, are also high in taurine content (Fletcher and Kalloniatis 1996). However, the pigment epithelium and distal parts of glial cells have much reduced immunostaining (Lake 1989; Lake and Verdone-Smith 1989). In the retina of the immature rat (postnatal day 14), taurine-like immunoreactivity was observed primarily in the photoreceptor layer (Lake 1994). However, in the mature rat retina (postnatal week 12), the cellular location of taurine-like immunoreactivity was found to be the opposite, present primarily in the inner segments of the photoreceptor cell layer but with only weak reactivity observed in the outer plexiform layer (Kuriyama et al. 1982; Fletcher and Kalloniatis 1996).

The taurine content of the mammalian retina changes with age.

This is due to photoreceptor development being predominantly postnatal and the concentration of taurine increasing in parallel with the maturation of these cells (Gupta et al. 1981; Heinämäki et al. 1986). For example, in the retina of the rat taurine levels increase dramatically until approximately day 30 of life (Macaione et al. 1974). The taurine levels then decrease between 3 and 6 months of age (Baskin et al. 1976; Baskin et al. 1977). Retinal maturity in primates, including man, occurs earlier than in the rat (Pycock 1985).

Taurine appears to be important for the structure and function of the mammalian retina (Lombardini 1991). Most evidence for this role comes from studies of taurine-deficient animal models. Hayes et al. (1975a) reported that cats fed a semipurified diet containing casein developed retinal degeneration visible on ophthalmoscopic examination. It was later determined that this degeneration was due to taurine deficiency (Hayes et al. 1975b). Cats have a restricted ability to synthesize taurine from endogenous precursors and rely on diet to obtain this nutrient (Knopf et al. 1978). A reduction in taurine below 50% of the normal tissue concentration results in retinal degeneration characterized first by a decrease in amplitude of the electroretinographic response (Berson et al. 1976) followed by severe morphological alteration of the photoreceptor cells (Hayes et al. 1975b). The otherwise highly ordered structure of membranous discs in the outer segments becomes

disarranged; the discs appear extremely disoriented, with twisting often extending through the entire length of the segment (Berson et al. 1976). As the pathology progresses, the entire photoreceptor cell population degenerates and eventually blindness occurs. Electron microscopy also reveals various degrees of disorganization in the tapetum lucidum (Sturman et al. 1981).

Retinal pathology associated with depleted taurine has also been reported in the albino rat. Treatment of rats with GES leads to a 30-40% decrease in the taurine content in the retina (Lake 1981; Lake 1982; Quesada et al. 1984). Recently Lombardini et al. (1996) reported an 80% decrease after extensive, long-term treatment with this analogue. As a consequence of this reduction in endogenous taurine, the characteristic pattern of retinal degeneration appears in rats. The properties are similar to those observed in cats, including reduced electroretinographic response (Cocker and Lake 1987; Hageman and Schmidt 1987; Lake 1986; Lombardini et al. 1996; Rapp et al. 1987) and a structural degeneration of the photoreceptors (Lake and Malik 1987; Pasantes-Morales et al. 1983).

Damage to the photoreceptors includes a reduction in the size of the layer, disarray of the discs and disc membranes, swollen inner segments and nuclei and accumulation of vesicular membrane-bound profiles within the basal region of the photoreceptors (Hageman and

Schmidt 1987; Lake and Malik 1987; Pasantes-Morales et al. 1983; Rapp et al. 1988). The rat eye does not contain tapetal cells. Subsequent research has shown that retinal taurine concentrations and ERG b-wave amplitudes are significantly increased when GES is discontinued and replaced with taurine in drinking water (Lombardini et al. 1996). The recovery implies that cell death or permanent function deficits were not obvious even after 16 weeks of GES treatment.

While it is well documented that taurine depletion produces morphologic changes, such as photoreceptor cell loss in the retina of the albino rat, similar changes are not observed in the retina of the pigmented rat, although a reduction in ERG amplitude does occur (Rapp et al. 1987). Thus, it has been suggested that in the albino rat light may increase the degenerative changes induced by taurine depletion (Lake and Cocker 1986; Lake and Malik 1987; Rapp et al. 1987). This theory has also been proposed to explain the unusual distribution of lesions in taurine deficiency retinopathy in the cat; however, unilateral tarsorrhaphy experiments exclude light damage as a major factor (Leon et al. 1995).

As has been described previously, fetal and neonatal animals may be at an increased risk of taurine deficiency. Kittens born to taurine-deficient mothers have abnormal visual cortex development, with neuroblasts failing to migrate properly (Palackal et al. 1986), photoreceptor outer segments that are reduced in length, and tapetal

cells distinguished by accumulations of electron-dense droplets (Imaki et al. 1986). Dietary taurine deficiency imposed on developing kittens restricts the normal developmental increase in retinal taurine concentration (Sturman 1993) promoting this photoreceptor degeneration (Imaki et al. 1986). Treatment of rats with 1% GES throughout gestation produces a degeneration of the photoreceptor layer of the retina in pups, similar to that observed in kittens (Bonhaus et al. 1985). Pups of nursing mothers treated with GES have retinal levels of taurine that are 50-70% of control values (Lake 1983).

In the infant rhesus monkey, taurine deficiency produced by feeding a taurine-free infant formula also produces retinal abnormalities (Lombardini 1991). The outer segments of photoreceptors are swollen or vacuolated in the case of rods and shrunken and condensed in the case of cones. At the ultrastructure level, cone outer segments appear shortened or retracted. In addition, cone ERG are depressed and a loss of visual acuity is apparent (Imaki et al. 1987; Neuringer et al. 1987a; Neuringer et al. 1987b; Sturman et al. 1984). A decrease in plasma (Neuringer et al. 1987b; Sturman et al. 1984) and retinal taurine concentrations are also noted (Sturman et al. 1988; Sturman et al. 1991). When maintained on this diet no significant difference in plasma and retinal taurine concentrations are apparent by 4 years of age; however, some photoreceptor damage persists suggesting that vulnerability to

taurine deficiency may be related to retinal maturity (Imaki et al. 1996).

Human infants and children fed totally by parenteral nutrition which was lacking in taurine also developed low plasma taurine levels accompanied by reduced amplitudes in the ERG and morphologic changes observed by ophthalmoscopy (Ament et al. 1986; Geggel et al. 1982; Geggel et al. 1985; Vinton et al. 1985). These abnormalities were corrected in some of the children when taurine was added to the infusate suggesting an exogenous requirement for taurine in this population.

These studies are indicative that taurine plays an important role in retinal health. It appears that inadequate taurine intake can affect the morphology and physiology of both the adult and developing retina. However, the exact mechanism responsible for the function of taurine has yet to be elucidated.

2.4 INTERACTION BETWEEN ZINC AND TAURINE

2.4.1 Biochemical functions of zinc

The biochemical basis for the physiological functions of zinc have been studied extensively (Vallee and Falchuk 1993). However, the clinical signs of altered zinc status are diverse and can not be adequately explained by the defect of a specific known function (Cousins 1996). For

example, zinc is known to be an essential component of over 300 enzymes (Vallee and Falchuk 1993). In spite of this, there is no clear evidence that the activity of any one limits physiological function during nutritional deprivation of zinc. Therefore, two additional hypotheses have been proposed to explain zinc deficiency pathology. One suggests that zinc exerts its critical role in gene expression (Hanas et al. 1983). The other proposes that zinc exerts its function at the level of the cell plasma membrane (Bettger and O'Dell 1981). Many nucleoproteins directly involved with replication and transcription of DNA are now known to contain functionally important zinc atoms (Berg and Shi 1996). However, there is little evidence that altered gene expression during dietary zinc deficiency significantly affects physiological outcome (Cousins 1996). There is mounting circumstantial evidence that zinc deficiency affects the structure and function of the plasma membrane (Bettger and O'Dell 1993). Because zinc is able to form protein-stabilizing cross-links without introducing undesirable chemical reactivity (Berg and Shi 1996), it may have a direct effect on the conformation of individual proteins and on protein-protein interactions (Bettger and O'Dell 1993).

Zinc is found in high concentrations in many cell membrane fractions (Vallee 1990) including those from bovine rod outer segment proteins (Tam et al. 1976). Though the precise locations are unknown, it is probable that zinc is located on the outer surface, within the aqueous

domain or channels and on the inner surface of the cell plasma membrane, bound to protein (Bettger and O'Dell 1993). The *in vitro* addition of zinc to plasma membranes provides evidence of these locations. Zinc has been shown to promote the binding of growth hormone to the cell surface prolactin receptor (Cunningham et al. 1990), to bind deep within the pores of a calcium channel in myotubes (Winegar and Lansman 1990) and to promote the binding of protein kinase C to receptor sequences on membrane skeletal proteins (Zalewski et al. 1990).

The physiological role of zinc in retinal membranes may involve similar mechanisms. It has been demonstrated that zinc stimulates the binding of azido-[α-^{32}P]ATP to the rim protein, a 240 kDa spectrin-like protein and a 220 kDa glycoprotein, in rat rod outer segment preparations (Shuster et al. 1988). Zinc has also been shown to bind directly and specifically to purified disc membranes (Shuster et al. 1992). In both studies, the investigators suggested that zinc may elicit a conformational change in specific proteins.

Research using zinc-deficient animal models contributes additional evidence that this metal is involved in the structure and function of biomembranes. Increases in fluidity of the lipid bilayer (Jay et al. 1987) and increased mobility of spin probes in protein (Kubow and Bettger 1988) and sialic acid residues on the cell surface (Jay et al. 1987) have

been reported in erythrocytes from zinc-deficient rats. Consequently, these cells have increased osmotic fragility (O'Dell et al. 1987) and sensitivity to haemolysis by sodium dodecyl sulfate (Paterson and Bettger 1985). Electron microscopic examination of the retina of severely zinc-deficient rats revealed degeneration of the photoreceptor outer segments (Leure-duPree and McClain 1982). Similar changes were observed in the retinas of marginally zinc-deficient pigs (Samuelson et al. 1992). The fragility of the outer segments observed in these models may be the result of zinc loss from membrane proteins.

2.4.2 Biochemical functions of taurine

Taurine is an essential nutrient for cats. Dietary deficiency in this species affects the functioning of the visual, reproductive, immune and cardiovascular systems (Sturman 1993). Taurine is also considered essential for primates during development; changes in retinal physiology associated with low intake have been reported in children receiving taurine-free parenteral nutrition (Arment et al. 1986; Geggel et al. 1985) and in rhesus monkeys raised on taurine-free formula (Imaki et al. 1987; Neuringer et al. 1987a). Treatment of rats with GES produces similar retinal changes (Cocker and Lake 1987; Rapp et al. 1987). Despite the mounting evidence that taurine plays an important role in retinal physiology, especially during development, the underlying mechanism is

not known (Lombardini et al. 1996).

A number of hypotheses have been proposed to explain the biochemical basis for taurine in this tissue. One possible function is in membrane stabilization (Pasantes-Morales et al. 1987a). Taurine may directly affect the physical state of membrane lipids possibly through its role as an antioxidant (Huxtable 1992; Schaffer et al. 1995). Alternatively, taurine may elicit a conformational change in membrane proteins which may explain its role in membrane binding and transport of Ca^{2+} (Lombardini 1991; Huxtable 1992). A second possible function for taurine in the retina is as an inhibitory neurotransmitter (Lombardini 1992) or as a regulator of intracellular signal transduction (Lombardini 1991). It is now known that phosphoproteins along with necessary protein kinases and/or protein phosphatases are enriched in the nerve terminal and thus synaptic transmission may be modulated or regulated by phosphorylation processes (Lombardini and Props 1996; Schaffer et al. 1995). Taurine has also been reported to stimulate rod differentiation when added to retinal cultures, perhaps as a mediator of intercellular signalling among developing retinal cells (Altshuler et al. 1993). A third possible function for taurine in the retina is as an osmoregulator (Pasantes-Morales and Cruz 1984b). Osmoregulation is the primary function of taurine in invertebrates and fish; there is increasing evidence that this is also an important role for taurine in mammals (Huxtable 1992).

Taurine meets the requirements for a biological osmoregulator almost ideally. It is transported by a system unique to amino acids, the transport is Na^+ dependent and responsive to other osmotic substances (Salceda and Saldaña 1993). Extremely high intra- to extracellular concentration gradients can be maintained due to its lipophobic properties. Additionally, the use of taurine as an osmoregulator "spares" metabolically important amino acids for protein synthesis (Huxtable 1992). Osmotic regulation by organic osmolytes such as taurine typically contribute 10-20% to the total intracellular osmolarity (Huxtable 1992). It is thought that taurine fulfills this role by modification of the movement of other osmotically active substances, such as ions or water (Huxtable 1992).

Schulze and Neuhoff (1983) reported that the loss of taurine from isolated retinas varies inversely with osmolarity. The greater the osmolarity the lower the efflux from this tissue. When primary cultures of human lens and RPE were exposed to hypertonic medium, the concentration of taurine was increased by 218% and 147%, respectively suggesting that taurine may have an osmoregulatory role in these tissues (Yokoyama et al. 1993). Further evidence is derived from research on isolated frog rod outer segments. Exogenous taurine has been shown to provide protection *in vitro* when these cells are subjected to light-induced peroxidative damage (Pasantes-Morales and Cruz 1984b). The authors

suggested that the protective effect of taurine may be due to an interaction with ion permeability and/or water accumulation in the membrane (Pasantes-Morales and Cruz 1984b).

Though depressed ocular taurine results in osmotic disturbances, it is not clear if these effects occur due to a loss of osmoregulatory function or to some other mechanism. It is important to note that disturbed ion-water balance can occur secondary to the other mechanisms described above. For example, a loss of membrane integrity can ultimately have this same effect.

2.4.3 Evidence for interaction

Reports that the nutrients zinc and taurine interact have prompted interest in the physiological significance and mechanisms responsible for this phenomenon (Pasantes-Morales et al. 1987a). The strongest evidence for an interaction comes from *in vitro* research on isolated frog rod outer segments. When these cells are exposed to ferrous sulfate they display extensive disruption of their structure, characterized by acute swelling and disc membrane disorganization. The addition of zinc or taurine alone to the culture medium is ineffective in protecting against this outer segment damage; however, zinc and taurine, added together, provide protection in a synergistic manner (Pasantes-Morales and Cruz 1984). Zinc and taurine do not prevent iron-induced lipid peroxidation but

appear to protect the outer segments from the subsequent disturbance in osmoregulation.

Whether zinc and taurine interact *in vivo* is unknown. A 50% reduction of retinal taurine in the cat produces degeneration of the photoreceptors (Hayes et al. 1975b) and depression of electroretinogram (ERG) a- and b-wave amplitudes (Berson et al. 1976). The addition of 200 μM zinc to the drinking water of these animals has been reported to partially prevent depression of the a-wave amplitude in some animals (Pasantes-Morales et al. 1987b) suggesting that zinc may influence taurine in the retina.

The relationship between zinc and taurine has also been explored in other tissues. Taurine-deficient cats exhibit disruption and disorganization of the membrane surrounding the tapetal rods with a subsequent loss of localized zinc (Sturman et al. 1981). Cultured human lymphoblastoid cells are protected from retinol-induced injury in the presence of zinc and taurine; however, the protection afforded by the addition of these nutrients is not synergistic (Pasantes-Morales et al. 1984). Urinary excretion of taurine (Anthony et al. 1971; Hsu and Anthony 1970) and plasma taurine concentrations (Griffith and Alexander 1972) are increased in zinc-deficient weanling rats. Interest in zinc and taurine in the human neurological disorders of epilepsy (Barbeau and Donaldson 1974) and Friedreich's Ataxia (Shapcott et al. 1984) has also

been reported.

2.4.4 Mechanism for interaction

The mechanism by which zinc and taurine may interact is unclear. It has been suggested that zinc-taurine complexes exist in living organisms (Sakurai and Takeshima 1983). Van Gelder (1983) proposed that zinc-taurine complexes modulate calcium movement in the central nervous system. Whether this is of physiological significance is not known. Direct association between these nutrients, however, appears unlikely due to the reported low log stability constant of a zinc-taurine complex of 4.6 (Wright et al. 1986). It is also unlikely that taurine alters the cellular availability of zinc as it is unable to displace zinc from albumin (Harraki et al. 1994). An alternative hypothesis proposes that zinc and taurine interact with cell membranes to provide a stabilizing effect, possibly through regulation of cell permeability (Pasantes-Morales et al. 1987a). This is thought to be a more viable theory and provides the basis for the work described herein.

Zinc has been proposed to play a physiological role in plasma membranes by its effects on membrane protein conformation and protein-protein interactions (Bettger and O'Dell 1993). While the precise biochemical mechanisms have not been fully elucidated, it has been suggested that the loss of zinc from specific proteins in the plasma

membrane alters water and ion channels. Ultimately intracellular ion and water concentration is changed (Bettger and O'Dell 1993). In its function as an intracellular osmoregulator (Huxtable 1992), taurine may be able to respond to these changes by regulating water and ion flow across cell membranes.

Chapter 3

OVERVIEW OF EXPERIMENTAL SECTIONS OF THESIS

The objective of this research was to explore the hypothesis that zinc and taurine interact in physiological functions *in vivo*. To carry out this work, a sensitive method was required to analyse taurine concentrations in biological tissues. **Chapter 4** describes the modification and validation of an analytical method for the HPLC analysis of taurine in biological tissues that is rapid, sensitive and reliable.

Initially, reproductive outcome and incidence of congenital malformations in the rat were to be used as physiologic measures of this interaction. These outcomes had been studied extensively in the zinc-deficient rat (Keen and Hurley 1989). While tissue taurine concentrations were known to be depressed when 1% GES, a taurine transport antagonist, was supplemented orally in this species (Huxtable et al. 1979), the effect of this decrease on fetal development had not been carefully explored. Additionally, it was not known if other doses of

this analogue would achieve similar outcomes. Therefore, a study was designed to assess pregnancy outcome in the rat in response to varying taurine status. The results of this investigation are presented in **Chapter 5**.

Based on the results of this work it was evident that taurine deficiency in the rat, as assessed by evaluation of fetal weight and incidence of congenital malformations at day 20 of gestation, was not similar to what had previously been observed in the taurine-deficient cat (Sturman et al. 1985; Sturman and Messing 1991). Therefore, it was concluded that incidence of fetal malformations might be an inadequate indicator with which to explore the proposed interaction between zinc and taurine *in vivo*.

Alternatively, the high concentration of zinc (Eckhert 1983) and taurine (Heinämäki et al. 1986) in ocular tissue and the purported importance of these nutrients in maintaining eye structure and function (Leure-duPree and McClain 1982; Sturman et al. 1986), suggested that the eye may be a unique physiological system in which to study this interaction. Therefore, a pilot study was designed to assess whether pre- and postnatal development of eye structure and function would be a more sensitive endpoint for demonstrating an interaction between zinc and taurine *in vivo*. These experiments are presented in **Chapter 6**.

Chapter 7 and **Chapter 8** present the results of subsequent research on retinal morphology and function in which a pre- and postnatal rat model was used to study the physiological interaction between zinc and taurine.

Chapter 4

MODIFICATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE HPLC ANALYSIS OF TAURINE

4.1 INTRODUCTION

Several methods are available for the analysis of taurine in biological fluids and tissues. Colorimetric (Anzano et al. 1978) and fluorometric (Yoneda et al. 1977) assays have been developed; however, these methods usually require time-consuming pretreatment of the sample and are less sensitive. Use of gas chromatography for taurine analysis, a more sensitive technique, has been confounded by the difficulty in preparing a suitable volatile derivative of this compound (Kataoka et al. 1984). Other chromatographic procedures utilizing an amino acid analyser or high performance liquid chromatography (HPLC) have also been developed (Lippincott et al. 1988; Yokoyama 1991); however, many use gradient elution to resolve taurine from other compounds in biological samples and, as such, require long run times making them impractical

for the measurement of a single amino acid. This chapter describes the modification of an analytical method for the HPLC analysis of taurine levels in tissues, that is rapid, sensitive and reliable.

Taurine was analysed by reverse-phase high performance liquid chromatography (HPLC) using precolumn derivatization with orthophthal-aldehyde (OPA) followed by electrochemical detection. This method is a modification of that reported by Donzanti and Yamamoto (1988). This procedure, which had originally been used to quantitate taurine concentrations in discrete brain areas, was adapted to analyse taurine levels in whole brain, liver, plasma, urine and eye. The chromatographic conditions were retained. Modifications involved changes to the tissue extraction procedure that were necessary to clean the samples prior to injection in the HPLC system. These included the use of a precolumn and the filtering of each sample prior to derivatization. The objective of this study was to validate this analytical method for precision, selectivity, linearity, accuracy and stability (Buick et al. 1990; Karnes et al. 1991).

4.2 MATERIALS AND METHODS

4.2.1 Chromatography

The HPLC system consisted of a Model 510 pump (Waters,

Mississauga, ON), with an SSI model LP-21 pulse dampner (Ranin Instruments Co., Woburn, MA) installed between the pump and Rheodyne 7125 injector (Cotati, CA). A 20 µL sample loop was used for all analyses. Chromatographic separation was accomplished using a 3 µm C18 HR-80 reverse-phase analytical column (8 cm X 4.6 mm i.d.; ESA Inc., Bedford MA) and a mobile phase of 0.1 mol/L sodium phosphate dibasic (Na_2HPO_4), 0.13 mmol/L disodium ethylenediamine tetraacetate (Na_2EDTA) and 28% methanol. The mobile phase was adjusted to pH 6.00 using 85% orthophosphoric acid and pumped at a rate of 1.2 mL/min. An uptight pre-column model C-135B (SPE Ltd., Concord, ON) preceded the analytical column. A high sensitivity analytical cell (Model 5011; ESA Inc., Bedford, MA) completed the system. A Shimadzu Model C-R3A integrator (Fisher Scientific, Edmonton, AB) or personal computer running Millennium software (Waters, Mississauga, ON) was used to capture the data. A model 5100A Coulometric electrochemical detector (ESA Inc., Bedford, MA) was used to monitor column effluent. The working electrode potentials for the coulometric-amperometric analytical cell were -0.4 V and +0.6 V, respectively. The guard cell potential was set at -0.7 V and the detector response time at 4 sec. To maintain baseline stability, mobile phase was recirculated for a minimum of 15 hours prior to tissue taurine analysis and discarded after a maximum 5 days of analysis.

4.2.2 Tissue extraction

All tissues were stored at -70°C prior to taurine analysis. Brain, eye, plasma or urine was homogenized in cold 0.5 mol/L perchloric acid and liver homogenized in cold 1.0 mol/L perchloric acid. Maternal brain was diluted 1:20 prior to analysis. Similarly, fetal brain, fetal liver, maternal liver and pup eyes were diluted 1:30; urine was diluted 1:50 and plasma 1:2. The samples were then centrifuged at 12,000 x g and 5°C for 30 minutes. The supernate was removed and filtered through 0.45 µm syringe filters. The resulting samples were stored at 4°C until analysis. Samples were analysed within 5 hours of extraction.

4.2.3 Derivatization procedure

The stock derivatization solution was prepared by dissolving 13.5 mg of OPA in 0.5 mL of methanol, followed by 2.5 µL of BME and 4.5 mL of 0.10 mmol/L sodium tetraborate (pH 9.3). This OPA/BME stock solution was stable for 5 days if kept tightly sealed in a foil covered bottle at room temperature. The working derivatizing solution was prepared by diluting 1 mL of the stock reagent with 3 mL of 0.10 mmol/L sodium tetraborate on the day of analysis.

Twenty µL of tissue supernate and 40 µL of working solution were mixed together and after exactly 60 seconds, 10 µL of the resulting mixture were injected into the HPLC system.

4.2.4. Standard solutions

Taurine stock solution (10 mmol/L) was prepared by dissolving 0.1251 g taurine in distilled deionized water. Taurine standards were prepared by diluting the stock solution with 0.05 mmol/L (brain, eye, plasma or urine) or 0.1 mmol/L (liver) $HClO_4$. Stock solutions were prepared monthly and standards prepared biweekly.

4.2.5 Validation of method

The analytical method was evaluated for selectivity, linearity, method and system precision, accuracy, stability and sensitivity (Buick et al. 1990; Karnes et al. 1991).

4.3 RESULTS AND DISCUSSION

4.3.1 Selectivity

Tissues were analysed according to the HPLC assay procedure. Total analysis time was 20 minutes with taurine eluting at approximately 13 minutes. Figure 4.1a shows a chromatogram of blank reagent which reveals no peaks. As shown in Figure 4.1b (liver) and 4.1c (eye), baseline separation of taurine was achieved with no chromatographic interferences.

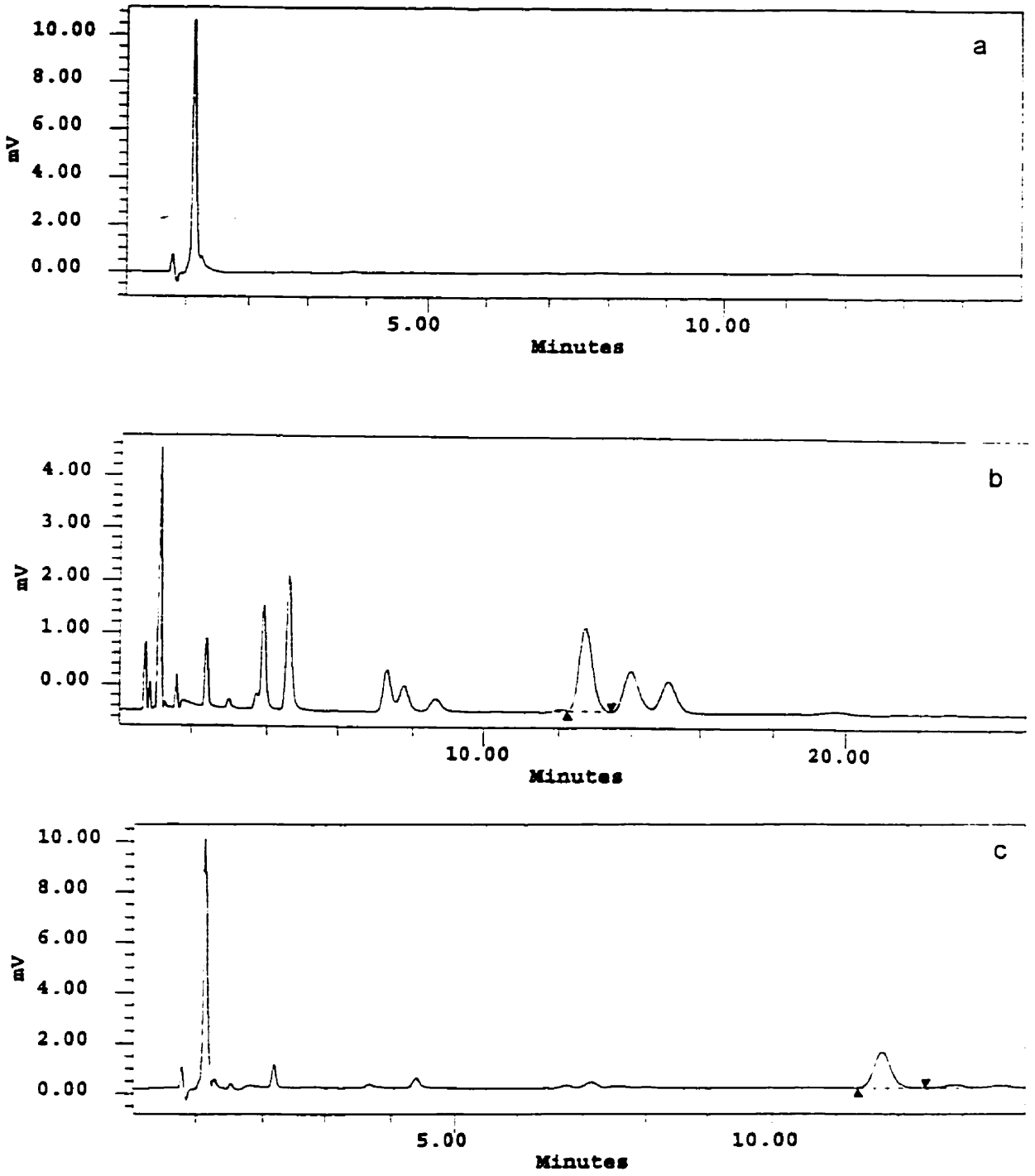

Figure 4.1

HPLC Chromatograms

A reagent blank (a), liver (b) and eye (c) tissues, from control animals, analysed for taurine.

4.3.2 Linearity

Linearity was evaluated using the integrated peak area response to 5 standard solutions (Karnes et al. 1991). Standards ranging from 0.01 to 0.5 µg/g taurine were used to encompass the range of taurine in tissue samples. A blank was included with each concentration curve. Standard concentration curves showed excellent detector linearity (Figure 4.2) with correlation coefficients between 0.9990-0.9999. Standard curves were generated daily. Correlation coefficients, slopes and intercepts from 10 standard curves are presented in Table 4.1.

4.3.3 System precision

Within-day precision of the peak area response for the HPLC system was determined by injection of 5 consecutive samples of the same taurine standard. The acceptance criteria that these tests produce a percent coefficient of variation (% CV) of no more than 5% is met (Buick et al. 1990). Between-day precision was determined by injection of taurine standard on 5 consecutive days. The %CV was less then 5% for each concentration evaluated.

4.3.4 Method precision

Within-day precision of the peak area response for tissue was determined by injection of 5 consecutive samples of the same tissue

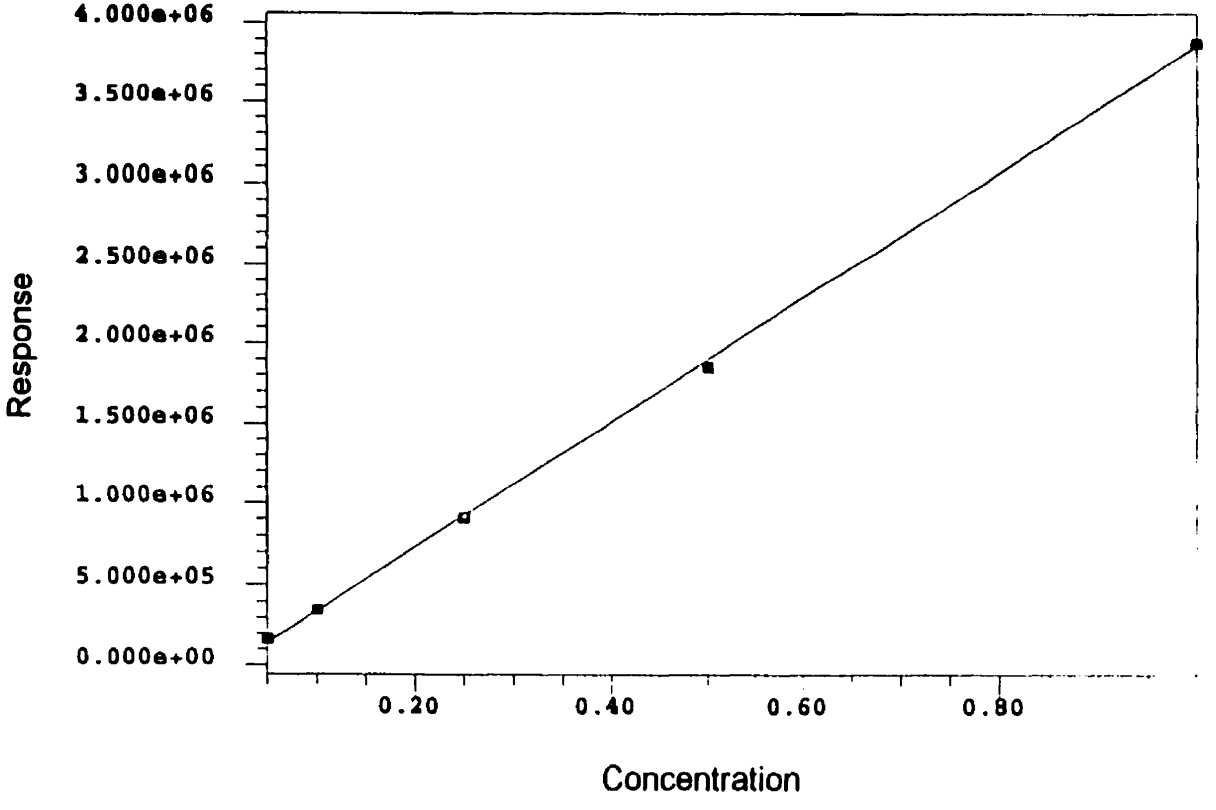

Figure 4.2
Standard Curve for the HPLC Analysis of Taurine in Biological Tissues

Table 4.1

Correlation Coefficients, Slopes and Y-Intercepts from Concentration Curves[1]

	Slope	Y-Intercept	r^2
1	3375156	-8795	0.9998
2	3286410	-7889	0.9999
3	3107200	-13512	0.9994
4	3165255	-6174	0.9998
5	3194334	-9433	0.9998
6	3233607	-9178	0.9999
7	2949842	-5585	0.9999
8	3097578	-6077	0.9999
9	3074674	-6663	0.9999
10	3064322	-8920	0.9999

[1]Concentration curves were created using a blank and 0.01, 0.05, 0.2, 0.25 and 0.5 µg/g taurine.

(Table 4.2). The acceptance criteria that these tests produce a percent coefficient of variation of no more than 5% is met. This reference is more conservative then the 10% range considered acceptable for analysis of compounds in biological tissues (Buick et al. 1990). A % CV of less then 5% was also chosen to ensure precision between duplicate tissue samples run for taurine analysis. Duplicates with a higher %CV were repeated. Between-day precision was determined by injection of samples on 5 consecutive days. The CV was less then 10% for each tissue evaluated.

4.3.5 Accuracy

Accuracy of the assay was determined by spiking the appropriate tissue with taurine. This method was chosen to eliminate any matrix effects. Five samples of a tissue were analysed for taurine content. Five samples of the same tissue were then spiked with an amount of taurine equivalent to the concentration measured in the original samples and re-analysed for taurine content. Taurine recovery from tissue ranged from 95-101% which meets the criteria for acceptable recovery. Tissue concentrations of taurine analysed in this study were comparable to those measured in previous research using other HPLC methods or amino acid analysers (Huxtable and Lippincott 1982; De La Rosa and Stipanuk 1984).

Table 4.2

Within-day Precision for Taurine Analysis of Brain Tissue[1]

Injection Number	Concentration (µg/g)
1	4.39
2	4.60
3	4.76
4	4.60
5	4.63
%CV	2.6%

[1]Brain tissue was forced through an 18G needle using a 10 cc plastic syringe. Aliquots of the homogenised tissue were used for this analysis.

4.3.6 Limit of detection and quantitation

The limit of detection for this assay is 0.1 ng/g. This concentration was determined by diluting and analysing a working standard solution until the taurine peak amplitude to noise ratio was 3. The limit of quantitation, 0.001 µg/g, was the lowest concentration of analyte that gave a % coefficient of variation of no more than 10% on a series of n=5 samples.

4.3.7 Analytical range

Concentrations from 0.0001 to 2.0 µg/g (n=5) were tested. Concentrations in the 0.001 - 2.0 µg/g range had % coefficient of variation below 5%. Tissue taurine concentrations, at the dilutions used in this assay, seldom measure above 0.5 µg/g; therefore, concentrations greater then 2.0 µg/g were not evaluated.

4.3.8 Stability of derivatizing agent

Using freshly made standards, data were generated to assess the stability of the derivatizing agent over a period 5 days. A standard curve was run on each day. The % coefficient of variation for each concentration (n=5) in this curve was calculated (Table 4.3). The % coefficient of variation was less then 5% for each concentration evaluated.

Table 4.3

Between-day Precision for Taurine Standards[1]

Concentration (µg/g)	%CV[2]
0.01	3.5
0.05	1.7
0.10	3.1
0.25	3.2
0.50	1.9

[1]Taurine standards were measured on 5 consecutive days. Working derivatizing solution was prepared daily from the same stock derivatizing solution.
[2]The %CV is calculated on n=5 injections.

4.3.9 GES interference detection

Samples of GES were analysed to determine whether this analogue would interfere with the measure of taurine in biological tissues and fluids. The HPLC method outlined in this chapter detects primary amines; therefore GES interference was not anticipated. When this compound was subjected to HPLC analysis of taurine no GES peak was observed.

4.4 CONCLUSION

The procedure described for the rapid determination of taurine in biological tissues and fluids has several advantages over other techniques available. The analysis time is shorter, gradient elution is not needed and samples do not require extensive clean-up prior to analysis. The modifications to this method allow for the determination of taurine in a range of biological tissues and fluids. This technique is sensitive in that it allows the measurement of taurine concentrations as low as 0.001 µg/g. Additionally, the method is reliable in that it consistently produces a CV below 5% when the taurine content of biological tissues is measured in duplicate. Concentrations of taurine analysed with this HPLC method are comparable to those reported in the literature (De La Rosa and Stipanuk 1984; Huxtable 1982).

Chapter 5

TAURINE DEFICIENCY ALTERS TISSUE TAURINE CONCENTRATIONS AND PREGNANCY OUTCOME IN THE RAT[1]

5.1 INTRODUCTION

Taurine biosynthesis varies with species and age. Cats, which have a low activity of the rate-limiting enzyme sulfinoalanine decarboxylase (SAD), are readily depleted when fed taurine-free diets (Knopf et al. 1978). Conversely, the rat, which has high sulfinoalanine decarboxylase activity and efficient renal taurine conservation, can be maintained on taurine-free diets for long periods of time without showing depleted taurine pools (Huxtable and Lippincott 1982). Instead, guanidinoethyl sulfonate (GES), a structural analogue of taurine, has been used to depress tissue taurine concentration in this species (Huxtable

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[1]A version of this chapter has been published [Gottschall-Pass et al. (1994) Effect of taurine deficiency on tissue taurine concentrations and pregnancy outcome in the rat. Can. J. Physiol. Pharmacol. 73: 1130-1135]. Reproduced with the permission of the NRC Research Press.

1982).

Fetal and neonatal animals may be at an increased risk of taurine deficiency. Sulfinoalanine decarboxylase activity and renal taurine conservation are lower (Kuo and Stipanuk 1984), translating into an increased dependence on exogenous taurine. In fact, it has been estimated that 60-70% of the total taurine present in rat pups at birth is derived from the mother's taurine pool (Huxtable and Lippincott 1982; Sturman 1982).

Taurine-deficient cats frequently abort or resorb their fetuses and have stillborn or live low-birth weight kittens with decreased tissue taurine levels (Sturman and Messing 1991). A number of neurological and morphologic abnormalities have been reported to occur; kittens born to taurine-deficient queens display abnormal hind leg development, thoracic kyphosis (Sturman et al. 1985), hydrocephalus (Sturman and Messing 1991) and failure of cells in the cerebellar external granule cell layer to migrate properly (Sturman et al. 1985). These kittens have also been reported to exhibit retinal degeneration (Sturman et al. 1986; Sturman and Messing 1991) and abnormal visual cortex development (Palackal et al. 1986).

Although the GES-induced taurine-deficient rat model has been used to study fetal (Ejiri et al. 1987) and neonatal growth (Bonhaus et al. 1985; De la Rosa and Stipanuk 1984), it has not been carefully explored as a model to study the developmental effects reported in the taurine-deficient cat. In one study describing fetal outcome in GES-treated rats (Ejiri et al. 1987), no congenital

malformations were reported. However, the analogue was not administered until day 11 of gestation although the susceptible period of organogenesis in the rat is from day 6-15 of gestation (Palmer 1978). Therefore, this study was designed to assess pregnancy outcome in the rat in response to varying taurine status instituted at day 0 of gestation.

5.2 MATERIALS AND METHODS

5.2.1 Animals and diet

Virgin female Sprague-Dawley rats (weighing 225-270 g) were obtained from Charles River, St. Constant, Quebec and housed in a room controlled for temperature (20-22°C) and light (12/12hr light/dark cycle). Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care 1984, 1993). All procedures used in this study were approved by the University Committee on Animal Care and Supply at the University of Saskatchewan.

Animals were fed a taurine-supplemented control diet[2] for at least 7 days and bred overnight. On day 0 of gestation, as determined by the

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[2]Taurine was added at a level approximating rat chow, which contains 1.5-5.0 µmol taurine/g chow (Huxtable 1982).

presence of sperm in vaginal smears, the females were placed individually in stainless steel cages and randomly assigned to 1 of 4 experimental groups. A modified AIN-based diet (American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, 1977, 1980) (Table 5.1) and distilled, deionized drinking water were provided, *ad libitum*, from day 0 to 20 of gestation. The basal diet, analysed by reverse-phase HPLC (Donzanti and Yamamoto 1988), contained <0.001 µmol taurine/g diet. Control animals received the basal diet with 2 µmol/g of added taurine and no GES in their drinking water. The 0.5, 1.0 and 2.0% GES animals received the basal diet and 0.5, 1.0 or 2.0% (w/v) GES, respectively, in drinking water. GES was synthesized and purified by POS Pilot Plant Corporation (Saskatoon, SK) according to the method of Morrison et al. (1958). Reverse-phase HPLC confirmed that the analogue contained 0.003% (w/w) taurine. Feed intake was recorded daily and dam weight recorded weekly.

5.2.2 Experimental protocol

On day 20 of gestation, dams were anaesthetized with methoxyflurane (Janssen Pharmaceutica, Mississauga, ON) and laparotomies were performed. Following hysterectomy, fetal position in the uterine horn, implantation sites, resorption sites and live and dead fetuses were recorded. Dams were killed by decapitation. Fetuses were

Table 5.1

Composition of Basal Diet[1]

INGREDIENTS	AMOUNT (g/kg diet)
Spray-dried egg white	200.0
Corn Oil	50.0
Canola Oil	50.0
Glucose hydrate	647.5
Mineral premix[2]	40.0
Vitamin premix[3]	10.0
Choline bitartrate	2.5

[1]AIN 76 diet. The basal diet contained <0.001 µmol taurine/g diet. The control diet was supplemented with 2 µmol taurine/g diet to approximate the taurine content of rat chow.

[2]Mineral premix at 4.0% of the diet supplied the following concentration of minerals (in g/kg diet): $CaHPO_4 \cdot 2H_2O$, 22.3; NaCl, 2.6; K citrate$\cdot H_2O$, 7.7; K_2SO_4, 1.8; MgO, 0.84; $MnCO_3$, 0.11; Fe citrate, 0.21; KIO_3, 0.00036; Na_2SeO_3, 0.00026; $CrK(SO_4)_2 \cdot 12H_2O$, 0.019; $CuCO_3$, 0.012; $ZnSO_4 \cdot 7H_2O$, 0.221.

[3]Vitamin premix at 1% of the diet supplied the following concentration of vitamins (mg/kg diet): thiamin HCl, 6; riboflavin, 6; pyridoxine HCl, 7; niacin, 30; Ca pantothenate, 16; folacin, 2; biotin, 2.2; cyanocobalamin (0.1% in mannitol), 25; retinyl acetate (beads) (500,000 IU/g), 8; dl-α-tocopheryl acetate (powder) (250 IU/g), 300; cholecalciferol (beads) (400,000 IU/g), 2.5; menadione, 0.5.

removed from the uterus, extra-embryonic tissues were separated and fetal and placental weights recorded. All live fetuses were examined for external malformations (Taylor 1986). Following euthanasia, one-half were placed in Bouin's fluid (BDH Inc., Edmonton, AB) for decalcification and subsequent examination for visceral malformations by Wilson's razor blade technique (Wilson 1965); crown-rump lengths were measured after 24 hours. The remainder were skinned, eviscerated and placed in 95% ethanol (Stanchem Inc., Winnipeg, MB) for subsequent clearing with potassium hydroxide (BDH, Inc., Edmonton, AB), staining with Alizarin red S (BDH Inc., Edmonton, AB) and examination for skeletal malformations (Taylor 1986). Pooled fetal liver and brain samples as well as maternal liver and brain were retained for taurine analysis. Protein was analysed by a modification of the Lowry procedure (Markwell et al. 1978). All tissues were stored frozen at -70°C.

5.2.3 Taurine analysis

Taurine was analysed by reverse-phase HPLC using precolumn derivatization with ortho-phthalaldehyde (Sigma Chemical Company, St. Louis, MO) and electrochemical detection and using a modification of the method of Donzanti and Yamamoto (1988). The protocol for this method and the validation procedures used are presented in Chapter 4.

5.2.4 Statistical analyses

Continuous variables, such as fetal weight and tissue taurine concentrations, were analysed by one-way analysis of variance followed by Tukey's test. Discrete developmental data, such as the number of live fetuses or percent resorptions, were analysed by the Kruskal-Wallis test. A probability of less than 0.05 was considered significant. The litter was used as the statistical unit for analysis of crown-rump lengths, placental and fetal weights (Haseman and Hogan 1975).

5.3 RESULTS

Maternal feed intake and weight gain data are given in Table 5.2. Treatment with low dietary taurine and varying doses of GES had no effect on feed intake. Dams receiving 2% GES gained significantly less weight (P=0.022) than control animals; however, dams receiving the two lower doses (0.5 and 1.0% GES) had weight gains that were not statistically different than controls.

Low dietary taurine and GES consumption during pregnancy depressed taurine concentrations in both maternal and fetal tissues (Table 5.3). Treatment with 0.5-2.0% GES significantly decreased liver taurine concentrations in the dams to approximately 15% of that of controls (P=0.0001); no differences were found among the three GES doses. Fetal liver showed a similar pattern with the

Table 5.2

Effect of Taurine Deficiency on Maternal Feed Intake and Weight Gain in the Rat

	Treatment Group[1]			
	CONTROL	0.5% GES	1.0% GES	2.0% GES
Feed Intake (g/20 days)	379.3±13.7[a]	356.6±13.5[a]	348.8±7.5[a]	351.6±16.2[a]
Weight Gain (g/20 days)	124.9±8.7[a]	104.0±5.5[a,b]	108.0±3.7[a,b]	96.3±5.6[b]

[1]Results expressed as mean ±SEM; n=8 for Control and 1.0% GES, n=7 for 0.5 and 2.0% GES. Statistical analysis was one-way ANOVA followed by Tukey's test. Values in a row not sharing a common superscript are significantly different (P<0.05).

Table 5.3

Tissue Taurine Levels in Dams and Fetuses Exposed to Low Dietary Taurine and Varying Doses of Guanidinoethyl Sulfonate

	Treatment Group[1]			
	CONTROL	0.5% GES	1.0% GES	2.0% GES
Maternal Tissue				
Liver (µmol/g ww)	12.3±0.8[a]	2.07±0.23[b]	1.76±0.20[b]	1.55±0.08[b]
Brain (µmol/g ww)	4.27±0.11[a]	2.72±0.18[b]	2.37±0.11[b,c]	1.99±0.08[c]
Fetal Tissue				
Liver (µmol/g ww)[2]	9.79±0.22[a]	5.45±0.11[b]	4.75±0.15[b]	4.58±0.35[b]
Brain (µmol/g ww)[2]	16.7±0.2[a]	13.6±0.5[b]	12.6±0.3[b,c]	11.9±0.2[c]

[1]Results expressed as mean±SEM; n=8 for Control and 1.0% GES, n=7 for 0.5% and 2.0% GES. Statistical analysis was one-way ANOVA followed by Tukey's test. Values in a row not sharing a common superscript are significantly different ($P<0.05$).
[2]Fetal tissue was pooled for taurine analysis.

taurine concentrations of treatment groups depressed to approximately 50% of that of control animals (P=0.0001). Brain taurine concentrations in the dam were significantly decreased in all taurine-deficient groups to approximately 55% of that of controls (p=0.0001). Fetal brain taurine was similarly depressed to approximately 75% of that of control animals (p=0.0001). The 0.5% and 2.0% GES treatment groups were statistically different from one another for both maternal and fetal brain taurine levels. Similar degrees of taurine depletion were found when data were expressed in nmol taurine/mg protein (Appendix A).

Reproductive outcome data are given in Table 5.4. The decreased maternal weight gain seen in the group treated with low dietary taurine and 2% GES can be attributed to the significantly smaller litter weights found in this group (P=0.0422). There were no significant effects of maternal taurine deficiency on male or female fetal weights, placental weights or fetal crown-rump lengths. Although not statistically significant, animals receiving 2% GES demonstrated a trend towards fewer implantation sites and live fetuses per litter then any of the other experimental groups; this resulted in an apparent higher percent of resorptions and late deaths. This trend was associated with unilateral pregnancies in four of seven dams from this group. Bilateral pregnancies were found in all dams from each of the other experimental groups.

Finally, gross assessment of fetuses revealed no significant external, visceral or skeletal malformations due to GES-induced taurine deficiency in any treatment group.

Table 5.4

Influence of Taurine Deficiency on Reproductive Parameters

	Treatment Group[1]			
	CONTROL	0.5% GES	1.0% GES	2.0% GES
LITTER WEIGHT (g)[2]	51.9±2.8[a]	47.6±3.4[a,b]	46.2±2.4[a,b]	35.8±6.1[b]
FETAL WEIGHT (g)[2]				
Males	3.76±0.10[a]	3.75±0.13[a]	3.59±0.06[a]	3.75±0.22[a]
Females	3.64±0.09[a]	3.53±0.11[a]	3.45±0.06[a]	3.43±0.21[a]
PLACENTAL WEIGHT (g)[2]	0.40±0.01[a]	0.39±0.01[a]	0.40±0.01[a]	0.47±0.05[a]
CROWN-RUMP LENGTH (mm)[2]	35.4±0.4[a]	35.6±0.5[a]	34.7±0.3[a]	35.1±1.0[a]
IMPLANTATION SITES PER LITTER[3]	15.0±0.9[a]	13.7±0.8[a]	14.0±0.7[a]	11.4±2.0[a]
LIVE FETUSES PER LITTER[3]	14.1±0.8[a]	13.0±0.8[a]	13.1±0.6[a]	10.1±1.8[a]
RESORPTIONS & LATE DEATHS[3]				
Percent of Implantation Sites	5.8±2.0[a]	5.0±2.0[a]	6.1±2.2[a]	11.6±3.2[a]
Number of Litters Affected	5/8	4/7	5/8	5/7
UNILATERAL PREGNANCY[4]				
Number of Litters Affected	0/8	0/7	0/8	4/7

[1]Results expressed as mean±SEM; n=8 for Control and 1.0% GES, n=7 for 0.5 and 2.0% GES.
[2]Statistical analysis was one-way ANOVA followed by Tukey's test. Values in a row not sharing a common superscript are significantly different ($P<0.05$). [3]Statistical analysis was a Kruskal-Wallis test. Values in a row not sharing a common superscript are significantly different ($P<0.05$). [4]Implantation sites confined to either left or right uterine horn.

5.4 DISCUSSION

Treatment of pregnant rats with taurine-deficient diets and varying doses of GES produced a sharp decline in maternal liver and brain taurine concentration to 15 and 55% of that of control levels, respectively. Similar depressions (to 35 and 30% of that of controls) have been reported in dams administered 1% GES for the final 10 days of gestation (Ejiri et al. 1987).

In the fetus, taurine is supplied primarily by the mother via the placenta (Huxtable and Lippincott 1982; Sturman 1982). Therefore, when maternal taurine status is depressed during gestation, fetal tissue taurine concentrations can also be compromised. Evidence for GES-induced fetal taurine deficiency in this study was demonstrated by a depression of both brain and liver taurine in fetal tissues to 50 and 75% of that of control levels, respectively. One-day-old pups from dams fed 1% GES two weeks prior to mating had similarly depressed liver and brain taurine concentrations, 60 and 65% of that of controls (Bonhaus et al. 1985). For both liver and brain, fetal taurine concentrations appear to be more highly conserved than maternal taurine concentrations. This trend is consistent with other studies that examined taurine concentrations of fetuses (Ejiri et al. 1987) and neonates (Bonhaus et al. 1985; De la Rosa and Stipanuk 1984) from dams receiving 1% GES for varying lengths of time prior to and during gestation.

In this study, fetal brain taurine is most highly conserved, with

approximately 75% being retained at day 20 of gestation. Turnover of taurine in fetal brain is slower than that in fetal liver (Sturman et al. 1977; Sturman 1982). which may account for this difference. Although the differences in maternal and fetal brain taurine concentrations between the 0.5 and 2.0% GES groups were statistically significant, physiologically the differences are small. In effect, very similar degrees of taurine depletion were achieved at all doses of the analogue.

Much of the depression in maternal weight gain in the group treated with 2% GES can be attributed to the smaller litter weights of these dams. This decrease in litter weight is due to smaller litter size and not to the intrauterine growth retardation reported in taurine-deficient cats (Sturman and Messing 1991). Fetal weights were similar among all experimental groups for both male and female fetuses. Maternal taurine deficiency did not influence placental weight or crown-rump lengths. In addition, no differences in organ to body weight ratios for fetal brain or liver were observed (Appendix B). In a previous study, treatment of dams with 1% GES in the drinking water from 2 weeks prior to mating and for the duration of gestation, produced no effect on the number of dams delivering litters, litter size or pup weight at birth (Bonhaus et al. 1985). Conversely, Ejiri et al. (1987) reported that rats treated with 1% GES from day 11 of gestation produced fetuses with significantly depressed whole body, liver and brain weights at day 21 of gestation. Unfortunately, the fetus rather than the litter was used as the unit for statistical analysis in that study; therefore, significant differences between groups may have been found where none

existed (Haseman and Hogan 1975). While no increase in intrauterine fetal death rate or malformations was noted (Ejiri et al. 1987), it is not clear how the latter were assessed. The gross assessment of fetuses in the present study, using standard teratological techniques, revealed no significant external, visceral or skeletal malformations due to GES-induced taurine deficiency.

Dams treated with 2% GES appear to have fewer implantation sites and live fetuses per litter than all other experimental groups. Although these animals appear to have a higher percent of resorptions and late deaths when expressed relative to the number of implantation sites, this is entirely attributable to fewer implantation sites and not to larger numbers of resorptions and late deaths. The decrease in litter size was specifically associated with confinement of implantation sites to either the left or right uterine horn in four of seven dams. While these trends are interesting, the necessity of utilizing nonparametric statistics for this type of data made it difficult to achieve statistical significance. The decrease in implantation sites and the presence of unilateral pregnancies in dams treated with 2% GES in this study are suggestive of increased reproductive loss possibly through preimplantation loss. Unfortunately, this possibility was not explored as corpora lutea were not counted. Preimplantation loss has also been implicated in the pregnancy failure seen in taurine-deficient cats (Dieter et al. 1993).

Although the role of taurine in mammalian embryonic development during the preimplantation stage is not clearly defined, this function has recently been

explored. Cultured two-cell stage mouse embryos in media containing > 1 mmol/L taurine (Dumoulin et al. 1992) and one- or two-cell pig embryos cultured in media containing 7 mmol/L taurine (Petters and Reed 1991) produce blastocysts significantly more often than embryos cultured in taurine-free media. In addition to this *in vitro* evidence, taurine has been found to comprise about 60% of the total free amino acid content in mouse oviduct flushings (Dumoulin et al. 1992) and is present in highest concentrations (11.7 mmol/L) in human uterine fluid during the luteal phase, during which implantation occurs (Casslén 1987).

It can be hypothesized that treatment of rats with GES prior to implantation may depress uterine taurine concentration thereby affecting preimplantation development. Though increasing the dose of analogue above 0.5% had little additional effect in depressing maternal liver and brain taurine concentrations in this study, uterine taurine levels were not measured. It is possible that treatment with 2% GES depressed uterine taurine concentrations to a greater extent than treatment with the two lower doses (0.5 or 1.0% GES) and that this hypothetical change in uterine taurine levels translated into an increase in preimplantation loss. Treatment of rats with this dose of analogue could be further explored as a model for the reproductive loss seen in the cat.

It can be concluded that the GES-induced taurine deficiency in the rat is not a good model for studying all developmental effects of taurine deficiency. No level of GES produced the intrauterine growth retardation or malformations

(Sturman and Messing 1991; Sturman et al. 1986) previously described in kittens born to taurine-deficient cats. This may be attributable to greater depletion in cats fed taurine-free diets (Sturman and Messing 1991) compared with the extent of depletion achieved with low dietary taurine and GES in the rat. However, pre- and postnatal exposure to GES in the rat is a useful model for studying the function of taurine in photoreceptors, as morphological changes in the retina are similar to those observed in taurine-deficient kittens (Bonhaus et al. 1985; Pasantes-Morales et al. 1983). In addition, the data from the current study suggest that treatment of rats with 2% GES could be further explored as a model for the reproductive loss seen in the cat.

Chapter 6

EXAMINATION OF THE INTERACTION BETWEEN ZINC AND TAURINE: A PILOT STUDY

6.1 INTRODUCTION

Based on the results presented in Chapter 5 it was evident that the effect of taurine deficiency on development in the rat, as assessed by evaluation of fetal weight and incidence of congenital malformations at day 20 of gestation, does not produce the degree of reproductive loss previously observed in the taurine-deficient cat (Sturman et al. 1985; Sturman and Messing 1991). Therefore, it was concluded that incidence of fetal malformations may be an inadequate indicator with which to explore the proposed interaction between zinc and taurine *in vivo*.

Alternatively, the high concentration of zinc (Eckhert 1983) and taurine (Heinämäki et al. 1986) in ocular tissue and the purported importance of these nutrients in maintaining eye structure and function (Leure-duPree and McClain

1982; Sturman et al. 1986), suggested that the eye might be a unique physiological system in which to study this interaction. As well, some developmental insults, which do not result in physical malformations, can induce significant and lasting functional defects postnatally (Tonkiss et al. 1993). Therefore, a pilot study was designed to assess whether pre- and postnatal development of eye structure and function would be a more sensitive endpoint for demonstrating an interaction between zinc and taurine *in vivo*. This study would allow confirmation of the incidence of congenital malformations at day 20 of gestation in marginal zinc, taurine and combined zinc and taurine deficiencies. In addition, the effectiveness of the protocol for inducing marginal zinc deficiency and obtaining ocular measurements in the rat could be explored.

6.2 MATERIALS AND METHODS

6.2.1 Animals and treatment

Virgin female Sprague-Dawley rats (weighing 220-250 g) were obtained from Charles River, St. Constant, Quebec and housed in a room controlled for temperature (20-22°C) and light (12/12hr light/dark cycle). Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care 1993). All procedures used in this study were approved by the

University of Saskatchewan Committee on Animal Care and Supply.

Animals were fed control diet for at least 7 days and bred overnight. On day 0 of gestation, as determined by the presence of sperm in vaginal smears, the females were placed individually in stainless steel cages and randomly assigned to 1 of 4 treatments. Treatments consisted of 4 diets formulated with 2 levels of zinc (**+Zn**, 50 µg Zn/g diet; **-Zn**, 9 µg Zn/g diet) and 2 levels of taurine (**+Tau**, 2 µmol taurine/g diet; **-Tau**, 0 µmol taurine/g diet) in the following combinations: +Zn/+Tau (control), +Zn/-Tau, -Zn/+Tau or -Zn/-Tau. The above groups were provided with free access to modified AIN diets (Table 5.1) and distilled deionized water. One percent (w/v) GES was added to the drinking water of the +Zn/-Tau and -Zn/-Tau groups. Feed intake was recorded daily and weight recorded weekly.

GES was synthesized and purified (POS Pilot Plant Corporation, Saskatoon, SK) according to the method of Morrison et al. (1958). Reverse-phase HPLC confirmed that a 1% (w/v) solution of GES contained <0.001 µmol/mL taurine; atomic absorption spectrophotometry revealed <0.1 µg/mL zinc.

6.2.2 Biomicroscopy and indirect ophthalmoscopy

Biomicroscopy and indirect ophthalmoscopy were completed on all dams prior to breeding. Animals with pre-existing eye disease were

excluded from the study. Similar examinations were to be performed on pups (4/dam) at 3 and 6 weeks postnatal age. The veterinary ophthalmologist who completed these examinations was blinded to the treatment groups.

6.2.3 Prenatal outcome

On day 20 of gestation, dams were anaesthetized with methoxyflurane (Janssen Pharmaceutica, Mississauga, ON) and laparotomies performed (+Zn/+Tau, n=2; +Zn/-Tau, n=1; -Zn/+Tau, n=3; -Zn/-Tau, n=1). Following hysterectomy, fetal position in the uterine horn, implantation sites, resorption sites and live and dead fetuses were recorded. Dams were killed by decapitation. Fetuses were removed from the uterus, extra-embryonic tissues were separated and fetal and placental weights recorded. Fetuses were killed by decapitation and placed in Bouin's fluid (BDH Inc., Edmonton, AB) for decalcification and subsequent examination for visceral malformations by Wilson's razor blade technique (Wilson 1965). Fetal eyes were assessed for malformations by light microscopy.

6.2.4 Postnatal outcome

On day 20 of gestation, the remaining dams were transferred to stainless steel cages fixed with plastic mesh bottoms (+Zn/+Tau, n=3;

+Zn/-Tau, n=3; -Zn/+Tau, n=3; -Zn/-Tau, n=2). The following describes the remainder of the protocol which was not carried out due to the mortality described below. Litters were to be culled to 8 pups at postnatal day 4 and maintained on their respective diets. At postnatal weeks 3 and 6, pups (4/dam) were to undergo measures of ERG. Animals were then to be anaesthetized with methoxyflurane, blood taken by cardiac puncture and the animals killed by decapitation. Liver, plasma and tibia were to be collected for analysis of zinc concentrations; liver was to be retained for analysis of taurine levels. Eyes were to be fixed in Bouin's fluid for subsequent examination by light microscopy.

6.2.5 Statistical analysis

Feed intake and weight gain in dams were analyzed by two-factor ANOVA with zinc and taurine as the independent variables (SuperANOVA, Abacus Concepts, Berkeley, CA). A probability of less than 0.05 was considered significant.

6.3 RESULTS

6.3.1 Outcome for the dams

Body weight and feed intake of all dams to day 20 of gestation are

presented in Table 6.1. No interaction was apparent for either parameter. No effect of marginal zinc deficiency or taurine deficiency on weight gain or feed intake was noted.

All dams in the -Zn/+Tau and -Zn/-Tau group had lesions on their paws at parturition, indicative of zinc deficiency (Clegg et al. 1989). These signs were not apparent in the zinc-adequate groups. In addition, those dams from the -Zn/+Tau and -Zn/-Tau groups which were allowed to deliver tended to have more difficult parturitions and to neglect their pups. As a result these pups died sooner then pups from the zinc-adequate group (see section on postnatal outcome).

Ophthalmic examination of all dams, just prior to day 20 of gestation, revealed a high incidence of incipient cortical cataracts which were most obvious on the suture lines in the -Zn/+Tau (5/6) dams as compared to dams from the -Zn/-Tau (2/5), +Zn/-Tau (0/5) and +Zn/+Tau (1/5) groups.

6.3.2 Prenatal outcome

No evidence of significant congenital malformations were observed in any experimental group. Assessment of intrauterine growth retardation was not possible due to the small numbers and the need to use the litter as the statistical unit (Haseman and Hogan 1975). Fetal weights (mean±SD) are as follows: +Zn/+Tau 3.48±0.16 g, n=2; +Zn/-Tau 4.49 g,

Table 6.1

Effect of Zinc and Taurine on Body Weight and Feed Intake in Dams to Day 20 of Gestation[1]

	Treatment Group						
	+Zn/+Tau	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
Feed Intake	381.7±13.7	362.0±5.5	387.1±13.5	351.1±26.1	P=0.8603	P=0.0889	P=0.6020
Weight Gain	130.1±3.0	104.1±17.9	108.5±3.8	96.4±11.4	P=0.1442	P=0.0636	P=0.4746

[1]Results expressed as mean±SEM; n=5 for +Zn/+Tau, n=3 for +Zn/-Tau, n=6 for -Zn/+tau, n=3 for -Zn/-Tau. Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables.

n=1 (based on n=1 dam with n=1 fetus); -Zn/+tau 3.40±0.24 g, n=3; -Zn/-Tau 2.80±0.17 g, n=1.

There were no obvious abnormalities in other determinants of pregnancy outcome except for the one +Zn/-Tau dam noted above which had only 1 fetus and no other visible implantation sites.

Histological assessment of fetal eyes at day 20 of gestation did not reveal ocular malformations in any experimental group.

6.3.3 Postnatal outcome

It was not possible to assess postnatal development of eye structure and function as an endpoint for exploring the zinc and taurine interaction as 100% pup mortality in 10 out of the 11 dams allowed to deliver was observed.

Ringtail was apparent in pups from all experimental groups suggestive of problems of low humidity in the animal housing unit (Dennis 1986).

Five pups from one +Zn/-Tau dam survived and were followed postnatally until week 10. The only eye abnormality detected with the slitlamp biomicroscope was cortical cataracts. These were most obvious on the suture lines and were observed in all five animals. No retinal changes were apparent by indirect ophthalmoscope.

6.4 DISCUSSION

The high incidence of lesions at parturition, on the paws of dams fed marginally zinc-deficient diets, are characteristic of zinc deficiency (Clegg et al. 1989). It was also found that dams from the -Zn/+Tau and -Zn/-Tau groups tended to have more difficult parturitions and to neglect their pups. Consequently, these offspring died sooner then those from the zinc-adequate group. Based on the latter observation, the zinc content of the marginal zinc-deficient diet was reevaluated. To improve pup survival, the decision was made to increase the zinc content of the marginally zinc-deficient diet to 15 µg/g throughout gestation, to be reduced to 7.5 µg/g at parturition, for subsequent experiments (Herzfeld et al. 1985; Oteiza et al. 1990; Rogers et al. 1985). Zinc status of pups in the pilot study was not assessed due to the high incidence of pup mortality.

As predicted, no evidence of significant congenital malformations were apparent in any experimental group at day 20 of gestation. Light microscopic assessment of fetal eyes at this timepoint similarly revealed no ocular malformations in any group studied. Unfortunately, the pup mortality in the postnatal group prevented an evaluation of eye morphology and function at 3 and 6 weeks postnatal age.

Premature pup death was observed in all groups including the control group. We also observed ring tail in pups from all experimental groups.

Subsequent experimentation has allowed confirmation that low humidity in the animal housing room was to blame.

The high incidence of cataracts in the zinc-deficient dams and taurine-deficient pups is interesting; however, assessment of cataracts was not a main objective of this experiment. It is possible that the cataract formation observed was secondary to retinal damage; however, no retinal changes on ophthalmoscopic examination in the dams in any experimental group were found. The cataracts may also have been due to environmental stress (low humidity) and/or zinc- and taurine-deficiency per se. The intent of this study was to assess retinal structure and function in pups made zinc- and taurine-deficient throughout gestation and postnatal life. Unfortunately, the high incidence of mortality made retinal assessment of pups impossible.

In summary, this study confirmed the lack of congenital malformations at day 20 of gestation in both marginal zinc and taurine deficiencies. These results support the earlier claim that incidence of fetal malformations may be an inadequate indicator with which to explore the proposed interaction between zinc and taurine *in vivo*. The high incidence of pup mortality made it impossible to evaluate pre- and postnatal development of eye structure and function as a more sensitive endpoint for demonstrating this interaction. The effectiveness of the protocol for obtaining ocular measurements in the rat was similarly not assessed. To improve pup survival in future studies, the zinc content of the marginally zinc-deficient diet will be increased to 15 µg/g throughout gestation.

At parturition, this level will be reduced to 7.5 µg/g to maintain a marginal model of zinc deficiency (Herzfeld et al. 1985; Oteiza et al. 1990; Rogers et al. 1985).

Chapter 7

ZINC INTERACTS WITH TAURINE IN THE DEVELOPING RAT RETINA: OSCILLATORY POTENTIALS AND LIGHT MICROSCOPIC CHANGES[1]

7.1 INTRODUCTION

Reports that the nutrients zinc and taurine interact have prompted interest in the physiological significance and possible mechanisms responsible for this phenomenon (Pasantes-Morales et al. 1987b). Isolated frog rod outer segments exposed to ferrous sulfate display extensive disruption of their structure, characterized by acute swelling and disc membrane disorganization. The

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[1]Versions of this chapter have been published [Gottschall-Pass et al. (1997) Oscillatory potentials and light microscopic changes demonstrate an interaction between zinc and taurine in the developing rat retina J. Nutr. 127: 1206-1213 and Paterson et al. (1997) Effect of zinc and taurine status during prenatal and postnatal periods on oscillatory potentials in the mature rat retina. In: Trace Elements in Man and Animals-9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals (Fischer, P.W.F. et al., Eds) pp. 89-90. NRC Press, Ottawa, Canada]. Reproduced with permission of the American Society for Nutritional Sciences and NRC Research Press.

addition of zinc or taurine alone to the culture medium is ineffective in protecting against this outer segment damage; however, zinc and taurine, added together, provide protection in a synergistic manner (Pasantes-Morales and Cruz 1984). *In vivo* experimentation contributes further evidence for this interaction. Plasma taurine concentration is increased in severely zinc-deficient rats (Griffith and Alexander 1972). Griffith and Alexander (1972) found no alteration in the urinary excretion of taurine in zinc deficiency; other researchers have described an increase (Anthony et al. 1971, Hsu and Anthony 1970). Interest in the interaction between zinc and taurine in neurological disorders has also been reported (Barbeau and Donaldson 1974, Shapcott et al. 1984).

The developing retina may be a sensitive tissue in which to explore the interaction between zinc and taurine. A 50% reduction in retinal taurine in the cat results in retinal degeneration characterized by a decrease in a- and b-wave amplitudes of the electroretinogram (Berson et al. 1976) and degeneration of the photoreceptor outer segments (Hayes et al. 1975). With prolonged taurine deficiency, blindness develops. The addition of 200 μM zinc to the drinking water of the taurine-deficient cat can provide partial protection to the a-wave amplitude of the electroretinogram (Pasantes-Morales et al. 1987a). During gestation and early postnatal life, taurine plays an important role in retinal development. Kittens born to taurine-deficient queens have shortened photoreceptors characterized by severely disorganized outer segments (Imaki et al. 1986).

Rats made taurine-deficient using guanidinoethyl sulfonate (GES), a structural analogue of taurine, develop similar retinal changes as those observed in cats. These include reduced a- and b-wave amplitudes of the electroretinogram (Cocker and Lake 1987, Hageman and Schmidt 1987, Lake 1986, Rapp et al. 1987) and degeneration of the photoreceptors (Lake and Malik 1987, Pasantes-Morales et al. 1983). Treatment of rats with 1% GES throughout gestation produces a degeneration of the photoreceptor layer of the retina in pups similar to that observed in kittens (Bonhaus et al. 1985).

The influence of zinc on the retina is less clear. Examination of fetuses from severely zinc-deficient dams, at day 12 and 14 of gestation revealed that invagination of the optic cup was often deficient and that closure of the choroid fissure did not occur. This resulted in retinal folding at the choroid fissure that was visible at term (Rogers and Hurley 1987). When zinc deficiency was instituted at parturition, eye lid opening was delayed but no morphological alterations were noted among photoreceptor cells at weaning (Sinning et al. 1984).

The influence of zinc and taurine on oscillatory potentials (OPs) has not been reported. Oscillatory potentials are a series of rhythmic consecutive discharges of retinal neurons found superimposed on the b-wave of the ERG (Speros and Price 1981). It is thought that these oscillations are generated independently from the mechanism producing the primary components of the ERG, the a- and b-waves, at a more proximal location, possibly the inner nuclear

layer (el Azazi and Wachtmeister 1990). The objective of this research was to investigate whether the proposed interaction between zinc and taurine influences the development of retinal structure and function in rats as examined by light microscopy and oscillatory potentials. In addition, the effect of this interaction on zinc and taurine status of the animals was determined.

7.2 MATERIALS AND METHODS

7.2.1 Animals and treatment

Virgin female Sprague-Dawley rats (weighing 220-250 g) were obtained from Charles River, St. Constant, Quebec and housed in a room controlled for temperature (20-22°C) and light (12-hr light:dark cycle). Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care 1993). All procedures used in this study were approved by the University of Saskatchewan Committee on Animal Care and Supply.

Rats were fed control diet for at least 7 days and bred overnight. On day 0 of gestation, as determined by the presence of sperm in vaginal smears, the females were placed individually in stainless steel cages and randomly assigned to 1 of 5 treatments. Treatments consisted of 4 diets formulated with 2 levels of zinc (**+Zn**, 50 µg Zn/g diet; **-Zn**, 15 µg Zn/g

diet) and 2 levels of taurine (**+Tau**, 2 µmol taurine/g diet; **-Tau**, 0 µmol taurine/g diet) in the following combinations: +Zn/+TauAL (control), +Zn/-Tau, -Zn/+Tau or -Zn/-Tau. The above groups were provided with free access to modified AIN-93G diets (Reeves et al. 1993) and distilled deionized water. A fifth group (+Zn/+TauPF) was fed control diet and individually pair-fed to their respective psirs in the -Zn/+Tau group. The basal diet presented in Table 7.1 contains <0.001 µmol/g taurine as determined by HPLC. One percent (w/v) GES was added to the drinking water of the +Zn/-Tau and -Zn/-Tau groups. Zinc concentration of the -Zn/+Tau and -Zn/-Tau diets was further reduced to 7.5 µg/g at parturition.

On day 20 of gestation, dams were transferred to stainless steel cages fixed with plastic mesh bottoms and provided with 30 x 50 cm of absorbent paper (VWR, Edmonton, AB) as nesting material. Litters were adjusted to 7-10 pups at postnatal day 4. Nesting material was removed within 7 days of birth. At postnatal day 23, male pups (10/treatment) were weaned onto their respective diets. Feed intake was recorded daily and weight recorded weekly for the pups.

GES was synthesized and purified (POS Pilot Plant Corporation, Saskatoon, SK) according to the method of Morrison et al. (1958). Reverse-phase HPLC confirmed that a 1% (w/v) solution of GES contained <0.001 µmol/ml taurine; atomic absorption spectrophotometry

Table 7.1

Composition of Basal Diet[1]

INGREDIENTS	AMOUNT (g/kg diet)
Spray-dried egg white	200.0
Soybean oil[2]	70.0
Glucose hydrate	632.486
Fiber (Solkafloc)	50.0
Mineral premix[3]	35.0
Vitamin Premix[4]	10.0
Choline bitartrate	2.5
Tert-butyl hydroquinone	0.014

[1]AIN 93G diet. To vary the zinc (zinc carbonate) and/or taurine concentrations in the diet, premixes of each were prepared in glucose.
[2]Phylloquinone at 0.91 mg/kg diet added to soybean oil.
[3]Mineral premix supplied the following concentration of minerals in g/kg mix: calcium carbonate, anhydrous, 80; calcium phosphate, dibasic, 377; potassium sulfate, 46.6; potassium citrate, tripotassium, 37; magnesium oxide, 24; ferric citrate, 6.06; manganous carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, anhydrous, 0.01367; ammonium paramolybdate, 4 hydrate, 0.00795; sodium meta-silicate, 9 hydrate, 1.45; chromium potassium sulfate, 12 hydrate, 0.275; lithium chloride, 0.0174; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; ammonium vanadate 0.0066.
[4]Vitamin premix supplied the following concentration of vitamins in g/kg mix: thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3; Ca pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.22; cyanocobalamin (0.1% in mannitol), 2.5; all trans-retinyl palmitate (250,000 IU/g), 1.6; all rac-α-tocopheryl acetate (250 IU/g), 30; cholecalciferol (400,000 IU/g), 0.25.

revealed <0.1 µg/ml zinc.

7.2.2 Biomicroscopy and indirect ophthalmoscopy

Biomicroscopy and indirect ophthalmoscopy were completed on all dams and males prior to breeding. Rats with preexisting eye disease were excluded from the study. Similar examinations were performed on the pups at 4 and 7 weeks postnatal age. The veterinary ophthalmologist who completed these examinations was not aware of treatment group during the examination.

7.2.3 Oscillatory potentials

Oscillatory potentials were recorded on pups at 7½-8½ weeks of age after overnight dark adaptation. Recordings were made within 5 hours of the usual onset of light. Rats were anaesthetized with an intramuscular injection of ketamine (30 mg/kg body wt; rogar/STB Inc., London, ON) and xylazine (7-8 mg/kg body wt; Chemagro Ltd., Etobicoke, ON). The right pupil was dilated with 1% tropicamide (Alcon Canada Inc., Mississauga, ON) and the cornea anaesthetized with proparacaine hydrochloride (Allergan Inc., Markham, ON). The eyelids were retracted with plastic clips. Oscillatory potentials were recorded differentially with a saline-soaked cotton wick electrode positioned on the cornea and fixed to a silver-silver chloride wire. A platinum needle reference electrode was

inserted subcutaneously 2 mm caudal to the lateral canthus; a platinum needle ground electrode was positioned at the base of the tail. The rats were placed in a Cadwell PA-10 Ganzfeld Full-Field Stimulator (Cadwell Laboratories, Inc., Kennewick, WA) at a premarked position to ensure consistent eye placement. All procedures were carried out under dim red light.

The light intensity used was 45 joules/cm^2 measured at approximate rat eye level, with a Pasco High Sensitivity Photometer model # OS-8020 (Pasco Scientific, Roseville, CA). Ten 1.2 millisecond pulses were presented at a rate of 3.6 flashes/minute. Oscillatory potential response was recorded using a low-cut filter setting of 30 Hz and a high-cut filter setting of 200 Hz. Data were averaged using a Cadwell 5200A (Cadwell Laboratories, Inc., Kennewick, WA) and stored on computer using the Cadwell 5200A Save/Recall Program (Cadwell Laboratories, Inc., Kennewick, WA). Amplitude and latency were determined for each oscillatory potential wavelet. OP_1 amplitude was measured from baseline; the amplitudes of OP_2, OP_3, OP_4, and OP_5 were measured from the lowest preceding deflection. Oscillatory potential latencies were measured from stimulus onset to the peak of each wavelet.

7.2.4 Zinc and taurine analysis

Rats were anaesthetized with methoxyflurane (Janssen

Pharmaceutica, Mississauga, ON), blood was taken by cardiac puncture and the rats killed by decapitation. The left eyes from 8 rats per group were reserved for zinc analysis. Eyes from 4 independent rats per group (2 left and 2 right) were retained for taurine analysis. Plasma, liver and tibia were also taken for zinc analysis. Liver was stored for taurine analysis. Tissues were collected on ice and stored at -70^{0}C for taurine analysis or -20^{0}C for zinc analysis. Tissues were collected in polypropylene vials, all glassware was acid washed and the necessary precautions were taken to prevent trace element contamination from the environment.

Liver and eye taurine concentrations were determined by HPLC using the method described in Chapter 4. Plasma zinc was determined by flame atomic absorption spectrophotometry (FAAS) after diluting the sample (1:4) with distilled, deionized water. Liver, tibia and eye zinc were determined by FAAS after wet ashing (Clegg et al. 1981). Two eyes were pooled for each analysis. All readings were conducted with the instrument in the absorbance mode. Zinc concentrations were calculated from the absorbance values by use of a linear regression equation, which was obtained with seven standard concentrations made in 0.1 mol/L Ultrex grade HNO_3 (PDI Joldon, Aurora, ON). National Institute of Standards and Technology (Gaithersburg, MD) bovine serum or bovine liver was included in sample runs as a standard reference material.

Recovery for these reference materials was 91.7% and 95.7-102.2%, respectively.

7.2.5 Light microscopy

The right eyes from 8 rats per treatment group were assigned for light microscopic examination. Eyes were enucleated via transconjunctival resection and the dorsal (12 o'clock position) was marked with ink immediately after killing. Four of these eyes were immersed in Bouin's fluid for 24 hours, rinsed in 70% ethanol and stored in 70% ethanol until sectioning. Sectioning was completed after the globe was orientated and the eye hemisected vertically through the optic nerve. Hemisected culottes were embedded in paraffin and sectioned and stained routinely with haematoxylin and eosin (Render 1992). Four eyes from each treatment group were immersed in 2.5% cacodylate buffered glutaraldehyde and 1 mm sections of the retina, choroid and sclera were harvested 1 mm below the optic disk. These specimens were washed, dehydrated and embedded in epoxy. Semi-thin sections were cut on a diamond knife and stained with toluidine blue (Render 1992).

7.2.6 Statistical analysis

Data from the +Zn/+TauAL, +Zn/-Tau, -Zn/+Tau and -Zn/-Tau groups were analysed by two-factor ANOVA with zinc and taurine as the

independent variables (SuperANOVA, Abacus Concepts, Berkeley, CA). Differences between groups were determined by least significant difference (Steel and Torrie 1980). To separate effects due to zinc deficiency from those resulting from the voluntary depression in food intake which usually accompanies zinc deficiency, data from +Zn/+TauPF and -Zn/+Tau animals were compared with paired t-tests; data from +Zn/+TauAL and +Zn/+TauPF animals were compared using unpaired t-tests (Statview 512+, Abacus Concepts, Berkeley, CA). A probability of less than 0.05 was considered significant.

7.3 RESULTS

7.3.1 Body weight and feed intake

Body weight and feed intake data are presented in Table 7.2. Marginally zinc-deficient pups tended to weigh less at weaning (P=0.0717) and week 1 postweaning (P=0.0516); however, no effect of zinc was evident at later time points. Marginal zinc deficiency also failed to significantly affect feed intake over the course of the study.

Taurine-deficient pups weighed significantly less at week 1 (P=0.0053), week 2 (P=0.0030), week 3 (P=0.0100), and week 4 (P=0.0292) postweaning. A similar trend was observed at the conclusion

Table 7.2

Effect of Zinc and Taurine on Body Weight and Feed Intake[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
Body Weight (g)							
Initial	49.9±1.0	44.2±2.2	43.2±2.2	43.9±1.8	P=0.0717	P=0.1888	P=0.1016
Day 7	83.2±1.8	69.2±3.3	71.9±3.6	68.9±2.5	P=0.0516	P=0.0053	P=0.0648
Day 14	132.4±3.1	109.7±4.8	119.3±5.9	112.1±4.5	P=0.2589	P=0.0030	P=0.1101
Day 21	185.9±5.3	156.6±5.7	169.2±8.0	163.7±6.3	P=0.4549	P=0.0100	P=0.0716
Day 28	237.6±8.1	202.8±7.1	222.8±11.2	217.7±8.2	P=0.9941	P=0.0292	P=0.1002
Final	279.6±10.4	234.5±7.1	266.4±18.8	267.5±10.5	P=0.4313	P=0.0860	P=0.0717
Feed Intake (g)							
Week 1	68.6±1.4	64.4±2.5	64.7±2.5	61.9±2.1	P=0.1580	P=0.1149	P=0.7560
Week 2	171.9±3.1	156.1±5.4	163.0±6.3	153.6±4.7	P=0.2692	P=0.0170	P=0.5247
Week 3	316.7±6.7	283.2±9.1	294.6±11.7	281.6±8.7	P=0.2066	P=0.0161	P=0.2714
Week 4	481.6±12.4	431.4±13.0	455.1±19.1	441.4±12.5	P=0.5723	P=0.0344	P=0.2170
Final	635.2±21.5	561.0±14.3	601.4±47.5	602.1±22.0	P=0.9014	P=0.2155	P=0.2080

[1]Results expressed as mean±SEM; n=10. Values with different superscripts differ significantly (P<0.05) . Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables. When a significant effect due to an interaction was found, differences between groups were determined by least significant difference.

of the experiment (P=0.0860). In addition, taurine deficiency depressed feed intake at week 2 (P=0.0170), week 3 (P=0.0161) and week 4 (P=0.0344); this difference was not apparent at the conclusion of the experiment. No interaction between zinc and taurine was detected at any time point.

The +Zn/+TauPF animals weighed significantly less than the +Zn/+TauAL animals at all time points measured (P<0.05; Appendix C); the +Zn/+TauPF animals had weights similar to those of the -Zn/+Tau group. Mean (±SEM) body weight for the +Zn/+TauPF group at the conclusion of the study was 234.8±10.2 g (n=10). In addition, the feed intake of the +Zn/+TauPF animals was significantly less than that of the +Zn/+TauAL animals at week 1, 2, 3 and 4 postweaning (P<0.05; Appendix C); a similar trend (P=0.0762) was observed at the conclusion of the experiment. The feed intake of the +Zn/+TauPF group was significantly less than that of the -Zn/+Tau group at all time points measured. Mean (±SEM) total feed intake for the +Zn/+TauPF group at the conclusion of the study was 553.4±37.8 g (n=10).

7.3.2 Tissue zinc concentrations

Tissue zinc concentrations are presented in Table 7.3. A significant interaction between zinc and taurine was observed for plasma zinc concentrations (P=0.0029). Feeding rats marginally zinc-deficient

Table 7.3

Influence of Zinc and Taurine Status on Tissue Zinc and Taurine Concentrations[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
Zinc Levels							
Plasma (µg/ml)	1.59±0.06[a]	1.49±0.05[a,b]	1.16±0.06[c]	1.41±0.05[b]	P=0.0001	P=0.1819	P=0.0029
Tibia (µg/g)[2]	303.7±3.2[a]	286.2±4.4[b]	165.2±3.4[c]	206.8±2.7[d]	P=0.0001	P=0.0014	P=0.0001
Liver (µg/g)[2]	95.2±2.6	92.8±1.9	85.2±1.9	84.8±1.5	P=0.0001	P=0.4895	P=0.6304
Eye (µg/g)[2]	19.7±0.5	18.4±0.6	17.8±0.2	16.3±0.3	P=0.0006	P=0.0082	P=0.8350
Taurine Levels							
Liver (µmol/g)[3]	6.76±0.82[a]	1.15±0.15[b]	9.20±0.61[c]	1.50±0.18[b]	P=0.0115	P=0.0001	P=0.0546
Eye (µmol/g)[3]	10.3±0.1	7.0±0.2	10.1±0.5	6.8±0.2	P=0.5245	P=0.0001	P=0.9867

[1]Results expressed as mean±SEM; n=10 for tibia and liver; n=4 for eye; n=9 for +Zn/+TauAL and -Zn/-Tau plasma and n=10 for +Zn/-Tau and -Zn/+Tau plasma. Values with different superscripts differ significantly (P<0.05) . Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables. When a significant effect due to an interaction was found, differences between groups were determined by least significant difference.
[2]Expressed on a dry weight basis.
[3]Expressed on a wet weight basis.

diets significantly depressed plasma zinc concentrations (+Zn/+TauAL vs -Zn/+Tau); this effect was reversed when the animals were also made taurine-deficient (-Zn/+Tau vs -Zn/-Tau).

A significant interaction between zinc and taurine was observed for tibial zinc concentration (P=0.0001). Zinc deficiency significantly depressed tibial zinc concentrations (+Zn/+TauAL vs -Zn/+Tau); however, this effect was less pronounced when the animals were also taurine-deficient (-Zn/+Tau vs -Zn/-Tau).

Marginal zinc deficiency (P=0.0006) and taurine deficiency (P=0.0082) both significantly depressed eye zinc concentrations; however, no statistical interaction between zinc and taurine was detected.

No interaction between zinc and taurine was observed in liver zinc concentration. However, marginal zinc deficiency did significantly depress liver zinc concentrations (P=0.0001).

Tissue concentrations of zinc in the +Zn/+TauPF group were similar to those of the +Zn/+TauAL group. Mean (±SEM) zinc concentrations for tissues from the +Zn/+TauPF group are as follows: tibia (322.3±4.8 µg/g; n=10), plasma (1.55±0.08 µg/ml; n=9), eye (19.5±1.6 µg/g; n=4) and liver (96.1±2.9 µg/g; n=10).

7.3.3 Tissue taurine concentrations

Tissue taurine concentrations are presented in Table 7.3.

Although a statistically significant interaction between zinc and taurine was not demonstrated for liver taurine concentration (P=0.0546), a trend was present. When animals were fed taurine-deficient diets and GES, liver taurine concentrations were significantly depressed independent of zinc status (+Zn/+TauAL vs +Zn/-Tau and -Zn/-Tau). Zinc deficiency, however, significantly elevated the liver taurine levels of taurine-adequate animals (+Zn/+TauAL vs -Zn/+Tau).

Taurine deficiency significantly depressed eye taurine concentrations (P=0.0001), but no interaction between zinc and taurine was observed (P=0.9867).

Tissue concentrations of taurine in the +Zn/+TauPF group were similar to those of the +Zn/+TauAL group. Mean (±SEM) taurine concentrations for tissues from the +Zn/+TauPF groups are as follows: liver (6.98±0.81 µmol/g; n=10) and eye (11.1±0.5 µmol/g; n=4).

7.3.4 Ophthalmoscopic assessment

Examination by indirect ophthalmoscope at 7 weeks postnatal age revealed focal retinal folds ventral to the optic disc in 5 out of 10 rats (Figure 7.1) and multiple coalescing patches of choroidal atrophy in 2 out of 10 rats from the -Zn/-Tau group. Retinal folds were first noted at 4 weeks in 4 out of 10 rats. No abnormalities were evident in animals from the +Zn/+TauAL, -Zn/+Tau, +Zn/-Tau or +Zn/+TauPF groups.

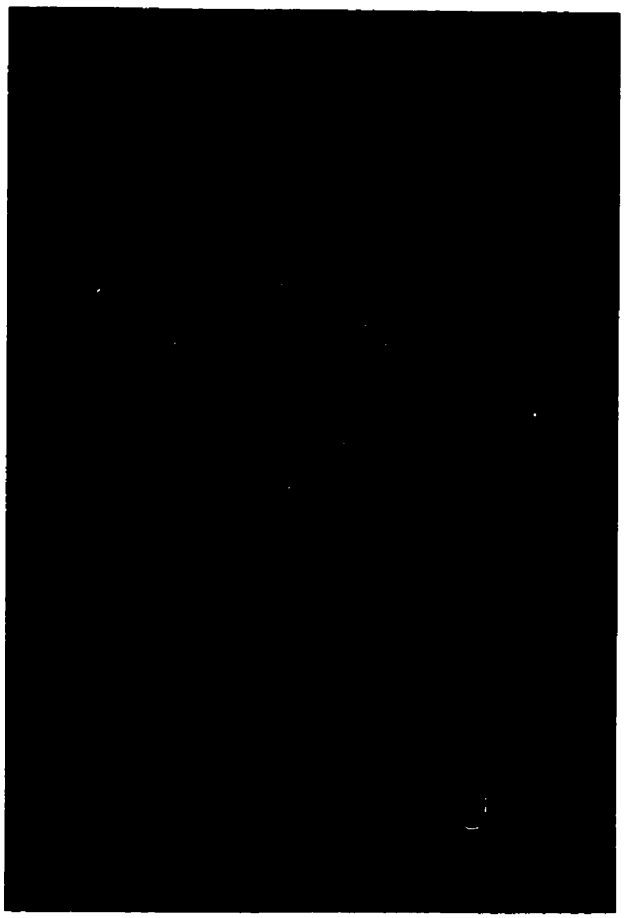

Figure 7.1

Fundus from a Rat Photographed Through a 20 Diopter Lens

Presents a fundus from an animal deficient in zinc and taurine (-Zn/-Tau). A focal retinal fold is visible (outlined by arrows).

Biomicroscopic examination at 7 weeks identified the unilateral development of uveitis, a focal keratitis and an anterior cortical cataract in one animal from the +Zn/+TauPF group and one animal from the +Zn/+TauAL group. These lesions were consistent with incidental trauma and the eyes were not included in electroretinographic or histologic assessments.

7.3.5 Oscillatory potentials

Oscillatory potentials from a control animal are shown in Figure 7.2. Individual OP were variably affected by the treatments (Table 7.4). Interactive effects between zinc and taurine were observed in OP_2 (P=0.0346) and OP_3 (P=0.0357) amplitudes; marginal zinc deficiency decreased the amplitude of the oscillatory potential, but only when animals were also taurine deficient (+Zn/+TauAL vs -Zn/-Tau).

Feeding animals marginally zinc-deficient diets significantly depressed the amplitude (P=0.0145) and increased the latency (P=0.0236) of OP_1. The latency of OP_3 was also significantly increased (P=0.0367). Mean (±SEM) OP_1 amplitude of the -Zn/+Tau group was not significantly different from that of the +Zn/+TauPF group when compared by paired t-test (90.6±8.6 µvolts vs 81.9±7.4 µvolts; n=9; P=0.2632); mean (±SEM) OP_1 amplitude of the +Zn/+TauPF group was significantly less than that of the +Zn/+TauAL group when compared by unpaired t-test

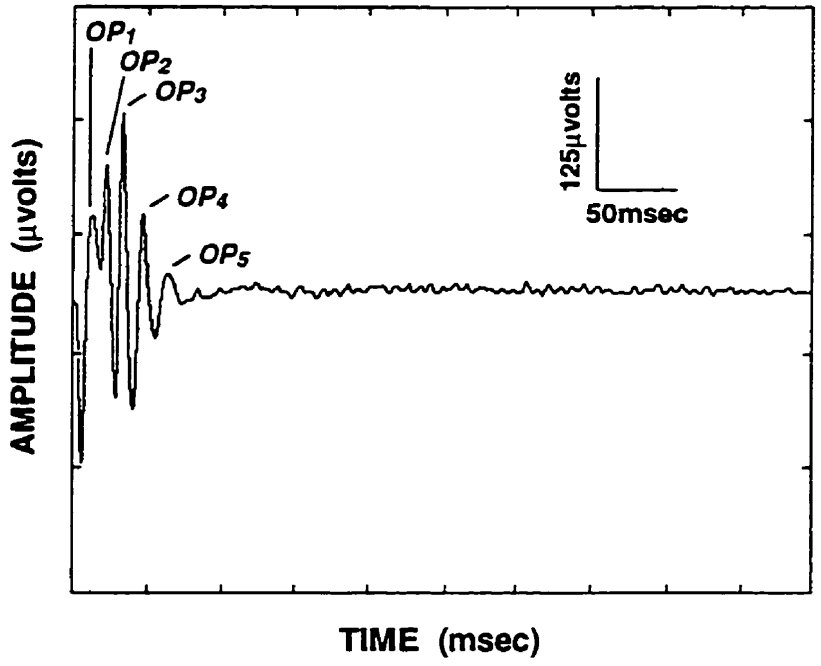

Figure 7.2

Oscillatory potentials recorded from a control rat (+Zn/+TauAL) after overnight dark adaptation. This tracing is an average of 10 responses. Oscillatory potentials are labelled OP_1 to OP_5.

Table 7.4

Oscillatory Potential Amplitudes and Latencies in Response to Varied Zinc and Taurine Status During Gestation and Postnatal Life[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
OP 1							
Amplitude (µvolts)	117.0±11.4	97.2±11.5	94.7±8.7	64.2±11.2	P=0.0145	P=0.0252	P=0.6238
Latency (msec)	12.9 ± 0.3	12.1±0.3	13.1±0.3	13.4±0.4	P=0.0236	P=0.3684	P=0.0716
OP 2							
Amplitude (µvolts)	110.4±11.7^{a}	110.6±13.1^{a}	127.5±12.4^{a}	74.0±11.0^{b}	P=0.4282	P=0.0360	P=0.0346
Latency (msec)	20.3±0.3	19.8±0.6	20.6±0.5	20.6±0.4	P=0.2573	P=0.5192	P=0.5517
OP 3							
Amplitude (µvolts)	299.2±17.1^{a,b}	256.9±18.9^{a}	330.9±26.4^{b}	195.8±20.0^{c}	P=0.4938	P=0.0002	P=0.0357
Latency (msec)	30.7±0.7	29.5±0.7	31.4±0.6	31.9±0.8	P=0.0367	P=0.6101	P=0.2338
OP 4							
Amplitude (µvolts)	223.3±20.3	174.4±13.8	228.1±19.1	148.3±13.4	P=0.5351	P=0.0006	P=0.3675
Latency (msec)	43.9±0.8	41.8±1.1	44.7±0.7	44.6±1.1	P=0.0688	P=0.2556	P=0.3075
OP 5							
Amplitude (µvolts)	93.1±11.6	74.1±9.2	88.4±8.3	72.6±6.6	P=0.7392	P=0.0639	P=0.8647
Latency (msec)	59.2±0.9	56.2±1.1	59.7±1.0	59.6±1.4	P=0.0815	P=0.1765	P=0.1964

[1]Results expressed as mean±SEM; n=9 for +Zn/+Tau and -Zn/-Tau, n=10 for +Zn/-Tau and -Zn/+Tau. Values with different superscripts differ significantly (P<0.05). Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables. When a significant effect due to an interaction was found, differences between groups were determined by least significant difference.

(81.9±7.4 μvolts vs 117±11.4 μvolts ; n=9; P=0.0204). Similarly, mean (±SEM) OP_1 latency of the -Zn/+Tau group was not significantly different from that of the +Zn/+TauPF group (13.2±0.3 msec vs 14.1±0.3 msec; n=9: P=0.1038); mean (±SEM) OP_1 latency of the +Zn/+TauPF group was significantly greater than that of the +Zn/+TauAL group (14.1±0.3 msec vs 12.9±0.3 msec; n=9; P=0.0167).

The amplitudes of OP_1 (P=0.0252) and OP_4 (P=0.0006), in taurine-deficient animals were also significantly depressed; the amplitude of OP_5 demonstrated a similar depression (P=0.0639). However, no significant effects of taurine deficiency were seen on the latencies of the oscillatory potentials.

7.3.6 Light microscopy

Light microscopy revealed degeneration of photoreceptor outer segments and confirmed choroidal atrophy and retinal dysplasia in the -Zn/-Tau group (Figure 7.3). Generalized loss of the outer segments of the photoreceptors was apparent (Figure 7.4a); in some areas only blunted inner segments remained and the retinal pigment epithelium appeared hyperplastic and hypertrophied. These abnormalities and atrophy of the choroid were most prominent in areas of retinal dysplasia.

Examination of retinas from +Zn/-Tau animals revealed mild degeneration of the photoreceptors; the outer segments were shorter and

Figure 7.3

Haematoxylin and Eosin Stained Retinal Sections

Presents a retinal section from a rat deficient in both zinc and taurine (-Zn/-Tau). This section was cut through the retinal fold shown in Figure 7.1 and confirms the presence of retinal dysplasia (arrows). Compare with figure 7.4a on the following page. Bar equals 10 μ.

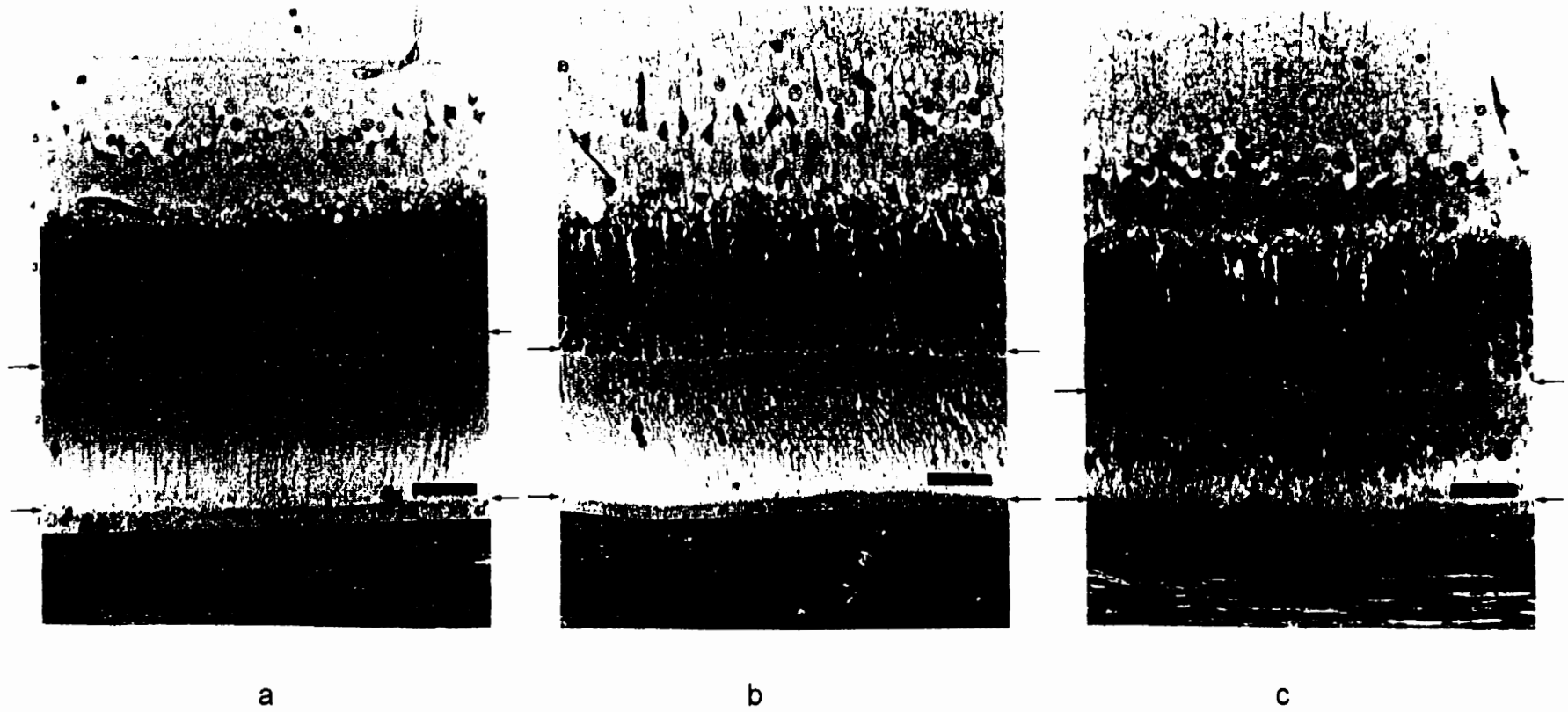

Figure 7.4

Toluidine Blue Stained Retinal Thin Sections

Arrows mark external limiting membrane and retinal pigment epithelium-photoreceptor junction. (1) Retinal pigment epithelium, (2) photoreceptor layer, (3) outer nuclear layer, (4) outer plexiform layer, (5) inner nuclear layer. See page 21 for a description of the various retinal layers. Bar equal 30 µm. a) Representative section from a control rat (+Zn/+TauAL). b) Representative section from a rat deficient in taurine (+Zn/-Tau). Mild degeneration of the photoreceptors is apparent. Note the inner and outer segments are decreased in length (arrows). c) Representative section from a rat deficient in zinc and taurine (-Zn/-Tau). Generalized loss of the outer segments of the photoreceptors is apparent. Note the inner and outer segments are reduced in length (arrows).

contained numerous vesicles, disorientated discs and discontinuous cell membranes (Figure 7.4b).

Retinas from -Zn/+Tau, +Zn/+TauPF and +Zn/+TauAL groups were similar and no abnormalities were noted (Figure 7.4c).

7.4 DISCUSSION

The results of this study provide morphological and functional evidence that zinc interacts with taurine in the developing rat retina. Rat pups were exposed to variable zinc and taurine status throughout the period of retinal development which begins prenatally and extends until approximately 6 weeks of age (Braekevelt and Hollenberg 1970). Light microscopic examination at 7½-8½ weeks postnatal age demonstrated marked photoreceptor degeneration and confirmed retinal dysplasia, detected with the ophthalmoscope at 4 weeks, in animals deficient in both zinc and taurine. These findings provide strong evidence for an interaction as the photoreceptors from zinc-deficient rats appeared histologically normal and only mild photoreceptor degeneration was seen in the taurine-deficient group. Retinal folds have previously been reported in term fetuses of severely zinc-deficient Long-Evans rats (Rogers and Hurley 1987). Spontaneous retinal dysplasia of unknown etiology has also been described in normal Sprague-Dawley rats (Hubert et al. 1994). However, no

evidence of this condition was observed in control animals.

Oscillatory potentials provide further evidence of an interaction between zinc and taurine; zinc deficiency depressed the amplitude of OP_2 and OP_3 only when the animals were also taurine-deficient. The presence of an interaction in only two of the five oscillatory potentials suggests that zinc and taurine may function synergistically in only specific areas of the inner retina. Each of the five oscillatory potentials is believed to originate independently from one another possibly in different areas of the inner nuclear layer (el Azazi and Wachtmeister 1990). The reversibility of these changes is not known. Interaction was also evident in the depressing effects of taurine deficiency on whole eye zinc concentration. However, it is difficult to relate these changes to the morphological and functional changes in the retina; whole eye may not be representative of retinal concentrations because the levels of these nutrients in different eye tissues are variable (Eckhert 1983; Heinämäki et al. 1986).

The retinal degeneration observed in combined zinc and taurine deficiency is particularly important given the marginal degree of zinc restriction employed in this study. This model was chosen to represent the degree of zinc deficiency that could occur in the human population. Although zinc deficiency was confirmed by significantly depressed plasma, tibia, liver and whole eye zinc concentrations, the animals showed no clinical signs. The small decrease in feed intake in zinc-deficient rats did not reach statistical significance although intake was consistently lower than that of control rats at all timepoints. Pups

born to dams fed low zinc diets weighed less at weaning and week 1 postweaning. No significant growth depression was apparent at later timepoints. suggesting that the effects of zinc deficiency may have been greater during the perinatal period.

The model of taurine deficiency used in this study is similar to that described previously (Bonhaus et al. 1985, Lake 1983, Pasantes-Morales 1983). Rats fed taurine-deficient diets and GES had depressed liver and whole eye taurine concentrations consistent with other reports (Bonhaus et al. 1985). Depressed retinal taurine has also been described using this model (Cocker and Lake 1989, Lake 1981, Rapp et al. 1987). Other investigators have reported limited data on food intake and weight gain. In the present study, taurine deficiency tended to depress food intake and weight gain in the postweaning period, whereas weight gain at weaning was similar to that of control rats. Previous research that found no intrauterine growth retardation in fetuses of rats made taurine-deficient throughout gestation (Chapter 5) and no differences in pup weights during lactation up to postnatal day 17 (Lake 1983) are consistent with this observation.

The biochemical mechanisms responsible for the interaction of zinc with taurine are unknown. Zinc has been proposed to play a physiological role in plasma membranes by its effects on membrane protein conformation and protein-protein interactions (Bettger and O'Dell 1993). While the precise biochemical roles in the membrane have not been fully elucidated, it has been

suggested that the loss of zinc from specific proteins in the plasma membrane alters water and ion channels. Ultimately intracellular ion and water concentration is changed (Bettger and O'Dell 1993). It was hypothesized that taurine, through its role as an intracellular osmoregulator (Huxtable 1992), can respond by regulating water and ion flow across the photoreceptor and inner retinal membrane. The photoreceptor degeneration in combined zinc and taurine deficiency may represent a loss of membrane integrity in the outer retina exacerbated by the loss of an osmoregulatory mechanism. Depressed OPs may reflect decreased integrity of neuronal membranes and altered retinal conduction in the inner retinal regions.

In addition to retinal pathology, evidence of focal choroidal atrophy in rats deficient in both zinc and taurine was also observed. This anomaly was initially detected on ophthalmoscopic assessment and confirmed by light microscopy. These data provide additional evidence of an interaction between zinc and taurine in another eye structure. Choroidal atrophy has been reported to occur spontaneously in normal Sprague-Dawley rats (Huber et al. 1994); the etiological significance is unknown. No evidence of this condition was found in any other experimental group.

Plasma and tibial zinc concentrations provide evidence that zinc and taurine interact in other tissues. Zinc deficiency depressed plasma and tibial zinc; however, the effect was less pronounced when animals were also taurine-deficient suggesting that taurine influences the zinc status of the animal. Zinc

apparently also influences taurine metabolism because liver taurine concentration was elevated by zinc deficiency. Other evidence for an interaction outside retinal tissue is the trend observed for body weight. Taurine deficiency decreased body weight but this effect was less pronounced when the animals were also deficient in zinc. This is an intriguing finding given the well described growth-depressing effect of zinc deficiency.

The main treatment effects observed in this study also deserve consideration. Independent effects of taurine deficiency on specific oscillatory potentials were evident with reductions in the amplitudes of OP_1 and OP_4. Although previous research has identified the importance of taurine in development of normal photoreceptor morphology and function (Bonhaus et al. 1985, Imaki et al. 1986), the results of this research suggest that taurine also functions in the inner retina. These findings support the work of Lake and Malik (1987) who reported a reduction in inner retinal width on light microscopic examination in rats treated with GES for the last week of gestation and for 8 weeks following parturition. The photoreceptor disruption in taurine-deficient animals that has been previously reported (Bonhaus et al. 1985, Lake and Malik 1987, Pasantes-Morales et al. 1983) was also observed in this study.

The main treatment effect of zinc on certain oscillatory potentials suggests additional independent biochemical functions for zinc in the retina. Marginal zinc deficiency independently depressed the amplitude and increased the latency of OP_1, and increased the latency of OP_3. Decreased photoreceptor

zinc content has previously been observed by histochemical localization (Hirayama 1990) and morphological changes have been reported in the photoreceptors and retinal pigment epithelium of severely zinc-deficient rats (Leure-duPree and McClain 1982). Little information is available on the influence of marginal zinc deficiency, particularly in regions of the inner retina. While no morphological alterations were detected in marginally zinc-deficient rats, future studies using electron microscopy may detect ultrastructural damage that was not apparent on gross histological assessment. The similar depressing effect of pair-feeding on OP_1 suggests that the retinal damage is due to the small but not statistically significant depression in feed intake that accompanied the zinc deficiency. These findings are difficult to interpret, however, as feed intake of the pair-fed animals was not equal to but significantly less than that of the zinc-deficient group. This limitation was related to the inaccuracy of pair feeding and collection of feed spillage in a extremely marginal zinc deficiency model. The effects of zinc deficiency on retinal electrophysiology and morphology and the mechanism by which zinc exerts these effects deserves further investigation.

In summary, morphological and physiological evidence has been obtained that zinc and taurine interact in the developing rat retina. This has been demonstrated by severe photoreceptor damage, retinal dysplasia and depressed oscillatory potentials in combined zinc and taurine deficiency. Evidence of an interaction in other tissues was noted in plasma zinc, tibia zinc and liver taurine concentrations. Choroidal atrophy was also apparent in the combined deficiency

group. The mechanism responsible for this interaction deserves investigation. In addition, independent effects of zinc and taurine on oscillatory potentials were demonstrated. These have not been previously identified and they suggest additional independent functions for each of these nutrients in the inner retina.

Chapter 8

ELECTRORETINOGRAPHIC CHANGES IN RATS DEFICIENT IN ZINC AND TAURINE DURING PRENATAL AND POSTNATAL LIFE[1]

8.1 INTRODUCTION

A 50% reduction of retinal taurine in the cat produces degeneration of the photoreceptors (Hayes et al. 1975) and depression of electroretinogram (ERG) a- and b-wave amplitudes (Berson et al. 1976). With prolonged taurine deficiency, blindness develops. The addition of 200 μM zinc to the drinking water of the taurine-deficient cat has been reported to partially protect the a-wave amplitude of the ERG (Pasantes-Morales et al. 1987) suggesting that zinc

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[1]A version of this chapter has been published [Gottschall-Pass et al. (1997) Intensity-response functions of the electroretinogram in rats deficient in zinc and taurine throughout gestation and into postnatal life. In: Trace Elements in Man and Animals - 9: Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals (Fischer , P.W.F. et al., Eds) pp. 91-92, NRC Press, Ottawa, Canada]. Reproduced with permission of the NRC Research Press.

and taurine may interact in the retina. In *in vitro* studies, zinc and taurine have been shown to protect frog rod outer segments from ferrous sulfate-induced disruption in a synergistic manner (Pasantes-Morales and Cruz 1984).

Rats made taurine-deficient with guanidinoethyl sulfonate (GES), a structural analogue of taurine, develop retinal changes similar to those observed in taurine-deficient cats. These include reduced a- and b-wave amplitudes of the ERG (Cocker and Lake 1987, Hageman and Schmidt 1987, Lake 1986, Rapp et al. 1987) with a reduction in Vmax (Cocker and Lake 1987). The latter represents the maximum amplitude attained when b-wave amplitudes are plotted as a function of log stimulus intensity.

The role of zinc in the retina is less clear. Electron microscopic examination of the retinas from severely zinc-deficient rats shows degeneration of the photoreceptor outer segments and accumulation of osmiophilic inclusion bodies in the retinal pigment epithelium (Leure-duPree and McClain 1982). Photoreceptor loss and degeneration of the retinal pigment epithelium has also been described in patients with acrodermatitis enteropathica, an inborn error of zinc metabolism in which zinc absorption is impaired (Cameron and McClain 1986).

Despite evidence of structural damage, retinal function has not been extensively studied, particularly in a marginal zinc deficiency state as might be observed in the human population. Cats fed diets containing less than 7 ppm zinc for 16-20 weeks showed a reduction in the b-wave amplitude of the dark-

adapted ERG that was reversible upon zinc repletion. Unfortunately, the number of animals examined in this study was small (Jacobson et al. 1986). Karcioglu et al. (1984) described depressed plasma zinc concentrations in retinitis pigmentosa patients who had no recordable ERG. It has also been reported that the elevated dark adaptation thresholds in patients with alcoholic cirrhosis and depressed serum zinc concentrations are improved with zinc supplementation (Morrison et al. 1978; Russell et al., 1978).

The objective of this research was to investigate whether the interaction between zinc and taurine influences the electroretinogram of the rat. The developing retina should be particularly sensitive to this interaction as these nutrients are critical to normal pre- and postnatal development (Lönnerdal 1988; Sturman 1988). It was hypothesized that marginal zinc deficiency imposed throughout gestation and postnatal life would act synergistically to worsen the electrophysiological response that occurs in taurine deficiency. In addition, the independent effect of marginal zinc deficiency on the electrophysiology of the retina was studied.

8.2 MATERIALS AND METHODS

8.2.1 Animals and Treatment

Data for this experiment were obtained from the animal model used

in Chapter 7. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care 1993). All procedures used in this study were approved by the University of Saskatchewan Committee on Animal Care and Supply.

Animals were fed control diet for at least 7 days and bred overnight. On day 0 of gestation, as determined by the presence of sperm in vaginal smears, the females were placed individually in stainless steel cages and randomly assigned to 1 of 5 treatments. Treatments consisted of 4 diets formulated with 2 levels of zinc (**+Zn**, 50 µg Zn/g diet; **-Zn**, 15 µg Zn/g diet) and 2 levels of taurine (**+Tau**, 2 µmol taurine/g diet; **-Tau**, 0 µmol taurine/g diet) in the following combinations: +Zn/+TauAL (control), +Zn/-Tau, -Zn/+Tau or -Zn/-Tau. Zinc content of the -Zn/+Tau and -Zn/-Tau diets was reduced to 7.5 µg/g at parturition. The above groups were provided with free access to modified AIN-93G diets (Table 7.1) and distilled deionized water. One percent (w/v) GES was added to the drinking water of the +Zn/-Tau and -Zn/-Tau groups. In order to separate the influence of lack of zinc from that due to the depression in food intake that accompanies zinc deficiency, a fifth group (+Zn/+TauPF) was fed control diet and pair-fed to the -Zn/+Tau group.

On day 20 of gestation, dams were transferred to stainless steel cages fixed with plastic mesh bottoms and provided with 30 x 50 cm of

absorbent paper (VWR, Edmonton, AB) as nesting material. Litters were adjusted to 7-10 pups at postnatal day 4. Nesting material was removed within 7 days of birth. At postnatal day 23, male pups (10/treatment) were weaned onto their respective diets.

8.2.2 Electroretinograms

The ERG were recorded on pups at 7½-8½ weeks of age after overnight dark adaptation. Recordings were made within 5 hours of the usual onset of light. All procedures were carried out under dim red light. Animals were anaesthetized with an intramuscular injection of ketamine (30 mg/kg body wt; rogar/STB Inc., London, ON) and xylazine (7-8 mg/kg body wt; Chemagro Ltd., Etobicoke, ON). The right pupil was dilated with 1% tropicamide (Alcon Canada Inc., Mississauga, ON) and the cornea anaesthetized with proparacaine hydrochloride (Allergan Inc., Markham, ON). The eyelids were retracted with plastic clips. Electroretinograms were recorded differentially with a saline-soaked cotton wick electrode positioned on the cornea and fixed to a silver-silver chloride wire. A platinum needle reference electrode was inserted subcutaneously 2 mm caudal to the lateral canthus; a platinum needle ground electrode was positioned at the base of the tail. The animals were placed in a Cadwell PA-10 Ganzfeld Full-Field Stimulator (Cadwell Laboratories, Inc., Kennewick, WA) at a premarked position to ensure consistent eye

placement.

Maximum light intensity used was 45 joules/cm^2 measured with a Pasco high sensitivity photometer model # OS-8020 (Pasco Scientific, Roseville, CA). Light flashes were attenuated with neutral density filters over 8 log units (ND0 - ND8). Ten 1.2 millisecond pulses were presented at each intensity at a rate of 3.6 flashes/minute, beginning with the dimmest stimuli. ERG response was recorded using a low-cut filter setting of 1 Hz and a high-cut filter setting of 3000 Hz. Data were averaged using a Cadwell 5200A (Cadwell Laboratories, Inc., Kennewick, WA) and stored on computer using the Cadwell 5200A Save/Recall Program (Cadwell Laboratories, Inc., Kennewick, WA). To avoid adaptation effects, subsequent ERG were performed after 5 minute intervals.

The ERG a- and b-wave amplitudes and latencies were determined for each recording. Amplitude of the a-wave was measured from baseline; b-wave amplitude was measured from baseline or from the peak of the a-wave when present. Latencies were measured from stimulus onset to the peak of each wave.

8.2.3 Intensity-Latency Analysis

ERG b-wave latencies were analysed by finding the best linear fit to log relative intensity through linear regression analysis (SuperANOVA,

Abacus Concepts, Berkely, CA). At illuminances below -2.8 log, implicit time is no longer a linear function of log intensity (Massof et al. 1984); therefore, only the four highest light intensities were used in this analysis. Latency-intensity functions were not included in further analysis if the r value ≤ 0.8.

8.2.4 Naka-Rushton Analysis.

Electroretinogram b-wave amplitudes as a function of log stimulus intensity were analysed by finding the parameters of the best fit modified Naka-Rushton function using nonlinear analysis (Winnonlin, Scientific Consulting Inc., Apex, NC):

$$V=V_0+((Vmax \times I^n)/(I^n+\sigma^n)) \quad (1)$$

where V represents b-wave amplitude, V_0 is the nonzero baseline effect, Vmax is maximum b-wave amplitude, I is intensity, σ is the intensity required to produce half maximal response and n is an exponent describing the slope of the function. The original Naka-Rushton equation forces the b-wave amplitude towards zero. We did not measure zero amplitude at low light intensity; the resulting poor curve fit overestimated

Vmax. The addition of the non-zero baseline parameter V_0 to this function resulted in a fit that better defines the data generated and in particular the parameter Vmax. This curve fitting procedure was applied to the data obtained from each animal. Best fit for each amplitude-intensity curve was determined by visual inspection, a correlation coefficient ≥ 0.95 and comparison of each parameter generated from the Naka-Rushton function to ensure that it fell within mean(±2SD) for the group. If 2 or more of these conditions were not met, the animal was not included in further analysis.

8.2.5 Statistical Analysis.

Data from the +Zn/+TauAL, +Zn/-Tau, -Zn/+Tau and -Zn/-Tau groups were analysed by two-factor ANOVA with zinc and taurine as the independent variables (SuperANOVA, Abacus Concepts, Berkeley, CA). Differences between groups were determined by least significant difference. To separate effects due to zinc deficiency from those resulting from the voluntary depression in food intake which usually accompanies zinc deficiency, data from +Zn/+TauPF and -Zn/+Tau animals were compared with paired t-tests; data from +Zn/+TauAL and +Zn/+TauPF animals were compared using unpaired t-tests (Statview 512+, Abacus Concepts, Berkeley, CA). A probability of less than 0.05 was considered significant.

8.3 RESULTS

8.3.1 Zinc and Taurine Status

Data on food intake, weight gain and tissue zinc and taurine concentrations of the pups have been reported previously (Chapter 7). Zinc deficiency was confirmed on the basis of depressed plasma, liver, tibia and eye zinc concentration; taurine depletion was demonstrated by decreased liver and eye taurine. Marginal zinc deficiency failed to significantly affect food intake or body weight of the pups over the course of the study; tissue zinc concentrations in the +Zn/+TauPF group were similar to those of the +Zn/+TauAL group.

8.3.2 Maximum Intensity.

Electroretinograms from a control animal measured over varying light intensities are shown in Figure 8.1. Data on ERG a- and b-wave amplitudes and latencies in response to maximum light intensity are presented in Table 8.1. No interaction was found between zinc and taurine for ERG a-wave ($P=0.6137$) or b-wave ($P=0.3838$) amplitudes or for a-wave ($P=0.6577$) or b-wave ($P=0.1716$) latencies at maximal light intensity. In addition, no interaction was observed for these parameters at any other light intensity measured (Appendix D & E).

Feeding animals marginally zinc-deficient diets significantly

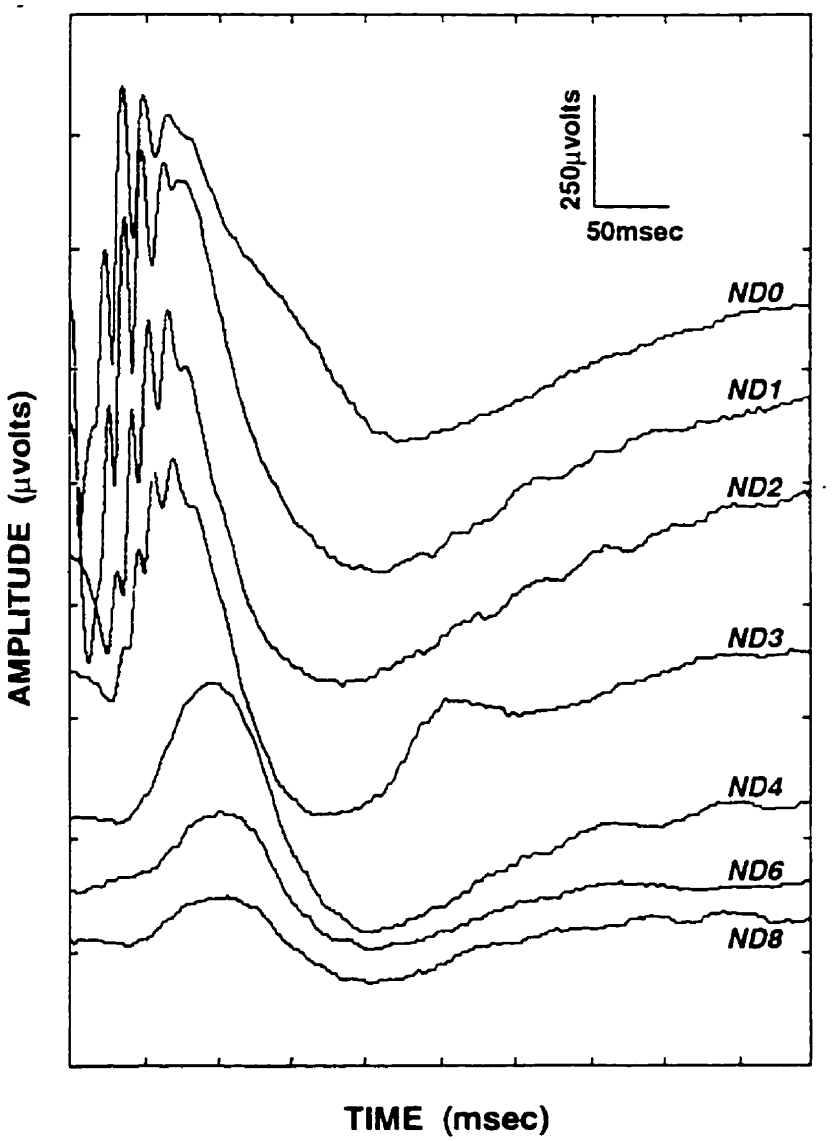

Figure 8.1

Electroretinogram recorded from a control rat (+Zn/+TauAL) measured over varying light intensities (ND is neutral density filter). Each tracing is an average of 10 responses.

Table 8.1

Latency and Amplitude of ERG a- and b-wave Peaks to the Brightest Stimulus in Response to Varied Zinc and Taurine Status During Gestation and Postnatal Life[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
a-wave							
Amplitude (µvolts)	475.0±43.4	356.9±39.7	391.7±36.6	234.7±31.6	P=0.0110	P=0.0010	P=0.6137
Latency (msec)	6.2±0.0	6.2±0.0	6.5±0.4	6.7±0.3	P=0.1892	P=0.6577	P=0.6577
b-wave							
Amplitude (µvolts)	889.6±77.1	668.8±60.1	818.1±71.3	479.2±56.9	P=0.0596	P=0.0002	P=0.3838
Latency (msec)	38.4±2.3	32.1±1.1	34.2±2.0	34.2±2.0	P=0.2368	P=0.5995	P=0.1716

[1]Results expressed as mean±SEM; n=9. Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables.

depressed both the a-wave (P=0.0110) and b-wave (P=0.0596) amplitudes of the ERG in response to maximum light intensity. No effect of zinc deficiency on a-wave (P=0.1892) or b-wave (P=0.2368) latencies, in response to maximal light intensity was noted. In addition, no effect of zinc restriction was observed for these parameters at other light intensities measured (Appendix D & E).

Mean (±SEM) a-wave amplitude of the -Zn/+Tau group was not significantly different from that of the +Zn/+TauPF group when compared by paired t-test (395.5±34.2 µvolts, n=7 vs 375.9±30.5 µvolts, n=7; P=0.4791); mean (±SEM) a-wave amplitude of the +Zn/+TauPF group was not significantly different from that of the +Zn/+TauAL group when compared by unpaired t-test (391.4±30.6 µvolts, n=8 vs 475.0±43.4 µvolts, n=9; P=0.1452). Similarly, mean b-wave amplitude of the -Zn/+Tau group was not significantly different from that of the +Zn/+TauPF group (844.6±71.0 µvolts, n=7 vs 765.2±63.6 µvolts, n=7; P=0.2179); mean (±SEM) b-wave amplitude of the +Zn/+TauPF group was not significantly different from that of the +Zn/+TauAL group when compared by unpaired t-test (783.6±58.0 µvolts, n=8 vs 889.6±77.1 µvolts, n=9; P=0.2992). Mean (±SEM) values for the +Zn/+TauPF group differ in the paired and unpaired t-tests. This is a result of differing sample size due to the need to use complete pairs for paired t-test analysis. Although no statistically significant differences were found

among the three groups, there is a trend toward a depressing effect of pair-feeding on ERG a- and b-wave amplitudes.

Electroretinogram a-wave (P=0.0010) and b-wave (P=0.0002) amplitudes in response to maximum light intensity were also significantly depressed in taurine-deficient animals. However, no significant effects of taurine deficiency were seen on the latencies of the a-wave (P=0.9318) or b-wave (P=0.5011).

8.3.3 Latency-Intensity.

No interaction or treatment effects were apparent for the slope when b-wave latencies were plotted as a function of log stimulus intensity (Appendix F). Mean (±SD) slopes for each group are as follows: +Zn/+TauAL (-8.21±1.81; n=9); +Zn/-Tau (-8.76±1.31; n=8); -Zn/+Tau (-9.52±1.44; n=9); -Zn/-Tau (-8.40±2.49; n=8); +Zn/+TauPF (9.23±2.25; n=8). These data must be interpreted with caution as the Cadwell 5200A used to measure latency was not sensitive in detecting very small changes in this parameter.

8.3.4 Amplitude-Intensity.

Average b-wave amplitude plotted as a function of log stimulus intensity from 4/5 groups is presented in Figure 8.2. Data on ERG b-wave amplitude as a function of log stimulus intensity generated from the

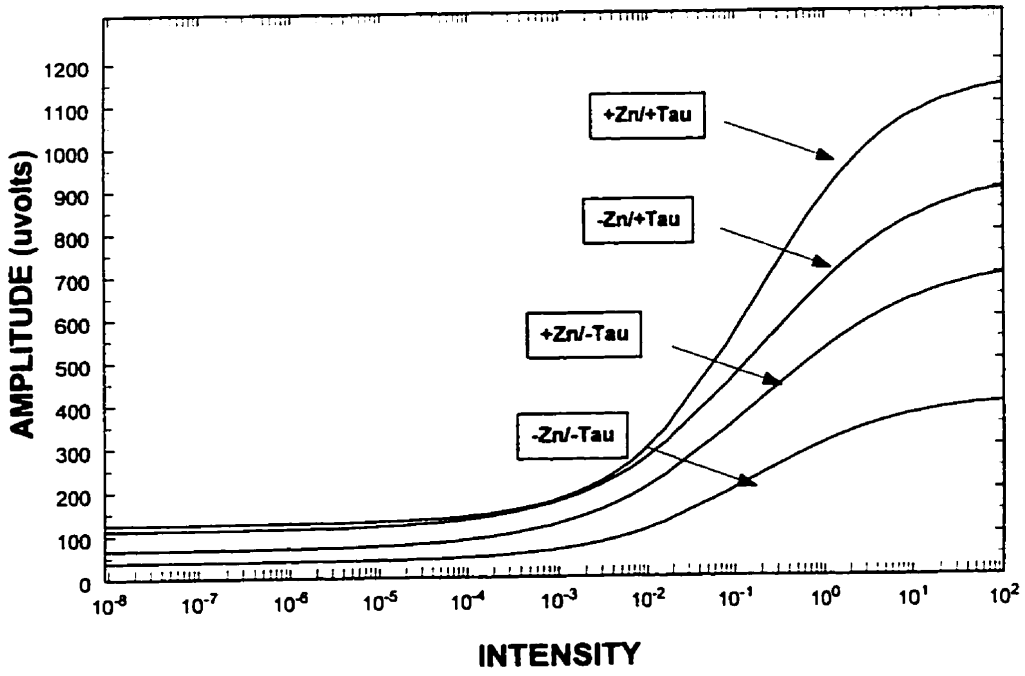

Figure 8.2

The Amplitude of the b-wave Plotted as a Function of log Stimulus Intensity from 4/5 Groups

Table 8.2

Trough to Peak b-wave Amplitude as a Function of log Stimulus Intensity in Rats Made Zinc- and Taurine-deficient Throughout Gestation and Postnatal Life[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
Vmax (μvolts)	1048.8±98.5	827.8±88.8	961.9±101.9	542.6±73.0	P=0.0498	P=0.0014	P=0.2854
log σ	-1.60±0.21	-1.21±0.20	-1.49±0.18	-1.54±0.19	P=0.5840	P=0.3987	P=0.2602
n	0.51±0.05	0.43±0.04	0.53±0.06	0.64±0.12	P=0.1241	P=0.8381	P=0.2227

[1]Results expressed as mean±SEM; n=9. Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables.

modified Naka-Rushton function are presented in Table 8.2. No interaction was found between zinc and taurine for Vmax (P=0.2854), σ (P=0.2602) or n (P=0.2227).

Feeding animals marginally zinc-deficient diets significantly depressed Vmax (P=0.0498); σ (P=0.5840) and n (P=0.1241) were not affected by the zinc restriction. Mean (±SEM) Vmax of the -Zn/+Tau group was not significantly different from that of the +Zn/+TauPF group when compared by paired t-test (974.2±96.9 μvolts, n=7 vs 852.8±66.8 μvolts, n=7; P=0.2047); mean (±SEM) Vmax of the +Zn/+TauPF group was not significantly different from that of the +Zn/+TauAL group when compared by unpaired t-test (878.6±63.4 μvolts, n=8 vs 1048.8±98.5 μvolts, n=9; P=0.1787). Although no statistically significant difference was found among the three groups, there was a trend toward a depressing effect of pair-feeding on Vmax.

Vmax was also significantly depressed in taurine deficiency (P=0.0014). However, no significant effect of taurine deficiency was seen on σ (P=0.3987) or n (P=0.8381).

8.4 DISCUSSION

The results of this study suggest that zinc and taurine do not interact

during the period of retinal development as assessed by electroretinographic measures in the mature rat retina. The ERG is comprised of an a-wave produced when light strikes rhodopsin in the photoreceptor outer segments and a b-wave generated at a more proximal location, possibly in the Müller cells of the inner nuclear layer (Berson 1992). Taurine deficiency depressed the amplitude of the a- and b-wave of the ERG as previously described (Cocker and Lake 1987, Hageman and Schmidt 1987, Lake 1986, Rapp et al. 1987). The decrease in Vmax has also been previously documented (Cocker and Lake 1987). While zinc deficiency similarly reduced the amplitude of the a- and b-wave, the two nutrients did not act synergistically in their depressing effects on the ERG.

In contrast to these results, it has been shown that deficiencies of zinc and taurine are synergistic in there depressing effects on specific oscillatory potentials (Chapter 7). Light microscopic evidence of marked photoreceptor degeneration found only in animals deficient in both zinc and taurine (Chapter 7) support this type of nutrient-nutrient interaction. The electrophysiological data indirectly support the theory that oscillatory potentials are generated independently from the mechanism producing the ERG (el Azazi and Wachtmeister 1990). While oscillatory potentials and the ERG b-wave may both arise in the inner nuclear layer, they respond differently to combined zinc and taurine deficiencies. This may be the result of differing zinc and taurine concentrations in the various cell types of the inner nuclear layer. Future

studies may resolve these discrepancies as the biochemical mechanism by which these nutrients interrelate is explored.

The main treatment effect of zinc on the ERG suggests an independent biochemical function for zinc in the retina. Marginal zinc deficiency independently depressed the amplitude of the a- and b-wave of the ERG at maximum light intensity suggesting that zinc exerts an effect in both the inner and outer retina. Vmax also declined, suggesting a decrease in the area of functioning retina (Wu et al. 1983). It has been reported previously (Chapter 7) that marginal zinc deficiency significantly depresses the amplitude and increases the latency of the first oscillatory potential, providing additional evidence that zinc has a function in the inner retina.

Morphological changes have been described in the photoreceptors and retinal pigment epithelium of severely zinc-deficient rats (Leure-duPree and McClain 1982). Samuelson et al. (1992), in an attempt to model human zinc deficiency, fed pigs diets marginally deficient in zinc for a 12 month period. Histological examination of their retinas revealed a decrease in cone photoreceptors and an increase in phagosomes and residual bodies in the retinal pigment epithelium that appeared to progress over time. Despite the depression of the a-wave, light microscopic alterations in the photoreceptor outer segments of marginally zinc-deficient rats were not detected (Chapter 7). The a-wave is a cumulative response of the entire photoreceptor population; therefore, it is possible that photoreceptor damage was present in other

unsectioned areas of the retina. It is also possible that the dampened ERG response is the result of ultrastructural damage not detectable on light microscopic examination. Future studies using electron microscopy should address this hypothesis.

The ERG changes observed in marginal zinc deficiency are particularly interesting given the degree of zinc restriction employed in this study. Although zinc deficiency was confirmed by significantly depressed plasma, tibia, liver and whole eye zinc concentrations, the animals showed no clinical signs of zinc deficiency (Chapter 7). This model was chosen to represent the degree of zinc deficiency which can occur in the human population. Recent research has examined the involvement of zinc in human eye diseases such as age-related macular degeneration, a condition known to affect the sensory retina and retinal pigment epithelium (Newsome et al. 1988). In a heterogeneous group of patients with age-related macular degeneration, a daily zinc sulphate supplement of 200 mg provided for a two-year period in a double-blind, randomized, placebo-controlled design, increased serum zinc and significantly reduced visual loss (Newsome et al. 1988). In contrast, a followup study in a homogeneous group of patients with unilateral exudative age-related macular degeneration showed no improvement in functional eye tests in response to the same dose of zinc supplementation (Stur et al. 1996). Unfortunately, the sample size was insufficient to determine whether zinc had a protective effect against the development of exudative lesions in the second eye. Both of these studies

are limited to conclusions based on short-term trials. The Eye Diseases Case-Control Study Group (1992) was also unable to demonstrate a positive relationship between serum zinc levels and incidence of age-related macular degeneration; however, this finding may be of limited significance as serum zinc concentration is not a sensitive or specific indicator of zinc status (Gibson 1990).

Despite its high concentrations in the retina (Eckhert 1983), the biochemical functions of zinc in this tissue have not been completely defined. This information would be beneficial for designing future studies of the role of zinc in human eye diseases. Zinc has been proposed to exert a critical physiological role in the structure and function of biomembranes by its effects on membrane protein conformation and protein-protein interactions (Bettger and O'Dell 1993). In zinc deficiency, the loss of zinc from these proteins may result in a loss of membrane integrity that could affect electrophysiological measurements in both the inner and outer retina. Other investigators have suggested that zinc may exert its effect on the retina through its involvement in vitamin A metabolism, and the impaired dark adapation of zinc deficiency has been attributed to decreased rhodopsin formation. However, conflicting results have been obtained in severely zinc-deficient rats as to whether this is a consequence of a decreased rate of oxidation of retinol to retinal by zinc-dependent alcohol dehydrogenase (Dorea and Olson 1986, Huber and Gershoff 1975, Smith 1980). The findings of this current research do not support a decrease in rhodopsin formation with the degree of zinc deficiency examined in

this study. Marginal zinc deficiency did not alter the parameter σ generated from the Naka-Rushton analysis. A change in σ reflects a change in retinal sensitivity (Wu et al. 1983). If rhodopsin content of the photoreceptors was depressed, more light would be required to elicit the same retinal response and σ would increase. Thus this work suggests that zinc has biochemical functions in the retina independent of vitamin A. Kraft et al. (1987) fed rats diets deficient in zinc and vitamin A, and reported decreased retina sensitivity that improved to a similar degree whether the animals were repleted with vitamin A or both zinc and vitamin A.

These data can not be used to draw conclusions as to whether depressed feed intake in zinc deficiency also contributes to the deleterious effects on the retina. Although the results did not reach statistical significance, pair feeding to the zinc-deficient group did tend to depress Vmax and the amplitude of the a- and b-waves at maximum light intensity. These findings are difficult to interpret, however, as feed intake of the pairfed animals was not equal to but significantly less than that of the zinc-deficient group. This limitation was related to the inaccuracy of pair feeding and collection of feed spillage in an extremely marginal zinc deficiency model.

In summary, evidence was not obtained that zinc and taurine interact in their depressing effects on the electroretinogram. While taurine deficiency has been previously shown to depress the ERG, this investigation has shown that zinc also exerts a role in the development of the electrophysiological response in

both the inner and outer rat retina. This has been demonstrated by significantly reduced ERG a- and b-wave amplitudes and Vmax in marginally zinc-deficient rats. The effects of zinc deficiency on retinal structure and function and the mechanism by which zinc exerts these effects deserve further investigation.

Chapter 9

GENERAL DISCUSSION AND FUTURE DIRECTIONS

Zinc has been reported to interact with taurine *in vitro* (Pasantes-Morales et al. 1984a). The research described herein was undertaken to determine the physiological significance of this interaction *in vivo*. Studies in rats have provided clear evidence of the importance of zinc during pregnancy (Keen and Hurley 1987). Likewise, taurine is considered essential in the perinatal period (Arment et al. 1986; Ghisolfi 1987). Consequently, deficiencies of zinc or taurine during periods of development make this timepoint a particularly sensitive one in which to study the interaction of these nutrients.

To examine the effects of a deficiency of zinc and taurine on development, an appropriate model was required. The teratogenicity of zinc deficiency in the rat is well established and results in a variety of congenital defects including skeletal abnormalities (Da Cunha Ferreira et al. 1989) and internal soft tissue malformations affecting the brain, heart, lungs and urogenital systems (Hurley 1981; Keen and Hurley 1987). However, the rat had not been

carefully assessed as a model to study the fetal abnormalities associated with taurine deficiency. In the cat, these include neurological and morphological abnormalities, such as abnormal hind leg development, thoracic kyphosis and hydrocephalus (Sturman et al. 1985; Sturman and Messing 1991).

Treatment of pregnant rats with low dietary taurine and varying doses of GES produced a sharp decline in both maternal and fetal tissue taurine concentrations. However, these depressions did not translate into developmental defects in the fetuses at day 20 of gestation. Instead, fetal weights were unchanged and gross assessment of fetuses revealed no significant external, visceral or skeletal malformations due to GES-induced taurine deficiency. A pilot study subsequently confirmed these results and established the absence of significant congenital malformations at gestational day 20 in combined zinc and taurine deficiencies.

Consequently, it was concluded that GES-induced taurine deficiency in the rat is not a good model for studying all developmental effects. No level of GES produced the intrauterine growth retardation or malformations previously described in kittens born to taurine-deficient cats (Sturman and Messing 1991; Sturman et al. 1986). This may be attributable to greater depletion in cats fed taurine-free diets (Sturman and Messing 1991) compared with the extent of depletion achieved with low dietary taurine and GES in the rat. However, the results do suggest that treatment of rats with 2% GES could be further explored as a model for the reproductive loss seen in the cat. Preimplantation loss has

been implicated in the pregnancy failure seen in taurine-deficient cats (Dieter et al. 1993). Treatment of rats with GES prior to implantation may depress uterine taurine concentration thereby affecting preimplantation development.

Alternatively, the high concentration of zinc (Eckhert 1983) and taurine (Heinämäki et al. 1986) in ocular tissue and the purported importance of these nutrients in maintaining eye structure and function (Leure-duPree and McClain 1982; Sturman et al. 1986), suggested that the eye may be a unique physiological system in which to study this interaction. The use of GES as a taurine depletor has gained wide-spread acceptance in studying the role of taurine in the rat retina and both electrophysiological deficits and morphological changes, including degeneration of photoreceptors and shrinkage of the inner retinal layer, have been observed in these animals (Lake 1981; 1983, 1986, 1989; Lake and Malik 1987; Pasantes-Morales et al. 1983; Quesada et al. 1984). These retinal changes are similar to what has been described in the cat which has a clear dietary requirement for taurine (Sturman 1993). Therefore, use of GES during pre- and postnatal development in the rat appeared to be a useful model for studying the interaction of zinc and taurine in the retina.

When eye structure and function were assessed using this model, morphological and functional evidence that zinc interacts with taurine in the developing rat retina were observed. Light microscopic examination at 7½-8½ weeks postnatal age demonstrated marked photoreceptor degeneration and confirmed retinal dysplasia in animals deficient in both zinc and taurine. These

findings provide strong evidence for an interaction as the photoreceptors from zinc-deficient rats appeared histologically normal and only mild photoreceptor degeneration was seen in the taurine-deficient group. Further evidence of a physiological interaction was observed when oscillatory potentials were measured; zinc deficiency depressed the amplitude of OP_2 and OP_3 only when the animals were also taurine-deficient. The presence of an interaction in two of the five oscillatory potentials suggests that zinc and taurine may function synergistically in only specific areas of the inner retina as each OP is thought to originate independently (el Azazi and Wachtmeister (1990).

It is well established that a reduction in retinal taurine concentrations in the rat results in severe morphological alteration of the photoreceptor cells (Hageman and Schmidt 1987; Lake and Malik 1987; Pasantes-Morales et al. 1983; Rapp et al. 1988). Over time the RPE becomes affected. Conversely, the effects of a severe zinc deficiency in the rat (Leure-duPree and McClain 1982) and marginal zinc deficiency in the pig (Samuelson et al. 1991) appear to originate in the RPE with an accumulation of osmiophilic inclusion bodies that progresses over time. Vesiculation and degeneration of the photoreceptor outer segments follows. It appears that zinc deficiency may exert its influence on the RPE whereas a deficiency of taurine preferentially affects the photoreceptors. It is possible that a combination of zinc and taurine deficiencies affect both the RPE and photoreceptors resulting in the synergistic relationship observed in the current study.

In addition to retinal pathology, evidence of focal choroidal atrophy in rats deficient in both zinc and taurine was also observed. This anomaly was initially detected on ophthalmoscopic assessment and confirmed by light microscopy. Rogers et al. (1987) had previously observed a lack of invagination of the optic cup and closure of the choroidal fissure that resulted in retinal folding, when fetuses from severely zinc-deficient dams were examined. In the present study, the added stress of taurine deficiency was required to produce this defect in marginally zinc-deficient rats.

Zinc and taurine also interact in other tissues. Zinc deficiency depressed plasma and tibial zinc; however, the effect was less pronounced when animals were also taurine-deficient suggesting that taurine influences the zinc status of the animal. An interaction was also apparent for liver taurine. Animals fed no dietary taurine and GES had significantly depressed liver taurine regardless of zinc status; however, zinc deficiency elevated liver taurine in taurine-adequate animals. It is apparent that the nature of the interaction between zinc and taurine is not consistent across all tissues examined suggesting that more than one mechanism may be involved in the expression of this interaction.

Previously, it had been suggested that zinc may influence the retinal changes observed in the taurine-deficient cat. A 50% reduction of retinal taurine in this species produces degeneration of the photoreceptors (Hayes et al. 1975b) and depression of electroretinogram (ERG) a- and b-wave amplitudes (Berson et al. 1976). The addition of 200 μM zinc to the drinking water of these

animals partially prevented the depression of the a-wave amplitude in some animals (Pasantes-Morales et al. 1987b). Whether feeding additional zinc to taurine-deficient rats can protect from the photoreceptor degeneration and subsequent electrophysiological alterations observed is not known. Future research is required to address this question.

A relationship between zinc and taurine in the cat has also been suggested in other eye tissues. Taurine-deficient cats exhibit disruption and disorganization of the membrane surrounding the tapetal rods with a subsequent loss of localized zinc (Sturman et al. 1981). Whether the loss of zinc is due to an interaction or simply to photoreceptor degeneration has not been established. However, it is interesting to note that the tapetum arises from similar embryonic tissues as the choroid (Gelatt 1991). The evidence of focal choroidal atrophy in rats deficient in both zinc and taurine in the current study suggests that the zinc loss from the tapetum of taurine-deficient cats may be the result of an interaction. The cat should be explored as a model to study the combined effects of zinc and taurine on eye development.

Independent effects of taurine deficiency on specific oscillatory potentials were evident with reductions in the amplitudes of OP_1, OP_4 and OP_5. While previous research has identified the importance of taurine in development of normal photoreceptor morphology and function (Bonhaus et al. 1985, Imaki et al. 1986), these results confirm that taurine also functions in the inner retina. Previous research has shown that retinal taurine concentrations and ERG b-

wave amplitudes are significantly increased when GES is discontinued and replaced with taurine in drinking water (Lombardini et al. 1996). Similar improvements were observed when parenterally fed children were provided with taurine in their infusate (Ament et al. 1986; Geggel et al. 1982; Vinton et al. 1985). The effect of re-feeding taurine on OP amplitude in the GES-induced taurine-deficient rat needs to be addressed.

The limitations of this research must be considered. Though zinc was observed to interact with taurine, other explanations are possible and have not been ruled out. First, it is well known that the tissues of animals treated with GES take up GES in place of taurine (Lake et al. 1987). Thus it is possible that the presence of GES in the retina rather than the lack of taurine is responsible for the depressing effect of taurine deficiency on the amplitude of oscillatory potentials and the interactive effects of taurine with zinc. However, the similarities in retinal changes observed between the GES-induced taurine-deficient rat and the cat suggest that the lack of taurine and not the presence of GES is responsible for the results observed. Use of a cat model to test the interaction will confirm this hypothesis.

The finding that zinc also independently affects ERG a- and b-wave amplitudes, Vmax and certain OP in the developing rat retina provides additional support for the importance of zinc in pre- and postnatal development. These data also suggest that zinc exerts an effect in both the inner and outer retina. Little information is available on the influence of marginal zinc deficiency in the

developing eye, particularly in the retina. Microphthalmia in 1/3 of the fetuses of Long Evans rats has been observed when a severe zinc restriction was imposed throughout gestation (Rogers et al. 1984). When zinc deficiency was instituted at parturition, eye lid opening was delayed but no morphological differences were noted among photoreceptor cells at weaning (Sinning et al. 1984). Similar observations were made in the current study. Despite the depression of the a-wave amplitude, morphologic alterations in the photoreceptor outer segments of marginally zinc-deficient rats were not detected with the light microscope. Zinc deficiency appeared to have a minimal effect on the histology of the developing retina; however, ultrastructural alterations may have been present. Subsequent research using electron microscopy may detect differences not apparent on histological assessment.

The results of this research are particularly interesting given the marginal nature of zinc restriction employed in this study. Although zinc deficiency was confirmed by significantly depressed plasma, tibia, liver and whole eye zinc concentrations, the animals showed no clinical signs of zinc deficiency. Developmental defects are known to occur in approximately 3% of all infant births (Centres for Disease Control 1989). Despite intensive research efforts over the past two decades, causative factors have been identified in only 35-60% of these cases (Schaffer 1993). Maternal dietary zinc intake is thought to be a significant factor in human pregnancy outcome (Simmer et al. 1991; Scholl et al. 1993). However, it is unlikely that poor maternal intake of a single nutrient

is responsible for reproductive defects in the human population. Instead, it is more likely that the compromised status of an individual nutrient increases the risk to other potential teratogens or that there is a synergistic interaction between nutrients that results in abnormal development (Keen 1992). The interaction of zinc with taurine during development could be responsible for some of the physiological and morphological defects reported (Keen 1992). Zinc is known to interact with other nutrients. A marked increase in plasma copper and decrease in plasma zinc have been reported in pregnant women (Hambridge et al. 1983). Hambridge et al. (1983) reported an inverse relationship between the level of daily iron supplement and the plasma zinc level in the first and third trimester of pregnancy. An interaction has also been observed between zinc and folate in pregnant women (Simmer et al. 1987) and between zinc and vitamin A in pregnant rats (Duncan and Hurley 1978; Peters et al. 1986).

It has been suggested that zinc may exert its effect on the retina through its involvement in vitamin A metabolism. The impaired dark adaptation of zinc deficiency has been attributed to decreased rhodopsin formation. However, conflicting results have been obtained in severely zinc-deficient rats as to whether this is a consequence of a decreased rate of oxidation of retinol to retinal by zinc-dependent alcohol dehydrogenase (Dorea and Olson 1986, Huber and Gershoff 1975, Smith 1980). The findings presented here do not support a decrease in rhodopsin formation with the degree of zinc deficiency

examined in this study. Marginal zinc deficiency did not alter the parameter σ generated from the Naka-Rushton analysis. A change in σ reflects a change in retinal sensitivity (Wu et al. 1983). If rhodopsin content of the photoreceptors was depressed, more light would be required to elicit the same retinal response and σ would increase. Future research should examine retinal concentrations of rhodopsin, retinal alcohol dehydrogenase and vitamin A to substantiate this observation.

The mechanism by which zinc and taurine interact is not known. Zinc has been proposed to play a physiological role in plasma membranes by its effects on membrane protein conformation and protein-protein interactions (Bettger and O'Dell 1993). While the precise biochemical mechanisms have not been fully elucidated, it has been suggested that the loss of zinc from specific proteins in the plasma membrane alters water and ion channels. Ultimately intracellular ion and water concentration is changed (Bettger and O'Dell 1993). It may be speculated that taurine as an intracellular osmoregulator (Huxtable 1992) can respond to these changes by regulating water and ion flow across cell membranes. Now that an interaction between zinc and taurine has been observed *in vivo*, the mechanism by which these nutrients interact needs to be elucidated. Fragility studies (Szuts and Cone 1977) on the isolated photoreceptors of rats deficient in zinc and taurine may provide evidence in support of this hypothesis. For example, isolated rod outer segment membranes from these animals could be subjected to osmotic shock and observed for

volume changes. Additional studies to identify specific membrane proteins or other membrane components whose structure and function are also modified by zinc and/or taurine are required.

Chapter 10

GENERAL CONCLUSIONS

The research presented herein support the following conclusions:

1. The effect of GES-induced taurine deficiency on development in the rat, as assessed by evaluation of fetal weight and incidence of congenital malformations at day 20 of gestation, does not produce the degree of reproductive loss previously observed in the taurine-deficient cat.

2. Morphological and physiological evidence has been obtained that zinc and taurine interact in the developing rat retina. This has been demonstrated by severe photoreceptor degeneration, retinal dysplasia and depressed OP amplitudes in combined zinc and taurine deficiency.

3. Plasma zinc, tibial zinc and liver taurine concentrations provide evidence that zinc and taurine interact in other tissues. Choroidal atrophy was also apparent only in the combined deficiency group, suggesting interactions

in the choroid of the eye.

4. Zinc also exerts an independent role in the development of the electrophysiological response in both the inner and outer rat retina. This has been demonstrated by significantly reduced ERG a- and b-wave amplitudes, Vmax and OP_1 amplitude in marginally zinc-deficient rats.

5. Taurine exerts an independent effect on the development of the electrophysiological response in the inner and outer rat retina. This has been demonstrated by significantly reduced OP and ERG a- and b-wave amplitudes.

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Appendix A

Tissue Taurine Levels in Dams and Fetuses Exposed to Low Dietary Taurine and Varying Doses of Guanidinoethyl Sulfonate

	Treatment Group[1]			
	CONTROL	0.5% GES	1.0% GES	2.0% GES
Maternal Tissue				
Liver (nmol/mg pro)	74.8±4.3[a]	12.6±1.4[b]	11.0±1.0[b]	9.3±0.4[b]
Brain (nmol/mg pro)	45.5±1.6[a]	27.7±2.3[b]	24.7±1.3[b,c]	21.3±0.7[c]
Fetal Tissue				
Liver (nmol/mg pro)[2]	98.3±2.7[a]	56.1±1.8[b]	48.6±2.4[b]	44.0±2.9[b]
Brain (nmol/mg pro)[2]	350.5±8.9[a]	280.4±11.9[b]	257.8±9.4[b,c]	226.5±9.2[c]

[1]Results expressed as mean ±SEM; n=8 for Control and 1.0% GES, n=7 for 0.5 and 2.0% GES. Statistical analysis was one-way ANOVA followed by Tukey's test. Values in a row not sharing a common superscript are significantly different ($P<0.05$).
[2]Fetal tissue was pooled for taurine analysis.

Appendix B

Organ to Body Weight Ratios in Dams and Fetuses Exposed to Low Dietary Taurine and Varying Doses of Guanidinoethyl Sulfonate

	Treatment Group[1]			
	CONTROL	0.5% GES	1.0% GES	2.0% GES
Maternal Tissues				
Liver/Body Weight	36.0±1.0[a]	36.7±0.9[a]	36.0±1.5[a]	36.6±1.5[a]
Brain/Body Weight	4.56±0.15[a]	4.86±0.14[a]	4.61±0.17[a]	4.87±0.19[a]
Fetal Tissues				
Liver/Body Weight	85.4±1.9[a]	81.3±1.8[a]	87.4±2.2[a]	82.7±2.9[a]
Brain/Body Weight	46.4±1.9[a]	42.9±1.0[a]	43.1±1.6[a]	44.5±1.5[a]

[1]Results expressed as mean ±SEM; n=8 for Control and 1.0% GES, n=7 for 0.5% and 2.0% GES. Statistical analysis was one-way ANOVA followed by Tukey's test. Values in a row not sharing a common superscript are significantly different ($P<0.05$).

Appendix C

Comparison of Body Weight and Feed Intake Data from Zinc-deficient Rats with Control and Pairfed Animals

	Treatment Group[1]		
	+Zn/+TauAL	-Zn/+Tau	+Zn/+TauPF
Body Weight (g)			
Weaning	49.9±1.0	43.2±2.2	44.4±1.5[2]
Week 1	83.2±1.8	71.9±3.33.6	71.1±2.7[2]
Week 2	132.4±3.1	119.3±5.9	115.8±3.6[2]
Week 3	185.9±5.3	169.2±8.0	162.1±4.7[2]
Week 4	237.6±8.1	222.8±11.2	207.6±7.3[2]
Final	279.6±10.4	266.4±18.8	234.8±10.2[2]
Feed Intake (g)			
Week 1	68.6±1.4	64.7±2.5	57.2±2.2[2,3]
Week 2	171.9±3.1	163.0±6.3	149.0±5.1[2,3]
Week 3	316.7±6.7	294.6±11.7	272.2±8.8[2,3]
Week 4	481.6±12.4	455.1±19.1	417.5±13.3[2,3]
Final	635.2±21.5	601.4±47.5	553.4±37.8[3]

[1]Results expressed as mean±SEM; n=10.
[2]Significantly different than +Zn/+TauAL ($P<0.05$) when compared by unpaired t-tests.
[3]Significantly different than -Zn/+Tau ($P<0.05$) when compared by paired t-test.

Appendix D

Amplitudes of ERG a- and b- wave Peaks to Varying Stimulus in Response to Varied Zinc and Taurine Status During Gestation and Postnatal Life[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
a-wave (µvolts)							
ND 1	430.6±36.8	310.0±33.3	368.1±25.7	204.9±24.2	P=0.0094	P=0.0001	P=0.4878
ND 2	141.0±14.2	93.1±14.4	115.6±5.5	59.0±11.5	P=0.0172	P=0.0001	P=0.7147
ND 3	45.8±6.4	28.7±6.3	34.4±4.8	22.9±6.8	P=0.1643	P=0.0249	P=0.6464
b-wave (µvolts)							
ND 1	910.4±73.7	646.9±56.2	813.8±46.0	467.4±46.4	P=0.0194	P=0.0001	P=0.4667
ND 2	550.7±47.5	353.8±30.0	450.0±26.9	255.6±35.2	P=0.0078	P=0.0001	P=0.9718
ND 3	442.4±41.9	291.9±30.9	355.6±35.3	218.8±37.7	P=0.0350	P=0.0004	P=0.8528
ND 4	192.4±25.0	157.5±15.6	179.4±17.6	123.6±23.2	P=0.2576	P=0.0327	P=0.6110
ND 6	125.7±24.2	97.5±10.8	115.6±13.8	70.1±9.5	P=0.2319	P=0.0222	P=0.5776
ND 8	83.3±7.9	68.8±12.9	82.5±9.2	61.1±15.6	P=0.7210	P=0.1355	P=0.7741

[1]Results expressed as mean±SEM; n=9. Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables.

Appendix E

Latencies of ERG a- and b- wave Peaks to Varying Stimulus in Response to Varied Zinc and Taurine Status During Gestation and Postnatal Life[1]

	Treatment Group						
	+Zn/+TauAL	+Zn/-Tau	-Zn/+Tau	-Zn/-Tau	Zn effect	Tau effect	Zn x Tau effect
a-wave (msec)							
ND 1	12.9±0.3	12.3±0.2	12.7±0.2	12.7±0.2	P=0.7010	P=0.1843	P=0.1557
ND 2	23.3±0.5	22.7±0.5	23.5±0.4	23.1±0.6	P=0.5605	P=0.3056	P=0.7878
ND 3	25.9±0.7	21.4±4.1	22.7±2.5	16.9±4.3	P=0.2437	P=0.1268	P=0.8383
b-wave (msec)							
ND 1	45.8±2.3	47.2±4.9	45.8±4.0	50.2±4.0	P=0.7164	P=0.4706	P=0.7164
ND 2	57.5±2.3	50.8±2.0	56.8±1.7	55.0±2.5	P=0.4135	P=0.0498	P=0.2424
ND 3	61.7±2.4	56.2±1.5	58.4±2.0	59.6±2.6	P=0.9611	P=0.3090	P=0.1210
ND 4	87.4±2.5	80.7±1.4	85.5±1.8	86.9±2.5	P=0.2893	P=0.2001	P=0.0525
ND 6	88.3±2.8	81.5±1.5	86.1±2.3	85.7±1.6	P=0.6279	P=0.0960	P=0.1343
ND 8	89.2±2.9	73.2±8.3	85.1±2.2	77.2±9.8	P=0.9903	P=0.0806	P=0.5446

[1]Results expressed as mean±SEM; n=9. Statistical analysis was by 2 factor ANOVA with zinc and taurine as independent variables.

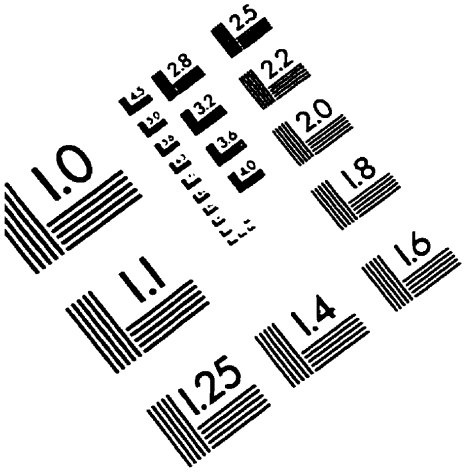
Appendix F

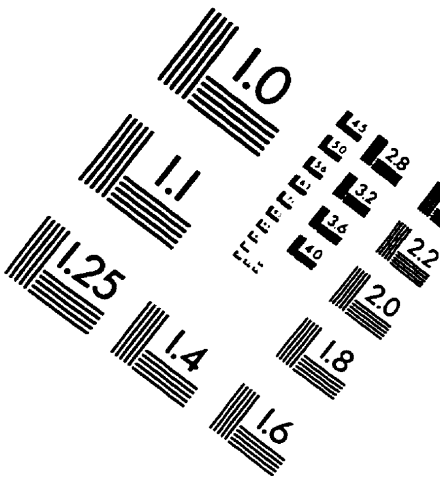
Intensity-latency Functions in Rats made Zinc- and Taurine-deficient Throughout Gestation and Postnatal Life

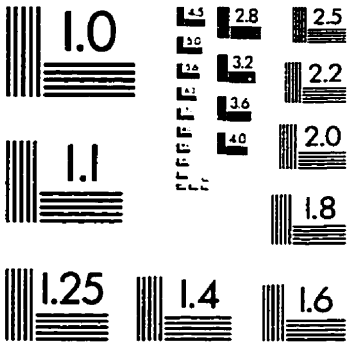
Animal #	P-value	R-value
10-1	0.0210	0.979
10-2	0.0444	0.956
10-3	0.0102	0.990
10-4	0.2148	0.785
10-5	0.0617	0.938
10-6	0.6698	0.330
11-1	0.1990	0.801
11-2	0.0418	0.958
11-3	0.0399	0.960
11-4	0.0480	0.952
2-2	0.0348	0.965
2-1	0.0903	0.910
2-3	0.0438	0.956
2-4	0.1134	0.887
4-2	0.0871	0.913
4-3	0.0871	0.913
4-4	0.1322	0.868
5-1	0.5616	0.438
5-2	0.0514	0.949
23-2	0.0481	0.952
23-3	0.0561	0.944
23-4	0.0838	0.916
25-1	0.0570	0.943
25-2	0.0871	0.913

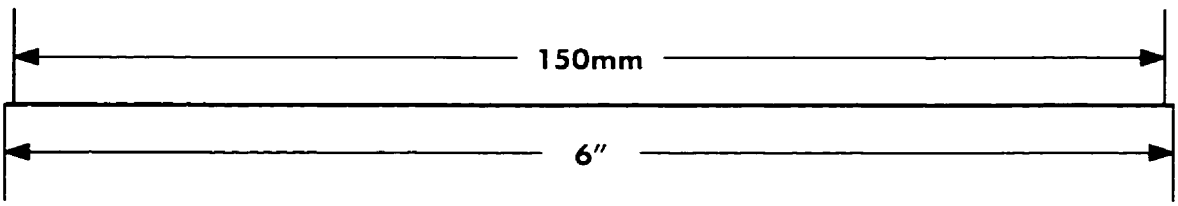
25-3	0.0798	0.920
25-4	0.0607	0.939
28-1	0.0101	0.990
28-2	0.0358	0.964
20-1	0.3529	0.647
20-2	0.0168	0.983
20-3	0.1920	0.808
20-4	0.4847	0.515
21-1	0.0379	0.962
21-2	0.0533	0.947
21-3	0.0431	0.957
21-4	0.0189	0.981
21-5	0.1526	0.847
21-6	0.0348	0.965
14-1	0.0846	0.915
14-2	0.1919	0.808
14-3	0.0379	0.962
14-5	0.1142	0.886
14-6	0.6220	0.378
16-1	0.1146	0.885
16-2	0.0056	0.994
16-3	0.0202	0.980
16-4	0.1322	0.868

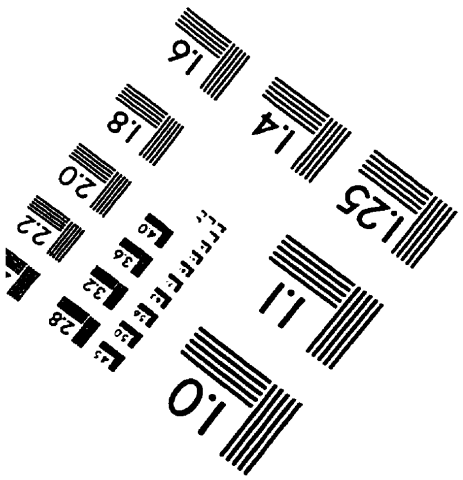
IMAGE EVALUATION
TEST TARGET (QA-3)

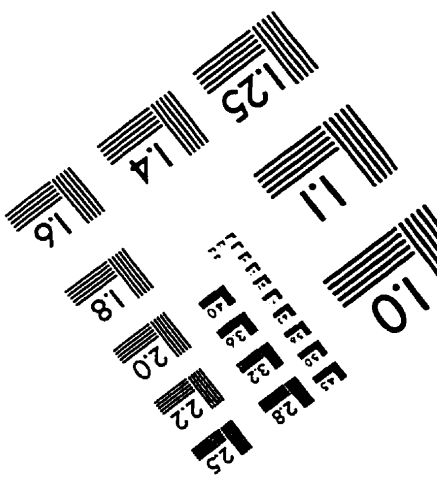












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