EFFECTS OF ALCOHOL AND TAURINE SUPPLEMENTATION ON BODY GROWTH, MATERNAL BEHAVIORS, AND OFFSPRING HIPPOCAMPAL DEPENDENT WATERMAZE LEARNING

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ABSTRACT

It is well known that maternal alcohol consumption is teratogenic, resulting in damages from structural deformity to mental retardation and disruption of learning and memory. This research investigated effects of alcohol on body growth, learning and memory of the affected offspring. Taurine, a conditionally essential amino acid, can protect nervous tissues against alcohol damage and is also crucial for development.

Pregnant mice received treatments of alcohol 0%, 5%, 10%, 20% and 30% prepared fresh daily in a nutritionally balanced liquid diet with 25% solid food and water ad libitum. Taurine at 1g/kg body weight was prepared in 10ml drinking water either to mothers until weaning or to offspring from weaning. Maternal food intake, weight and behaviors were monitored. Offspring body weights were recorded. Adult offspring were tested on learning and memory using a water maze during the course of a 6-day task. Percentage of escapes, escape latency and learning curves were analyzed.

It was found that alcohol retarded offspring body growth and disrupted the process of learning and memory. Taurine was not protective to body growth but was shown to protect the learning when available in early stages of the development.

It can be concluded that taurine is protective against alcohol damage when given early in life. Although offspring body growth was delayed, the offspring was able to catch up with the growth once having reached adult age. With taurine available intrauterine and through the mother’s milk during early development, the learning and memory processes were preserved.

KEY WORDS: MATERNAL ALCOHOL/TAURINE/DEVELOPMENT/LEARNING AND MEMORY

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ผลกระทบของแอลกอฮอล์และทอรีนต่อการเจริญเติบโตของลูก พฤติกรรมแม่ และการเรียนรู้ Water Maze โดยอาศัยสมองส่วนฮิปโปแคมปัส

EFFECTS OF ALCOHOL AND TAURINE SUPPLEMENTATION ON BODY GROWTH, MATERNAL BEHAVIORS, AND OFFSPRING HIPPOCAMPAL DEPENDENT WATERMAZE LEARNING

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บทคัดย่อ

การดื่มแอลกอฮอล์ของแม่ ในระหว่างที่ตั้งครรภ์ สามารถส่งผลกระทบในด้านลบต่อการเจริญเติบโตของลูก ตั้งแต่โครงสร้างทางร่างกายที่ผิดปกติ จนถึงพัฒนาการทางการเรียนรู้ที่ล่าช้าตลอดจนถึงปัญญาอ่อนได้ งานวิจัยชิ้นนี้ศึกษาถึงผลกระทบของการดื่มแอลกอฮอล์ในแม่ต่อการเจริญเติบโตทางร่างกาย การเรียนรู้ ตลอดถึงความทรงจำของลูกที่ถูกกระทบ ทอรีนเป็นกรดอะมิโนชนิดหนึ่งซึ่งสามารถช่วยปกป้องสมองและระบบประสาทจากผลกระทบของแอลกอฮอล์ อีกทั้งยังสำคัญต่อการพัฒนาการของลูกในวัยเจริญเติบโตอีกด้วย

แม่หนู Mouse ท้อง ถูกแบ่งออกเป็นกลุ่มต่างๆและได้รับแอลกอฮอล์ในระดับที่ต่างกัน ตั้งแต่ 0%, 5 %, 10%, 20% และ30% ที่ถูกผสมในอาหารเสริมเหลวสูตรครบถ้วน พร้อมกับอาหารหนูชนิดเม็ด 25 % และน้ำดื่มไม่จำกัด การให้ทอรีนกระทำโดยผสมในปริมาณทอรีน 1 กรัมต่อน้ำหนักตัว 1 กิโลกรัมในน้ำดื่ม 10 มิลลิลิตร โดยให้แม่หนูระหว่างตั้งครรภ์และระหว่างให้นมลูก หรือให้ลูกหนูจากระยะหย่านมจนถึงโตเต็มวัย การรวบรวมข้อมูลเริ่มตั้งแต่การกินอาหาร น้ำหนักตัวและพฤติกรรมแม่ และน้ำหนักตัวลูกหนูตั้งแต่แรกคลอดจนถึงโตเต็มวัย เมื่อลูกหนูโตเต็มวัย การวัดความสามารถการเรียนรู้ได้ศึกษาโดยทดสอบจาก Water Maze เป็นเวลา 6 วัน ข้อมูลที่ถูกรวบรวมได้แก่ อัตราการการเจอแท่นพักใต้น้ำ ระยะเวลาที่ใช้ในการหาแท่นพัก และกราฟการเรียนรู้ถูกนำมาวิเคราะห์

ผลการทดลองพบว่าการดื่มแอลกอฮอล์ในแม่ขณะตั้งครรภ์ส่งผลกระทบร้ายแรงต่อลูกตั้งแต่การเจริญเติบโตในระยะแรกๆ ทอรีนที่ใช้เสริมอาหารไม่สามารถป้องกันผลกระทบของแอลกอฮอล์ที่มีต่อการเจริญเติบโตทางร่างกายของลูกหนู แต่สามารถช่วยปกป้องระบบการเรียนรู้และความทรงจำของลูกได้ ถ้าหากได้รับการเสริมในอาหารตั้งแต่ระยะเริ่มต้นของการพัฒนาการ

จากผลการวิจัยสรุปได้ว่าทอรีนสามารถป้องกันผลกระทบที่เสียหายของการดื่มแอลกอฮอล์ ถ้าหากถูกให้ในช่วงแรกของการเจริญเติบโต ถึงแม้ว่าการเจริญเติบโตทางร่างกาย จะมีผลเสียหายร้ายแรงในระยะแรก ลูกหนูสามารถที่จะพัฒนาน้ำหนักตัวเทียบเท่ากลุ่มควบคุมเมื่อวัยโตเต็มวัยได้ และการให้ทอรีนเสริมในแม่ระว่างที่ตั้งครรภ์ และระยะให้นมลูกในช่วงแรกของการพัฒนาการสามารถปกป้องกระบวนการเรียนรู้และความทรงจำของลูกหนูได้

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CHAPTER I

INTRODUCTION

1.1 Maternal Alcohol Consumption

There is an increasing body of evidence denoting harms of alcohol consumption during pregnancy to the developing fetus (Fraser, 2006). It is well known that maternal alcohol consumption is teratogenic-exerting deleterious effects on the developing life within the womb. Moreover, alcohol may exert its devastative effects in a long term, permanently throughout the life of the affected offspring and survived children may suffer from serious cognitive deficits and behavioral dilemmas caused by alcohol-induced changes in certain brain structures. Developing nervous tissues are sensitive to intra-uterine exposure to alcohol as suggested by prevalent alcohol-related neuro-developmental disorders (Ikonomidou et al, 2000). The important mechanisms of such ranges of disorders are thought to be from various physiological changes, namely, the inhibition of proliferation during neurogenesis and the induction of apoptosis during the period of synaptogenesis (Gohlke, Griffith and Faustman, 2005). Nevertheless, it is still unclear how these two mechanisms are involved in alcohol dose and timing of exposure. Patterns of developmental delay as suggested by changes seen through the offspring lifespan can help predict such correlations. Alcohol also exerts its effects, leading to behavioral changes (Hannigan, Berman and Zajac 1993; Francesca et al, 2009). Prenatal alcohol exposure offers no benefits, regardless of the amount consumed, to the outcomes of pregnancy (Howard and Carrie, 2006), fetus growth and development (Ikonomidou et al, 2000) and as mentioned above, survived children may suffer from serious alcohol-related changes in brain physiological functions and intellectual impairment (Jacobson and Jacobson, 1996) which leads to varieties of damages including developmental delay, behavioral deviation (Jacobson, 1998; Riley and Mcgee, 2005) and cognitive deficits such as hyperactivity and attention deficit dilemmas, deficits in motor coordination, lack of regulation of social behavior or poor psychosocial functioning; deficits in other cognitions such as learning and memory (Reyes and Savage, 1989; Hall, Church and Berman, 1994; Gohlke, Griffith and Faustman, 2005). Alcohol is teratogenic but it has only recently been widely recognized by the scientific communities and the public (Australian Bureau of Statistics, 2007; Prayer for Berlin, 2008). Nonetheless, educating pregnant women in prevention of such damages has been a mere impossibility as people often think that humans have been drinking for thousands of years and they do not seem to acknowledge that drinking alcohol can be detrimental to their progeny. Therefore, additional to education, strategies in preventing, or at least, minimizing the effects of prenatal alcohol exposure should prove valuable. Such protective mechanisms begin to be explored. A number of evidence from the literatures supports the knowledge that maternal alcohol exposure causes various damages through multiple mechanisms depending on doses given, pattern and timing of exposure as well as maternal health status and genetic make-ups (Ernest, 1989; Leonard, Dwight and Louis 1997; Charles, Kristin and Feng, 2005), as well as developing tissues and target structures in question. Although there is not a specific research stating that low level of alcohol produces a strongly harmful result and that critical dosage of alcohol responsible for a wide range of abnormalities seen in affected children is still unknown, safe level of alcohol during pregnancy is neither indicated (Mukherjee et al, 2005). Animal models used in studying effects of prenatal alcohol exposure play a significant role in the effort to develop strategies in revealing deleterious impacts and preventing such occurrence. Simple animal models are useful in answering biological and behavioral questions not likely to be morally investigated by human studies due to limitations in small number of available autopsy findings, unreliable self-reports, interference from other drugs of abuse, variables from nutritional status and genetics and of course, in ethical issues. One of the most widely used animals in alcohol studies are rodents such as rats and mice. Advantages of using mice are that there is extensive literature on mice as they are quite inexpensive to acquire and maintain; they have a short gestational period, and that alcohol-induced facial dysmorphologic features have been successfully studied in mice (Timothy, 2005). Accordingly, mice were chosen as an animal model for this research.

1.2 Taurine

Taurine is a conditionally essential, sulfur-containing amino acid found in tissues such as liver, skeletal and cardiac muscles, retina and brain (Barbeau, 1975; Timothy, 1998) of most animals (Kendler, 1989). In conjunction with vitamin B6, taurine is synthesized from amino acids methionine and cysteine by an important enzyme called cysteine sulfinic acid decarboxylase (CSAD). Unlike other true amino acids, taurine contains a sulfonic acid group instead of a carboxylic acid moiety and it does not incorporate into proteins but is rather abundantly found free or as simple peptides within various tissues. Taurine is involved in various important physiological processes throughout the body, especially in electrically active tissues (Barbeau, 1975; Huxtable, 1992). As a conditionally essential amino acid, taurine exerts its vital roles during certain aspects of life, for instances, growth and development (Hayes, Carey and Schmidt, 1975; Chapman and Greenwood, 1988; Magnusson, 1994; Lake, 2003; Lima et al, 2004) as well as in cell-damaging conditions (Cruz and Pasantes-Morales, 1983; Kendler, 1989). Taurine is one of the most abundant free amino acids in the brain, being only lower than that of glutamate. The central nervous system (CNS) concentration of taurine is measured in the range of micro molar (mM) with its ubiquitous effects therein. Taurine is found to be positively involved in neuronal cell damage, exerting its neuroprotective actions in neural tissues. Such neuro-protection is observed in conditions like hypoxia, hypoglycemia, ischemia, oxidative stress as well as detrimental alcohol effects (Aragon and Trudeau, 1992; Dahchour, Quertemont and Witte, 1996).

Taurine is one of the most abundant free amino acids in the central nervous system. In many mammals, its concentrations even exceed that of glutamate during ontogenic development. Taurine is an essential nutrient, being vital for the development and survival of neuronal cells in cats as they unable to synthesize it (Hayes, Carey and Schmidt, 1975; Sturman et al, 1985). Taurine-deficient studies indicated that this simple yet genuine sulfonic amino acid is thought to exert its vital actions in developing brain tissues. Moreover, taurine is virtually proposed as having an important role in the process of learning and memory (Lee and Kesner, 2003), especially the long-term memory storage within the hippocampus, the long lasting potentiation (Galaretta et al, 1996; Olmo et al, 2000) and the late phase long-term potentiation (LTP) (Olmo et al, 2003; Olmo et al, 2004) in Schaffer collaterals of the CA1 region. Taurine also involves much with cell-damaging conditions and neuronal environmental alterations (Rea, Mcbride and Rohde, 1981) including those insulted by alcohol (Aragon and Trudeau, 1992; Dahchour et al, 2000) with its actions likely to be neuroprotective (Huxtable, 1992; Hussy et al, 1997). Interactions of alcohol and taurine within the brain (Olive, 2002) also suggest that taurine may protect the nervous tissues from vast environmental changes which lead to cellular damages caused by alcohol. Exposure of the developing brain to alcohol causes detrimental effects, leading to persistence in cognitive functional deficits and behavioral deviation. When taken together with the relationships among taurine, alcohol, learning and memory as well as neurodevelopment, the questions raised within such findings would lie in the functions of taurine involving in such complicated but vital aspects of the developing life.

Clinically, taurine is proved to be quite effective with very minimal side effects for treatments of certain diseases like cardiovascular diseases, hypercholesterolemia, seizure disorders, Alzheimer’s disease and alcoholism (Kendler, 1989). Apart from its clinical significance, taurine is used as a supplemented nutrient added to formula milk with the concentrations close to the amount found in breast milk, possibly to ensure optimal growth and development of the growing infants.

It was mentioned above that maternal alcohol consumption does no benefits to the developing life within the womb and also throughout the life of the affected offspring. If there are to be a form of preventative measures for the devastative outcomes, numerous innocent lives to be born of mothers drinking alcohol will be rescued to a large extent. It has been stipulated that taurine plays a vital role during development of the central nervous system and is also neuro-protective during damaging conditions such as alcohol exposure. Accordingly it is hypothesized that taurine may protect the developing brain against deleterious effects caused by maternal alcohol consumption.

1.3 Research Questions

1.3.1 Do various doses of alcohol differ in their teratogenic levels?

1.3.2 Does taurine play a protective role during development of the affected offspring from the mothers who have been prenatally consuming alcohol?

1.3.3 At adult age of the offspring, are there any long-term damages caused by maternal alcohol consumption in learning and memory processes and whether taurine is protective against such damages?

1.4 Hypotheses

1.4.1 Maternal alcohol consumption is teratogenic at all doses whether or not it can be physically conspicuous.

1.4.2 Taurine protects CNS tissues from alcohol insults during development and learning and memory of the affected offspring.

1.5 Objectives

In order to test the hypotheses, the research was aimed:

1.5.1 To measure changes in pregnancy outcomes and offspring body growth and also maternal behaviors resulting from different doses of maternal alcohol consumption.

1.5.2 To observe and compare changes caused either by only prenatal alcohol exposure or by prenatal alcohol exposure together with taurine supplementation.

1.5.3 To investigate changes of behavioral hippocampal-dependent watermaze learning caused by prenatal alcohol exposure with and without taurine supplementation.

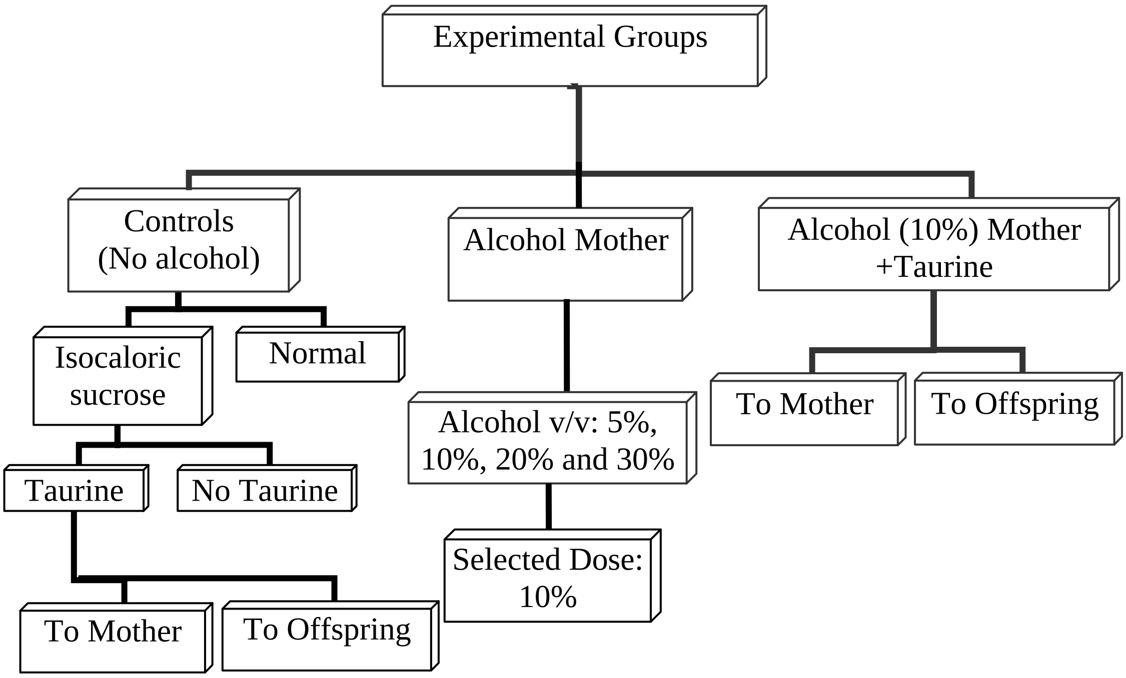
In order to pursue the objectives above, this research was designed to be comprised of 3 sections as follows:

1. Offspring body growth
2. Maternal behaviors
3. Hippocampal-dependent learning

1.6 Experimental Designs

All study groups in this research utilized the benefits of using laboratory animals: adult ICR outbred mice. Regardless of individual treatments, the experiment mice were randomly assigned to be in the following groups.

Figure 1-1 Experimental Designs



The following abbreviations were used throughout the research unless indicated otherwise.

Table 1-1 Research Abbreviations:

Control/Pair-fed	Treatment Groups
NM = Normal, non-treatment	
Suc =Isocaloric sucrose	Alc = Alcohol
SucTr = Isocaloric sucrose + taurine	AlcTr = Alcohol + taurine
SucTrPw = Isocaloric sucrose + taurine post-wean	AlcTrPw = Alcohol + taurine post-wean

CHAPTER II

LITERATURE REVIEWS

2.1 Alcohol Teratology

It has become clearer from human autopsies, human imaging studies, behavioral investigations and animal studies that prenatal alcohol exposure, at any levels, causes deleterious outcomes in the babies (Riley et al., 2004). Children indirectly exposed to even moderate levels of alcohol from the mother during pregnancy display growth deficits and intellectual as well as behavioral problems (Jacobson and Jacobson, 1996) Teratogens refer to any kinds of ontogenic damages in living organisms. In the case of alcohol, its teratogenic nature creates certain deleterious effects to the developing embryos which result, for instances, in growth retardation and physical abnormalities. In 2000, Ikonomidou and coworkers reported that exposure of rat brain to ethanol for a period of hours during specific developmental stages induces an apoptotic neurodegenerative reaction that delete large neurons from several developing sites. Moreover, cell adhesion molecules are inhibited even to exposure at low concentrations of ethanol (Charness et al., 1994). These reports suggest subsequent effects on neuronal migration, fasciculation, and synaptogenesis, all known to be vital to the developing brain. Experiments both in vitro and in vivo showed long term neuronal loss after developmental ethanol exposure. The inhibition of proliferation during neurogenesis and the induction of apoptosis during synaptogenesis all have implicated teratogenic effects of alcohol to the developing brain. Other possible cellular mechanisms of alcohol to the developing brain include the inhibition of insulin expression and action during development, thus impairing insulin-stimulated survival signals leading to inhibition of glucose transport and ATP production; and the loss of calcium-stimulated adenylate cyclase and cAMP levels resulting in neurodegeneration after administration of alcohol.

The literatures have postulated that there is no ‘safe’ level of alcohol during pregnancy. The child’s behaviors were adversely affected with as low as one single drink of alcohol per week during pregnancy. Children exposed to alcohol when compared to the non-exposed, showed at least 3 times greater odds of delinquent behaviors (Mukherjee et al., 2005). One of the deviations in behavioral patterns seen in alcoholics is antisocial personality disorder. This anti-social behavior is thought to have been associated with serotonin (5-HT) dysfunction (Francesca et.al, 2009). More research studying the tendency of female offspring from alcohol-treated mothers to have been more or less susceptible to alcoholic damages during their own pregnancy; the results suggested that offspring of alcohol-treated mothers who do not consume alcohol themselves during their own pregnancy may still have a tendency to have offspring of lower birth weight. On the other hand, if mothers prenatally exposed to alcohol do consume alcohol during their own pregnancy, the impact of fetal weight suppression is even greater than expected for in-utero alcohol exposure alone. It was also assumingly explained that mothers who were prenatally exposed to alcohol weighed less than controls at the time of becoming pregnant. As such, these data imply an increased risk for low birth weight offspring in children of alcoholic mothers who drink during their own pregnancy (Howard and Carrie, 2006).

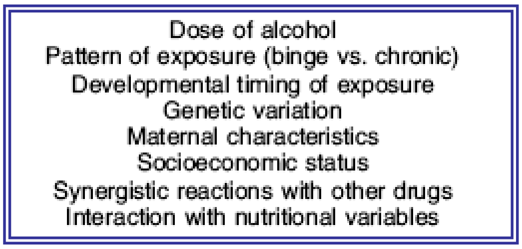
One study suggested that 2 peptides that are associated with novel glial proteins regulated by vasoactive intestinal peptide are shown now to provide protective intervention in a model of fetal alcohol syndrome. In this study, it was believed that death and growth abnormalities elicited by alcohol treatment during development are associated, in part, with severe oxidative damage. The peptides have been shown to exhibit antioxidative and antiapoptotic actions in vitro. Biochemical assessment of the fetuses indicated that the combination peptide treatment prevented the alcohol-induced decreases in reduced glutathione (Catherine et.al, 2001). These studies demonstrated that fetal death and growth restriction associated with prenatal alcohol exposure were prevented by combinatorial peptide treatment. As a result, alcohol-induced damages are caused, in part, by oxidative stress. Precise mechanisms in which alcohol mediates the developmental delays and deviations are extensively being studied.

In another recent study, it was found that activation of the glial cell line–derived neurotrophic factor (GDNF) pathway in the ventral tegmental area (VTA) reduces moderate alcohol (ethanol) intake in a rat operant self-administration paradigm (Sebastien, Ryoji and Dorit, 2009). Unlike the developed brain which has got its own protective mechanism when there is an invasive input as alcohol, the developing brain is very sensitive to deleterious effects on alcohol with little or no protective mechanisms to defend itself. Results from animal studies indicate that the fetotoxic effects of alcohol are more dependent on peak blood alcohol levels occurring in cases of heavy drinkers than upon the total amount of alcohol consumed throughout an entire pregnancy (Leonard, Dwight and Louis 1997).

2.1.1 Identifying Fetal Alcohol Spectrum Disorders

Alcohol exposure during pregnancy is associated with developmental problems in childhood ranges from severe to mild forms of physical, mental and behavioral defects. The term used to describe disorders affected by alcohol is “FASD-Fetal Alcohol Spectrum Disorder” which encompasses a wide range of deleterious effects that occur when an individual is exposed to alcohol indirectly from the mother during gestational period. All FASDs ranges from the most severe form, ‘FAS’ (Fetal Alcohol Syndrome) to ‘partial FAS’ and ‘ARND’ (Alcohol-Related Neuro-developmental Disorder). The most striking damages alcohol does to the babies are to the developing brain, causing various CNS deficits which lead to abnormal behaviors. FASDs constitute a major public health problem since the early 1970’s when reports on the long-term consequences of prenatal alcohol exposure have captured the public attention to care for children born of alcohol-abusing mothers (Jones and Smith, 1973).

Table 2-1 Risk Factors Associated With Alcohol Teratogenic Effects



Dose of alcohol
Pattern of exposure (binge vs. chronic)
Developmental timing of exposure
Genetic variation
Maternal characteristics
Socioeconomic status
Synergistic reactions with other drugs
Interaction with nutritional variables

Table 2-2 FASD Diagnostic Criteria

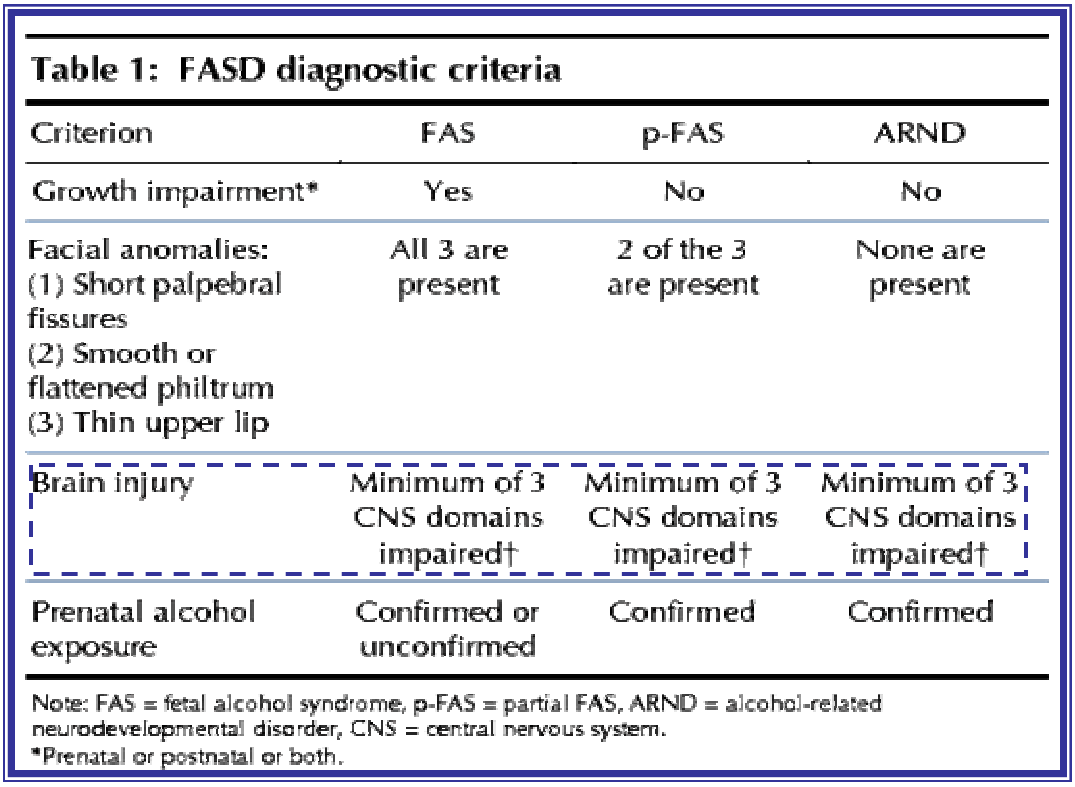


Table 1: FASD diagnostic criteria

Criterion	FAS	p-FAS	ARND
Growth impairment*	Yes	No	No
Facial anomalies: (1) Short palpebral fissures (2) Smooth or flattened philtrum (3) Thin upper lip	All 3 are present	2 of the 3 are present	None are present
Brain injury	Minimum of 3 CNS domains impaired†	Minimum of 3 CNS domains impaired†	Minimum of 3 CNS domains impaired†
Prenatal alcohol exposure	Confirmed or unconfirmed	Confirmed	Confirmed

Note: FAS = fetal alcohol syndrome, p-FAS = partial FAS, ARND = alcohol-related neurodevelopmental disorder, CNS = central nervous system.
*Prenatal or postnatal or both.

Loock et al., 2005

The table above illustrates how ranges of alcohol damages are classified and diagnosed. It has to be stressed out that it doesn't matter what spectrum the disorder falls into, the brain is the most heavily vulnerably affected as all disorders are presented with at least 3 kinds of CNS deficits. The key domains of CNS impairments are shown below.

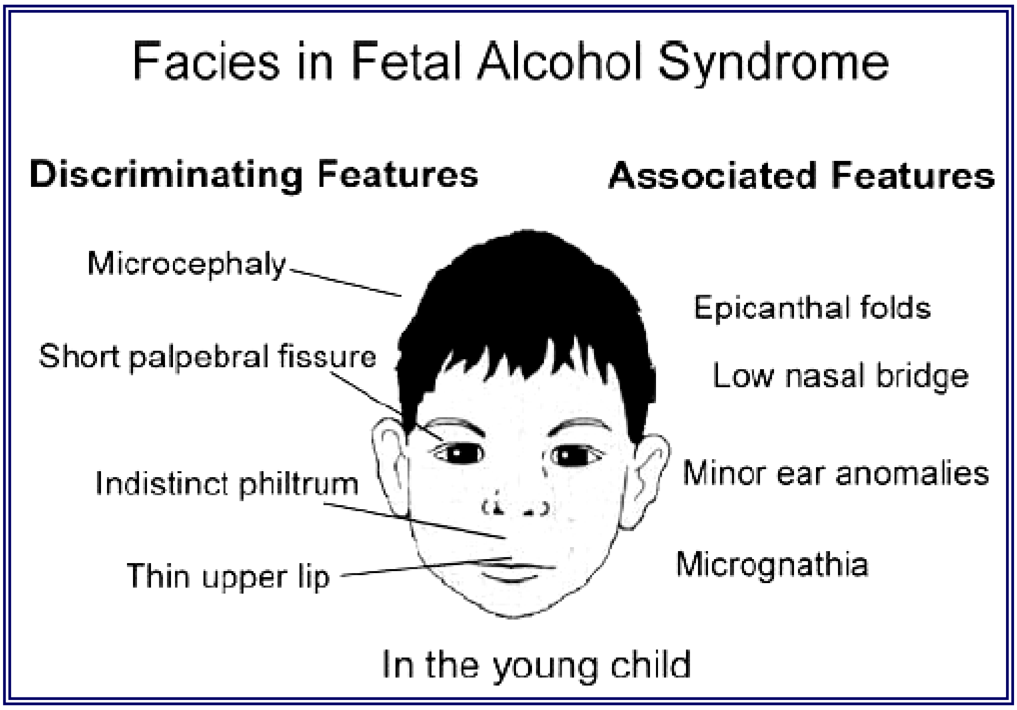
Table 2-3 Key Domains Of CNS Deficits
Adapted from Loock et al., 2005

Hard and soft neurological signs
Brain structure (including microcephaly)
Cognition
Communication
Academic achievement
Memory
Executive functioning and abstract reasoning
Adaptive behaviour, social skills, social communication
Attention span, activity level, distractibility

Fetal Alcohol Syndrome

FAS is the most widely recognized form of the consequences of prenatal alcohol exposure, first reported to have much effects on the developing brain in 1973 by Jones and Smith. They reported that the autopsy of an infant with FAS revealed a widespread damage throughout the brain. In addition to microcephaly, there were errors in migration, agenesis of the corpus callosum and anterior commissure, and cerebellar and brainstem anomalies. Subsequent autopsy confirmed the widespread disorganization of the CNS, for instances, neuroglial heterotopias and ventricle, corpus callosum, basal ganglia, and cerebellar anomalies (Jones and Smith, 1973; Symposium by Riley and McGee, 2005). The children diagnosed with FAS display at least three main abnormalities: growth retardation, specific abnormal facial appearance, and CNS dysfunctions. The typical facial characteristics of FAS are smooth philtrum, thin vermillion, and short palpebral fissures. Other less common features are epicanthal folds, strabismus, ptosis, low nasal bridge and ear anomalies.

Figures 2-1Facies In Fatal Alcohol Syndrome: *Riley and McGee, 2005*



FAS children are usually first diagnosed by their below-average head circumference (< 10th percentile). With the head circumference being below normal, FAS children display some neurological problems and a number of functional deficits including global cognitive or intellectual abnormalities. The behavioral and cognitive abnormalities cover such deficits in problem solving, planning, arithmic, language, motor functioning and other social behaviors.

Other FASDs

Unfortunately FAS is not the only consequence of prenatal alcohol exposure as evident from bodies of scientific and clinical reports. Gestational alcohol exposure expresses a continuum or spectrum of disorders. The effects of alcohol at any given level during pregnancy produce a life-long damage on physical, mental and behavioral aspects of the offspring. The major factors influencing the outcomes are the amount of alcohol that gets to the embryos and during which period of development. Genetic and nutritional factors also play a role in sensitivity of individuals to alcohol. More importantly, the time during which alcohol reaches the embryos determines which developing structures are the most susceptible, being the most vulnerably affected. This of course involves the critical period of exposure. In human, prenatal alcohol exposure during the first trimester interferes with migration, proliferation and organization of brain cells as also confirmed with mice experiments during the period equivalent to human period resulted in severe malformations of the face and brain (Reviewed in Symposium by Riley and McGee, 2005). The malformations are thought to be the results of cellular death. During the third trimester, alcohol can damage the cerebellum, hippocampus and prefrontal cortex.

2.1.2 Alcohol and Changes in Brain Structure

Although the facial characteristics of FAS are the most noticeable signs of heavy alcohol drinks during pregnancy, the more devastating consequences are unfortunately related to brain and behaviors. A symposium article by Riley and McGee in 2005 demonstrates that the overall brain size and shape are affected. At anatomic level, the most affected brain regions are cerebellum, corpus callosum and basal ganglia. The research, using MRI, found that there was an overall reduction of the cranial vault and the concomitant reduction in brain size. There also found a prominent abnormalities in the cortices in parietal and temporal lobes. Shapes of the affected brains are also found to alter in alcohol-affected children. There indicated the reduced brain growth in the ventral portions of the frontal lobes. The results may suggest the correlations of cognitive and behavior abnormalities seen in the children such as their difficulties in response inhibition, behavioral control and executive functions.

Cerebellum

Several autopsies and animal studies suggest that cerebellum is a structure particularly sensitive to prenatal alcohol (Riley et al., 2004). Cerebellum is involved in motor control and maintenance of balance as well as attention in certain types of learning. The most severe damage alcohol does to the cerebellum is in size reduction. It was found that in FAS, the reduction was more than 15 % of normal subjects while other forms of FASDs showed also significant reduction but not as high. Regional analysis indicated the anterior vermis was reduced in size while the posterior vermis was not affected.

Corpus Callosum

Structural analysis by Riley and coworkers demonstrates significant changes in this brain structure connecting the two hemispheres. Corpus callosum allows coordination of actions through interhemispheric communication. They found that one child with FAS showed agenesis of the corpus callosum while the other child had extremely thin corpus callosum. Some other subjects with FASDs was shown to have the structure displaced in someway, being more anterior and inferior than normal. This displacement may help to explain the correlations with difficulties of these children with performance on verbal learning tasks.

Basal Ganglia

This group of subcortical nuclei is also sensitive to alcohol effects. The basal ganglia involve voluntary limb movement, eye movement and cognition. Riley and coworkers, using quantitative structural magnetic resonance imaging, found that most of the nuclei including caudate and lenticular nucleus were reduced in size in FASDs compared to controls.

Hippocampus

Alcohol consumption during development affects the maturation of hippocampal circuits but mechanisms of how this happens are not yet fully understood. It was found that ethanol increases a giant depolarizing potentials in the CA3 hippocampal region of slices from neonatal rats. Ethanol is also found to induce potentiation of GABAergic activity. A recent finding from Clements and coworkers showed that ethanol exposure during brain growth spurt impairs spatial short-term memory in rates tested with Morris water maze (Clements et al., 2005). Apart from longer time spent in the maze, hippocampi from these rats express lower c-Fos expression than controls.

HPA Axis

Alcohol abuse is known to result in clinical abnormalities of endocrine function and neuroendocrine regulation. Influence of alcohol on endocrine function during pregnancy may contribute to etiology of fetal alcohol syndrome since alcohol crosses the placenta and directly affects the developing fetal cells and tissues. The alcohol-induced changes in maternal endocrine function may also disrupt the maternal-fetal hormonal interactions and affect the female’s ability to maintain a successful pregnancy. A research by Zhang and coworkers reported that alcohol can reprogram the HPA axis in such a way that the HPA activity increases in both the mother and offspring. This increase in HPA leads to increased endogenous glucocorticoids throughout the lifespan, thus altering behavioral and physiologic responsiveness later in life (Zhang et al., 2005).

2.1.3 Neuropsychological and Behavioral Changes

Heavy prenatal alcohol exposure is associated with a wide range of neuropsychological deficits including impairments in memory, language, attention, reaction time, visuo-spatial abilities, executive functioning, fine and gross motor skills and social/adaptive functioning. Children with or without physical features of FAS demonstrate similar deficits.

Learning and Memory

Both verbal and nonverbal learning and memory assessed in children with FAS appeared to be impaired (Symposium by Riley and McGee, 2005). FAS children demonstrate deficits in memorizing verbal information, resulting from difficulties with acquisition of the information rather than with the ability to remember the information. Also prenatal alcohol exposed individuals, both humans and animal models are shown with working memory deficits. Working memory refers to a measure of the individual's ability to mentally manipulate the information presented and to link the information with other information retrieved from memory. Several tests during childhood and adolescence demonstrate that these individuals are impaired in working memory (Jacobson, 1998). In laboratory animals, the results linked prenatal alcohol exposure to impaired performance in the Morris water maze which assesses the animals' abilities to find and recall the location of a platform submerged in an opaque liquid (Hannigan et al., 1993); and on the radial eight-arms maze which assesses the animals' abilities to retrieve food pellets from the end of all eight-arms of a maze without revisiting arms from which food has not been retrieved (Reyes et al., 1989; Hall et al., 1994).

Language

Children with FAS and FASDs are often found to have less word comprehension and problems with naming ability.

Attention

Children with FAS are often compared with those of ADHD in terms of their attention. Most of FAS are also diagnosed with ADHD, showing inattention and impulsivity. Deficits in visual attentions seem to be common while auditory attention is also impaired but with less degree of severity.

Visuospatial Abilities

Children affected by prenatal alcohol exposure have shown deficits in visuospatial processing and memory. Such deficits may be related to the difficulty in shifting attention and the reduced cerebellar vermis may just explained it (Courchesne et al., 1994).

Executive Functioning

Executive functioning is a complex construct defined as the ability to maintain an appropriate problem solving set for the attainment of a goal. Several cognitive domains in prenatal alcohol exposed children are affected. Such domains are for examples, planning, working memory, cognitive flexibility, response inhibition, concept formation and reasoning. These deficits are thought to be related to the reduction and thinning of frontal lobes and basal ganglia which are connected to frontal-subcortical circuits. These executive functioning deficits also relate to other deficits in spatial memory and attention problems.

Fine and Gross Motor Skills

Children with FAS often have delayed motor development and fine motor dysfunction including tremor, weak grasp and poor hand-eye coordination. In rats, impairments in balance are associated with damages to the cerebellum.

Adaptive and Social Skills

Prenatal alcohol exposed children often have behavior problems interfering with their participation in home, school, and social environments. These children are observed as hyperactive, disruptive, impulsive or delinquent. Their impairments are particular difficulties in social attention.

2.1.4 CNS Cellular Response to Teratogenic Effects of Alcohol

Much of what alcohol is done to the developing brain is to cause neuronal cell death or neuroapoptosis. It has been reported repeatedly that exposure of infant rats to ethanol causes a reduction in brain mass and loss of cerebellar Purkinje cells, but the mechanisms underlying these effects have remained elusive. It was proposed that ethanol-induced lengthening of the cell cycle of neural progenitor cells during rat neocortical neurogenesis and an increase in cell death rates may be the key factor. Rat synaptogenesis (P0–P14), when programmed cell death plays a major role in shaping the future neocortex is of course, more sensitive to effects of maternal alcohol consumption, assuming that there is a proper lactation while the mother is still given alcohol. A study (Gohlke, Griffith and Faustman, 2005) indicated that at a human blood ethanol concentration that occurs after 3–5 drinks (~150 mg/dl), the study model predicts a 20–30% neuronal deficit due to inhibition of proliferation during neurogenesis, while a similar exposure during synaptogenesis suggests a 7–9% neuronal loss through induction of cell death. Research also suggested that the mechanism of alcohol-induced structural changes resulting in developmental delay lays heavily on long-term neuronal loss after developmental exposure to ethanol (Gohlke, Griffith and Faustman, 2005). Another study (Rasmussen and Christensen, 1980) suggested significant growth retardation, malformation, and intrauterine death among the animals given alcohol. At the same time they found a clear dose-effect relation. The malformations were also found to be localized to the facial skeleton, the CNS, the eyes and lungs (Rasmussen and Christensen, 1980). In a recent series of studies, it demonstrated that exposure of infant rats or mice to ethanol on a single occasion during the synaptogenesis period of development causes neurons in many regions of the developing central nervous system to commit suicide. A recent finding by Dikranian and team (2005) reported that intrauterine exposure of rats and mice to alcohol causes neuronal cell death in the developing cerebellum and some brain stem structures like nucleus pontis, and inferior olivary complex.

A research by Wen and Kim in 2004 attempted to interpret the biochemical mechanisms underlying hippocampus-related functional deficits associated with prenatal ethanol exposure. In their work, using high performance liquid chromatography/electrospray ionization-mass spectrometry analysis, have investigated the effects of chronic ethanol exposure on the phospholipids profile in developing rat hippocampi. There, they found that ethanol lowered the levels of total phosphatidylserine by 15-20% at all ages. Ethanol exposure also leads to a decrease in phosphatidylcholine and an increase in phosphatidylethanolamine but the total phospholipids profile was not significantly changed. Alterations of the phospholipids profile in the hippocampus resulting from ethanol exposure during prenatal and developmental stages may have significant implications with respect to the cognitive dysfunction observed in alcohol affected children. Chronic prenatal exposure also alters hippocampal $GABA_A$ receptors (Iqbal et al., 2004). In adult guinea pig, alcohol exposure up-regulates $GABA_A$ receptor expression in the cerebral cortex and decreases functional synaptic plasticity in the hippocampus. It was found that in adult offspring of the alcohol exposed guinea pig, there are an increased expression of the β 2/3 subunits of the $GABA_A$ receptor in the hippocampus. These animals were also tested in an open-field and water maze for their changes in behaviors. It was found that there is an increase in spontaneous locomotor activity but an impaired task acquisition in Morris water maze. The data suggested that alterations in hippocampal $GABA_A$ receptor expression may partially contribute to hippocampal-related behavioral and cognitive deficits associated with chronic prenatal alcohol exposure.

In brief, prenatal alcohol exposure can cause permanent damages to the brain and no safe 'level of alcohol' is suggested at all during pregnancy. The brain changes affect behaviors. These alterations in brain are reduced brain size, alterations in shapes, tissue density and symmetry, volumetric reduction and specific abnormalities in cerebellum, basal ganglia, and corpus callosum. The shape and tissue abnormalities highlight the regional nature of brain morphologic differences and suggest that assessment of brain-behavior relationships may be region-specific. Brain growth continues to be adversely affected long after the prenatal insult of alcohol exposure to the developing brain, and the brain regions most implicated, frontal and parietal areas, may be consistent with the neurocognitive deficits, characteristics of individuals prenatally exposed to alcohol.

2.2 Exploring Taurine

2.2.1 History and Availability

Taurine was first isolated from ox bile in 1827 (Tiedemann and Gmelin, 1827). As being discovered in bovine, with a Latin name of 'Bos Taurus', hence its name: 'Taurine'. It was not until almost 150 years later that taurine was recognized to be nutritionally significant (Raiha et al., 1975) when it was found that formula-fed, pre-term infants were not able to retain normal plasma or urinary taurine levels. It was also found that in taurine-deficient cats, there occurred a retinal degeneration (Hayes et al, 1975). Since this sulfonated amino acid was discovered in ox bile, its functions in bile acid conjugation is probably the most well-known, yet, accounts for only a very small proportion of the total body pool of taurine especially in humans (Timothy, 1998). Other important functions of this sulfonated amino acid started being explored since its nutritional significance in 1975, and the more it becomes obvious as a conditionally essential amino acid abundantly found throughout the body tissues of most animal species.

Dietary Sources of Taurine

Taurine is present in high concentration in algae and in animal kingdom including mollusks and arthropods, but is generally absent or present in traces in the bacterial and plant kingdom (Huxtable, 1992). Taurine can be found as a high percentage of all amino acids in various carnivorous animal proteins in such food as meat, certain seafoods and poultry while cow milk and some plant kingdom, as mentioned, contain only traces of taurine. Taurine, if ever existing at all, in plants is measures in nano-mol only while animal taurine is measured in the range of micro-mol. The most abundant sources are found in human breast milk, indicating the vital roles of taurine in development of infants. Different species contain different proportions of taurine.

High levels of taurine were observed in some seafoods. However, not all sea creatures contain large amounts of this sulfonated amino acid. Shrimps, for instance, contain levels of taurine similar to that found in most meats. Taurine is also abundantly found in milk of some mammals. Details on nutritional taurine levels are as followed.

Table2-4*Taurine Contents In Certain Food*

Food	**Taurine content** mg/kg	**Food**	**Taurine content** mg/kg
Conch (Strombus gigas)	8500	Eel	910
Inkfish	6720	Pork meat	1180
Blood Clam	6170	Pork heart	2000
Shellfish	3320	Pork kidney	1200
Crab	2780	Pork liver	420
Prawn	1430	Chicken breast	260
Sole	2560	Chicken leg	3780
Crucial carp	2050	Quail muscle	95-280
Silver carp	900	Quail serum	0.50-0.9
Hairtail fish	560	Tuna canned	3320
Yellowfish	880	Low-fat plain yogurt (mg/l)	7.8
Octopus	3900	Shrimp	1150
Cat, entire body	2000	Cheetah serum	0.8-6.3
.	.	Cat serum	6-14

Adapted from "A compilation by Ch M Ruessheim", 2000

Cow's milk and milk products in general don't contain a lot of taurine, eggs virtually none. Taurine content in milk varies greatly between species and depends also on time of lactation. Rassin et al. has established an interesting overview in 1978 on various taurine concentrations in milk of diverse species:

Table 2-5 Taurine Concentration In Milk Of Diverse Species

Species	Concentration mmol/l	Amount of free AA %
Gerbil	5.95	43.8
Cat	2.87	71.8
Dog	1.91	75.5
Rhesus Monkey	0.56	33.1
Mouse	0.75	25.4
Human	0.34	13.0
Chimpanzee	0.26	6.6
Rat	0.15	9.6
Sheep	0.14	14.2
Rabbit	0.14	4.2
Cow	0.01	1.8
Horse	0.03	1.6
Guinea Pig	0.56	3.4

Rassin et al., 1978

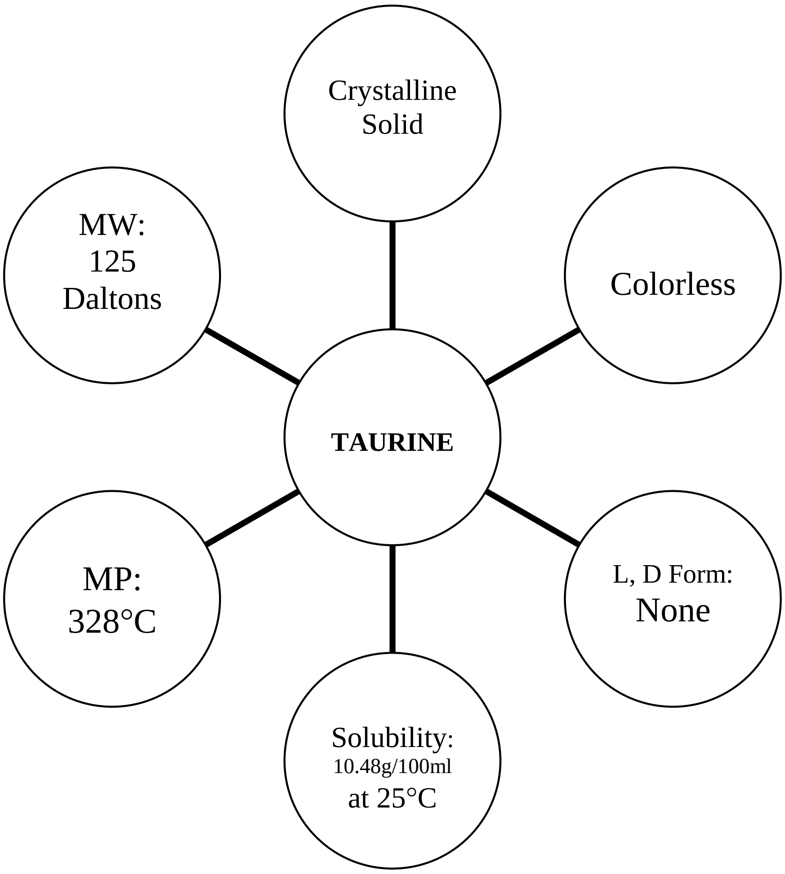
A 70-kg human can contain up to 70 g of taurine (Huxtable, 1992), suggesting that the compound is strongly conserved in such a way that it exhibits advantageous functions to the life forms containing it.

Taurine is now used widely in biochemical research and pharmaceuticals as well as an active ingredient in energy drinks such as “Red Bull” and pre- and post-alcohol consumption drinks such as “Hang”.

Bio-Physico-Chemistry of Taurine

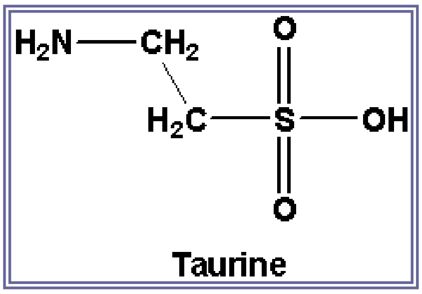
Structurally, taurine is a unique beta-aminosulfonic acid, widely distributed throughout the body although the highest concentrations are found in muscles and brain (Chapman and Greenwood, 1988). Taurine, 2-aminoethanesulfonic acid, with a molecular mass of 125 Daltons, is a colorless compound, crystallizing in tetragonal needles. Taurine holds a melting point at 328°C, and is water soluble in the ratio of 10.48g/100ml at 25°C (For review, see: Wright et al, 1986). Most amino acids possess the L- or D- configuration as it rotates light either to the left (Levo-L) or right (Dextro-D) in solution. Taurine, on the other hand, does not polarize light, thus possesses no L- or D- configurations.

Figures 2-2 Bio-Physico-Chemistry Of Taurine



Although frequently referred to as an amino acid, it should be noted that taurine contains a sulfonic acid group rather than carboxylic acid moiety likely found in other amino acids. Taurine does not incorporate into proteins like any other true amino acids, but rather found to be free within tissues. Its structure is similar to gamma-aminobutyric acid (GABA), leucine and choline, all known to be important in nervous tissues.

Figures 2-3 Structure Of Taurine ($C_2H_7NO_3S$)



The biological actions of certain compounds are surely the consequences of its physicochemical properties. Taurine is a unique amino acid in that it contains sulfonic instead of carboxylic group, and in being β-amino acid rather than α-amino acid. Compared to carboxylate, sulfonate is a strong acid, having an acidic dissociation constant (pK_a) equals to that of mineral acids (Huxtable 1992). By nature of its properties, taurine is highly water soluble and low lipophilic. As a consequence, comparing with carboxylic amino acids, taurine diffuses through lipophilic membranes very slowly. The impermeability of biological membranes to taurine probably underlies the extraordinarily high concentration gradients that are maintained across such membranes.

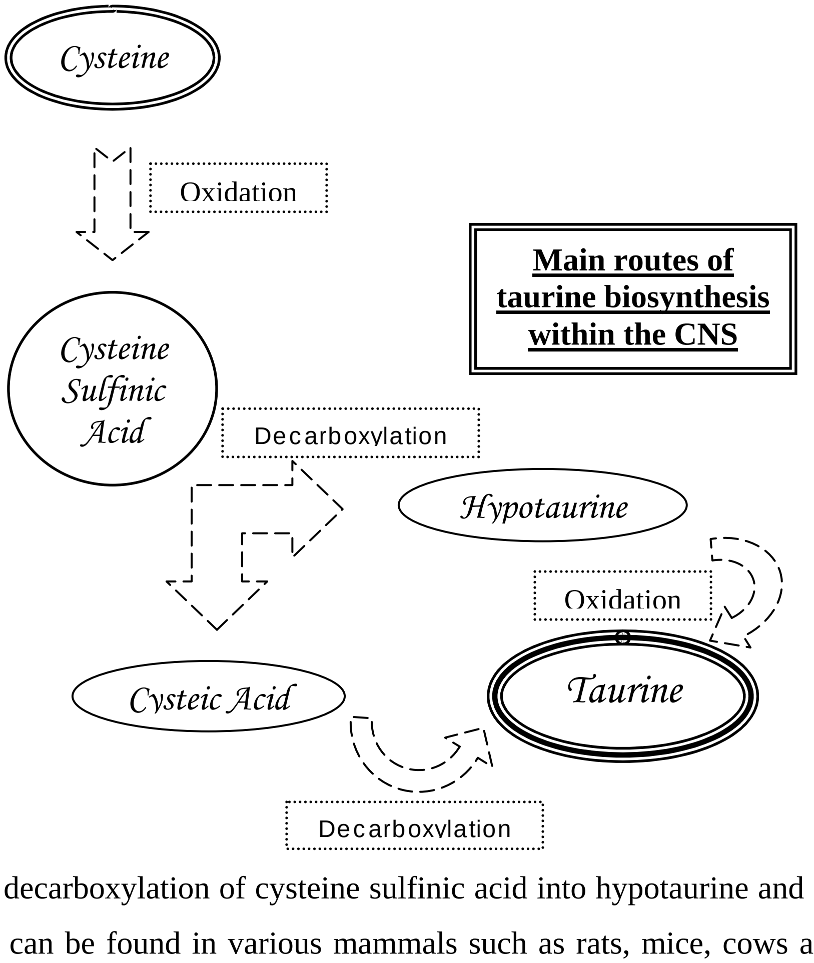
During transport, sodium ions are co-transported with amino acids. For each taurine molecule transported, between 1-3 Na^+ are carried. This Na^+ is pumped back out via the Na^+ - K^+ - ATPase, hydrolyzing 1 ATP per 3 molecules of Na^+.

Taurine possesses an isoelectric point that falls between that of carboxylic acids such as glycine, β-alanine and GABA, and that of acidic amino acids like aspartate and glutamate. The membrane modulatory actions of taurine and its interactions with Ca^{2+} and other cations probably are the consequences of its unique ionic properties.

Biosynthesis of Taurine

There are several pathways in which taurine can be synthesized but the main biosynthetic pathway in many mammalian tissues is through the metabolisms of the essential amino acid methionine and its related non-essential amino acid cysteine. However, all pathways require an active co-enzyme form of vitamin B6, pyridoxal-5-phosphate, as a co-factor (Timothy, 1998). Cysteine sulfinic acid decarboxylase (CSAD), an enzyme converting cysteine sulfinic acid and cysteic acid into hypotaurine and taurine, respectively, is thought to be the rate-limiting enzyme in taurine biosynthesis. The diagram below simplifies 2 main pathways of taurine being synthesized from cysteine into hypotaurine and cysteic acid and finally into taurine.

Figures 2-4 Main Routes Of Taurine Biosynthesis Within The CNS



The decarboxylation of cysteine sulfinic acid into hypotaurine and cysteic acid into taurine can be found in various mammals such as rats, mice, cows and humans. Tissues found with taurine synthesis (CSAD activities) are, for instance, intestines, liver, kidneys, heart and brain (Jacobsen and Smith, 1968). Tables below illustrate where such activities are found in various mammalian tissues.

Species	***Tissues***
Man	Liver
	Brain
	Kidney
Rabbit	Liver
	Brain
	Kidney
	Heart
Mouse	Liver
Rat	Liver
	Brain
	Kidney
	Heart
Guinea pig	Liver
Dog	Liver
	Brain
	Kidney
	Heart
Cat	Liver
	Brain
	Kidney
	Heart
Pig	Liver
Cow	Liver
Horse	Liver

Table 2-6 Locations Of Enzymatic Activity Of CSAD
Modified From Jacobsen And Smith, 1968

Distribution of Taurine

Taurine is one of the most abundantly free amino acids found throughout the body of diverse animal species. In general, taurine concentration is high in species having cells which lack cell wall (eg. animal cells) and is low or absent in cells having rigid cell wall (eg. plant cells). Highest concentrations being found in electrically active tissues suggest its vital roles in those areas. In this review, although taurine distribution within the body is also mentioned, it will be mainly focused within the CNS tissues.

Body Taurine Distribution in Man

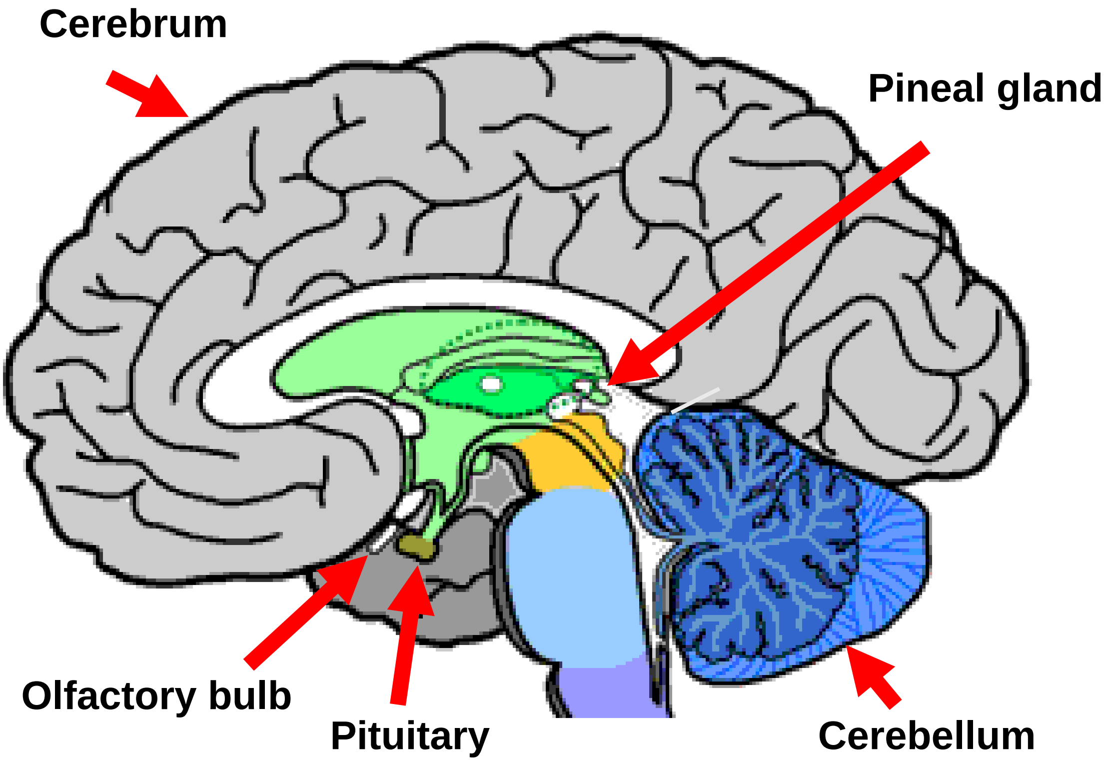
In human, taurine is found to be distributed throughout the body especially electrically active tissues such as heart, muscles, and brain. Some other parts of the body also contain high amount of taurine, for example, in body fluids like bile, milk, saliva, plasma, and cerebro-spinal fluid (CSF). The concentration of taurine in human body fluid can range from 25 μmoles / litter found in plasma (ranges between 25-150) to 337 μmoles / liter found in human breast milk. For review, see: Jacobsen and Smith, 1968.

Taurine Distribution in the CNS

There have been a number of reports on the CNS distribution of taurine (For review, see: Huxtable, 1989). There is a uniform microdistribution of taurine in various structures (Yoneda and Kuriyama, 1978). In all species, taurine level within the retina is found to be the highest of all structures in the CNS (Pasantes-Morales and Cruz, 1985). Two-third of the level in retina is concentrated in the photoreceptors. Other regions rich in taurine are cerebral cortex, cerebellum, olfactory bulbs, hippocampus (pyramidal and granular cells in CA1, CA3 regions and dentate gyrus), secretory structures like pineal, pituitary and neurohypophysis (For review, see: Huxtable, 1989).

It appears that all cell types within the CNS contain taurine but found to be high in glia and synaptosomes. Measurement of tissue taurine levels following selective destruction of cerebellar granule and stellate cells suggests that taurine is present in numerous cell types (Rea et al., 1981). Taurine is found abundantly in synaptosomes and synaptic vesicles contained within. However, taurine content found in these cellular structures account only for a small amount of the total whole brain taurine.

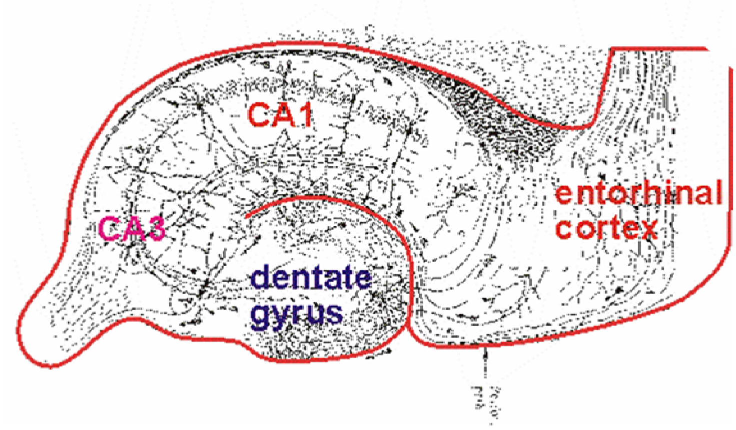
Figures 2-5 Gross CNS Distribution Of Taurine



Gross CNS Distribution of Taurine

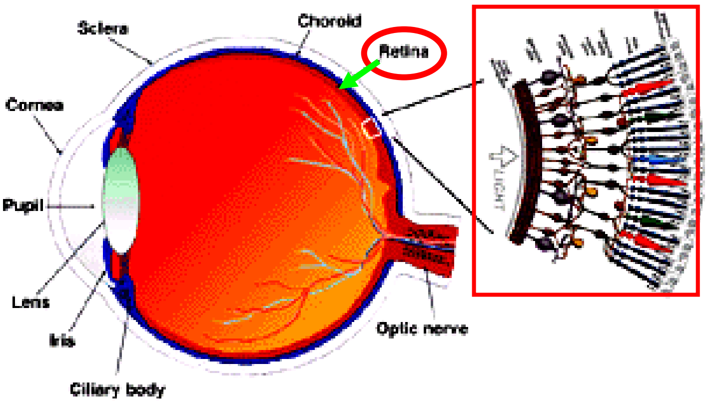
The techniques used to detect levels of taurine has been developed 20 years ago, one being immunocytochemistry (Storm-Mathisen et al, 1984; Madsen et al, 1985; Ottersen et al, 1985; Tomida et al, 1985; Ida et al, 1988) and the other brain microdialysis (Ungerstedt et al, 1982 and Max, 1987). By using immunocytochemistry, high distribution of taurine is found in cerebellum and hippocampus (Madsen et al, 1985). There also found the colocalization of immunoreactivity of both taurine and taurine synthesizing enzyme, CSA decarboxylase within cerebellar neurons and pyramidal and granular cells of the CA1, CA3 regions of the hippocampus. In 1988, Ottersen reported that there is an immunoreactivity overlap of taurine-like and GABA-like in Purkinje cell terminals. Another technique used to detect taurine is the brain microdialysis. Similar distribution has been found: dentate gyrus, striatum, cortex, olfactory bulb and hippocampus (For review, see: Huxtable, 1989).

Figures 2-6 Hippocampus



Hippocampus:
Pyramidal and granular cells in CA1, CA3 regions and dentate gyrus are found to contain high level of taurine.

Figures 2-7 Retina



Retina:
Highest concentration of taurine is found in retina, of all structures derived from CNS.

Taurine concentration in the CNS is maintained both by biosynthesis and transport from plasma. Little amount of taurine can be synthesized within the brain so it is mostly transported. As a result, many functions of taurine within the CNS is non-metabolic, unlike those observed in other tissues like kidney as bile salt conjugations. As mentioned earlier on the biosynthesis of taurine there are several routes that this sulfur amino acid can be produced. But only one main route is used within the CNS: the CSA decarboxylase route where cysteine is decarboxylated into hypotaurine first and to taurine. It was found that many species are capable of converting cysteine into taurine. In rat brain slices preloaded with [^{35}S] methionine, there found the radio-labeled production of cysteine, CSA, cysteic acid, hypotaurine and taurine (Peck and Awapara, 1967). Taurine transport appears to be more important in some structures. There is an evidence that taurine is transported into the retina across the pigment epithelium. In the review by Huxtable, it is clearly stated that synthetic enzymatic activities of CSA decarboxylase and the concentration of taurine is not correlated within the CNS, meaning that taurine level is obtained mainly through transport and the metabolic functions of taurine resulted from biosynthesis do not play as significant roles within the CNS as its non-metabolic ones. If there is a significance of its metabolic functions, it is so by the process, and not by product. In other words, one of the lesser known metabolic functions of taurine is there in a relationship between GABA and taurine biosynthesis being that cysteine sulfinic acid can be, on a different enzymatic route, converted into glutamic acid and to later to GABA, for instance.

Current methods for taurine chemical analysis include colorimetric method, fluorometric method, radiometric method, enzymatic method. HPLC, and Gas Chromatography.

Molecular Consideration of Taurine Availability

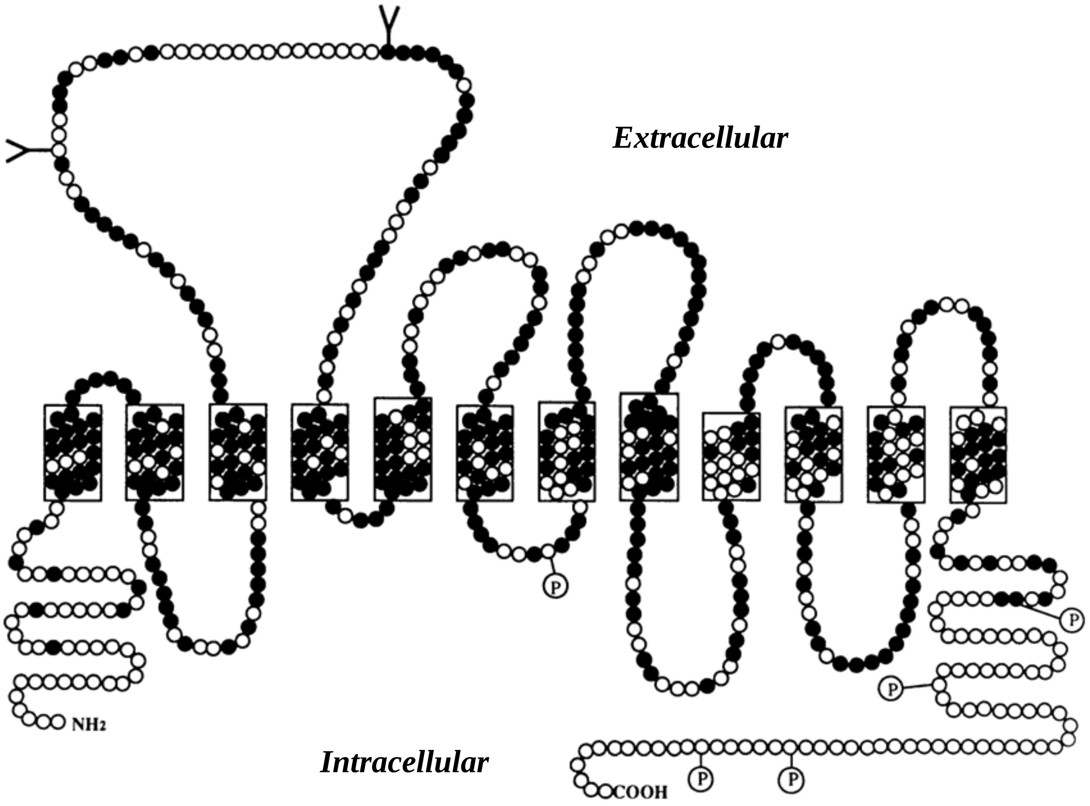
Taurine contents are found to be high in various tissues among diverse species. Many biological activities of taurine depend on its intracellular concentration. Its availability relies on two sources, namely, taurine transport and intracellular biosynthesis. Recent methods have been developed over the past decade for the molecular investigations of taurine availability. Such methods are, for instances, molecular probes, RT-PCR primers, antibodies against synthetic polypeptides or recombinant proteins, wild-type and mutation studies, etc.

Taurine Transporters

Taurine transport is accounted for the provision of taurine into the cells and is found to be more significant in maintaining the high levels within the cells than that of intracellular biosynthesis. Taurine is transported through a specific β-amino acid transporter called TAUT (***TAU***rine ***T***ransporter). TAUT is a Na^+ and Cl^- dependent transporter, a specific characteristic of various identified transporters for brain neurotransmitters (Uchida et al, 1992). Northern hybridization indicates the presence of TAUT mRNAs in various tissues like kidneys, intestines, liver, heart and brain. Mammalian species in which taurine transporter cDNAs have been cloned are rat, mouse, dog, bovine and human (For review, see: Tappaz, 2004).

Taurine transporter is a 12 trans-membrane protein composed of approximately 620 amino acids with a molecular weight of 70 kDa. Mammalian TAUTs show more than 90% amino acid sequence identity. TAUT has 12 hydrophobic domains that are thought to be the membrane spanning regions of the transporter. This makes TAUT a member of the larger family of the Na^+ and Cl^- dependent transporters such as those of neurotransmitter, amino acid and osmolyte transporters (Nelson, 1998). There are two potential N-glycosylation sites in the second intracellular loop and other potential phosphorylation sites at the fourth cytosolic loop, which is later found to be the specific site for PKC. TAUT is a high affinity and low capacity transporter requiring two Na^+ ions and one Cl^- ion for one taurine molecule transport across the cell membrane. TAUT allows a narrow specificity for β-amino acids: taurine, hypotaurine and β-alanine. Alignment of TAUT amino acid sequence with other members of the family showed that TAUT has 40% identity with glycine transporter, 42-46% identity with catecholamine transporters, 48% identity with proline tranporter and 52% and 62% identity with GABA transporters GAT1 and GAT2 respectively (Liu et al, 1992). Similar homology suggests that these transporters are from the common ancestors.

Figures 2-8 Proposed Membrane Topology Of TAUT



Adapted from Uchida et al, 1992

<u>Proposed Membrane Topology of TAUT</u>

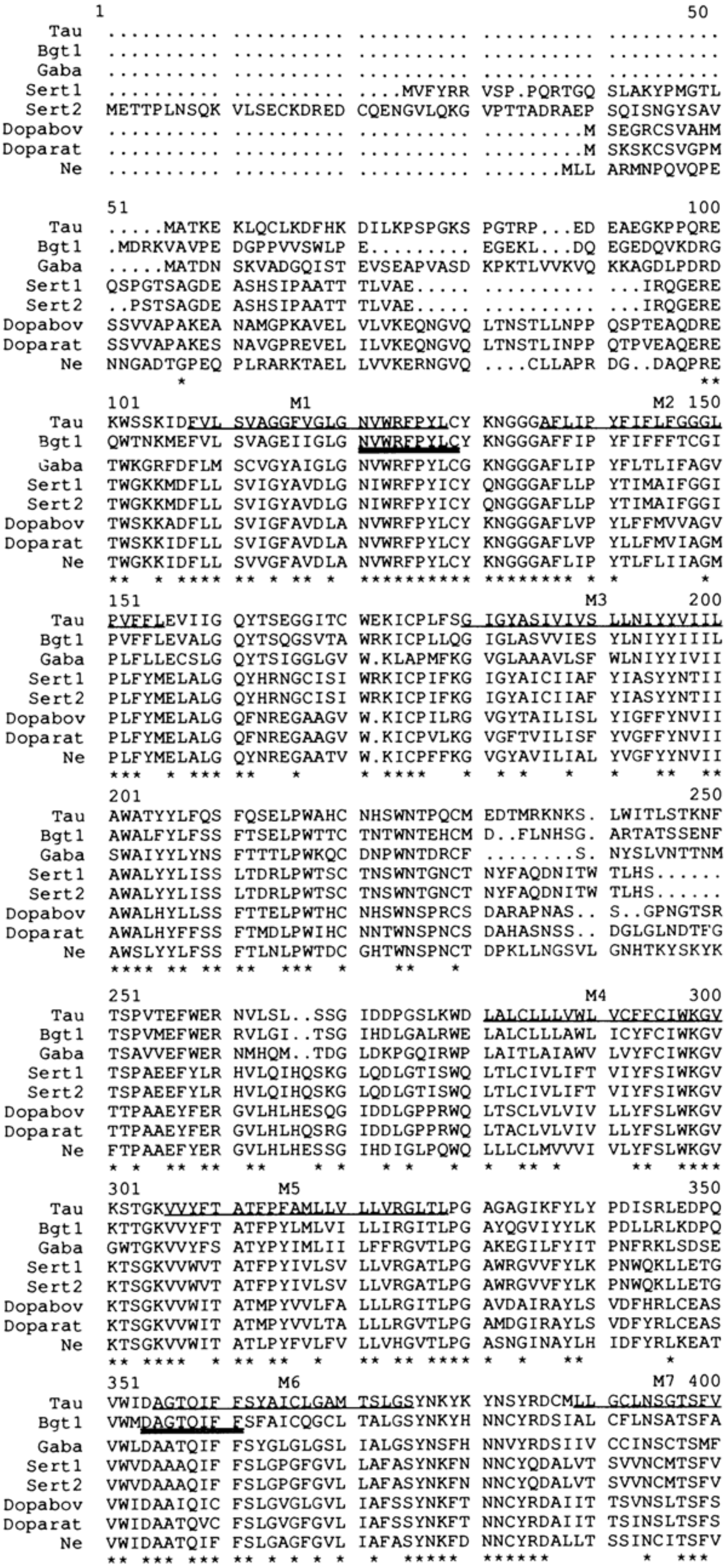
Each circle represent an amino acid. The dark, solid circles are identical amino acids of TAUT to those of MDCK cell betaine/GABA transporters. The putative N-glycosylation sites (Y) and phosphorylation sites (P)are indicated (amino acids 322, 581, 637 for Protein Kinase C; 607 and 643 for cAMP–dependent protein kinases).

TAUT cDNA in mouse brain has been cloned by Liu and coworkers in 1992 and it was found that TAUT possesses an isoelectric point of 5.98 which is more acidic than other cloned neurotransmitter transporters in which the pI falls above 8.0. The same group of researchers showed by using northern hybridization that a single transcript of 7.5 kilo bases is distributed evenly within various parts of the brain, with cerebellum being a little more abundant than that in cerebrum. In situ hybridization showed that the mRNA of TAUT is localized in corpus callosum, striatum and anterior commissure, cerebellum and brainstem (Liu et al, 1992). It was also shown that TAUT is distributed more in white matter and it is mostly glia.

The amino acid sequence of TAUT contains several putative consensus sites for the phosphorylation through Ca^{2+} / diacylglycerol-dependent protein kinase C (PKC) and cAMP-dependent protein kinase A (PKA) within the intracellular domains. With this idea, TAUT is assumed to be regulated by phosphorylation. PKC and PKA show different regulatory effects on TAUT. PKC was found to exert down-regulation for TAUT in astrocyte primary cultures (Tchoumkeu-Nzouessa and Rebel, 1996). Among several putative PKC phosphorylation sites, there found a highly conserved Serine-322 located at the intracellular segment of the TAUT polypeptide chain which was a critical site for PKC phosphorylation (Han et al, 1999). Ser-322 mutated TAUT showed a higher rate of taurine transport, indicating the phosphorylation of Ser-322 site as an inactivation of TAUT. On the other hand, PKA seems to be positively regulating TAUT mRNA level. Activation of PKA was reported to increase taurine transport in Ehrlich ascite tumor cells (Mollerup and Lambert, 1996).

Deduced amino acid sequence of MDCK cell taurine transporter (Tau) compared to deduced sequences of MDCK cell betaine/GABA transpoter (Bgt1), brain transporters of GABA (Gaba), Serotonin (Sert1 and Sert2, bovine and rat dopamine (Dopabov and Doparat), and norepinephrine (Ne). The proposed membrane spanning domains of the taurine transporter are underlined and numbered M1 to M12. Asterisks are placed below amino acids that are identical in six of the eight transporter sequences. (Below)

Table 2-7 Deduced Amino Acid Sequence Of MDCK Cell Taurine Transporter



```
         1                                                   50
     Tau .......... .......... .......... .......... ..........
    Bgt1 .......... .......... .......... .......... ..........
    Gaba .......... .......... .......... .......... ..........
   Sert1 .......... .......... ....MVFYRR VSP.PQRTGQ SLAKYPMGTL
   Sert2 METTPLNSQK VLSECKDRED CQENGVLQKG VPTTADRAEP SQISNGYSAV
 Dopabov .......... .......... .......... .........M SEGRCSVAHM
 Doparat .......... .......... .......... .........M SKSKCSVGPM
      Ne .......... .......... .......... .......MLL ARMNPQVQPE

         51                                                 100
     Tau .....MATKE KLQCLKDFHK DILKPSPGKS PGTRP...ED EAEGKPPQRE
    Bgt1 .MDRKVAVPE DGPPVVSWLP E......... EGEKL...DQ EGEDQVKDRG
    Gaba .....MATDN SKVADGQIST EVSEAPVASD KPKTLVVKVQ KKAGDLPDRD
   Sert1 QSPGTSAGDE ASHSIPAATT TLVAE..... .......... ...IRQGERE
   Sert2 ..PSTSAGDE ASHSIPAATT TLVAE..... .......... ...IRQGERE
 Dopabov SSVVAPAKEA NAMGPKAVEL VLVKEQNGVQ LTNSTLLNPP QSPTEAQDRE
 Doparat SSVVAPAKES NAVGPREVEL ILVKEQNGVQ LTNSTLINPP QTPVEAQERE
      Ne NNGADTGPEQ PLRARKTAEL LVVKERNGVQ ....CLLAPR DG..DAQPRE
               *                                             **
         101             M1                              M2 150
     Tau KWSSKIDFVL SVAGGFVGLG NVWRFPYLCY KNGGGAFLIP YFIFLFGGGL
    Bgt1 QWTNKMEFVL SVAGEIIGLG NVWRFPYLCY KNGGGAFFIP YFIFFFTCGI
    Gaba TWKGRFDFLM SCVGYAIGLG NVWRFPYLCG KNGGGAFLIP YFLTLIFAGV
   Sert1 TWGKKMDFLL SVIGYAVDLG NIWRFPYICY QNGGGAFLLP YTIMAIFGGI
   Sert2 TWGKKMDFLL SVIGYAVDLG NIWRFPYICY QNGGGAFLLP YTIMAIFGGI
 Dopabov TWSKKADFLL SVIGFAVDLA NVWRFPYLCY KNGGGAFLVP YLFFMVVAGV
 Doparat TWSKKIDFLL SVIGFAVDLA NVWRFPYLCY KNGGGAFLVP YLLFMVIAGM
      Ne TWGKKIDFLL SVVGFAVDLA NVWRFPYLCY KNGGGAFLIP YTLFLIIAGM
         **  * **** ** * ** *  ********** ******** * *       *
         151                                        M3      200
     Tau PVFFLEVIIG QYTSEGGITC WEKICPLFSG IGYASIVIVS LLNIYYVIIL
    Bgt1 PVFFLEVALG QYTSQGSVTA WRKICPLLQG IGLASVVIES YLNIYYIIIL
    Gaba PLFLLECSLG QYTSIGGLGV W.KLAPMFKG VGLAAAVLSF WLNIYYIVII
   Sert1 PLFYMELALG QYHRNGCISI WRKICPIFKG IGYAICIIAF YIASYYNTII
   Sert2 PLFYMELALG QYHRNGCISI WRKICPIFKG IGYAICIIAF YIASYYNTII
 Dopabov PLFYMELALG QFNREGAAGV W.KICPILRG VGYTAILISL YIGFFYNVII
 Doparat PLFYMELALG QFNREGAAGV W.KICPVLKG VGFTVILISF YVGFFYNVII
      Ne PLFYMELALG QYNREGAATV W.KICPFFKG VGYAVILIAL YVGFYYNVII
         ***  * *** **   *     * ****   *  * *   *   *   **  **
         201                                                250
     Tau AWATYYLFQS FQSELPWAHC NHSWNTPQCM EDTMRKNKS. LWITLSTKNF
    Bgt1 AWALFYLFSS FTSELPWTTC TNTWNTEHCM D..FLNHSG. ARTATSSENF
    Gaba SWAIYYLYNS FTTTLPWKQC DNPWNTDRCF ........S. NYSLVNTTNM
   Sert1 AWALYYLISS LTDRLPWTSC TNSWNTGNCT NYFAQDNITW TLHS......
   Sert2 AWALYYLISS LTDRLPWTSC TNSWNTGNCT NYFAQDNITW TLHS......
 Dopabov AWALHYLLSS FTTELPWTHC NHSWNSPRCS DARAPNAS.. S..GPNGTSR
 Doparat AWALHYFFSS FTMDLPWIHC NNTWNSPNCS DAHASNSS.. DGLGLNDTFG
      Ne AWSLYYLFSS FTLNLPWTDC GHTWNSPNCT DPKLLNGSVL GNHTKYSKYK
         **** ** ** **  ***  *    **   *
         251                                        M4      300
     Tau TSPVTEFWER NVLSL..SSG IDDPGSLKWD LALCLLLVWL VCFFCIWKGV
    Bgt1 TSPVMEFWER RVLGI..TSG IHDLGALRWE LALCLLLAWL ICYFCIWKGV
    Gaba TSAVVEFWER NMHQM..TDG LDKPGQIRWP LAITLAIAWV LVYFCIWKGV
   Sert1 TSPAEEFYLR HVLQIHQSKG LQDLGTISWQ LTLCIVLIFT VIYFSIWKGV
   Sert2 TSPAEEFYLR HVLQIHQSKG LQDLGTISWQ LTLCIVLIFT VIYFSIWKGV
 Dopabov TTPAAEYFER GVLHLHESQG IDDLGPPRWQ LTSCLVLVIV LLYFSLWKGV
 Doparat TTPAAEYFER GVLHLHQSRG IDDLGPPRWQ LTACLVLVIV LLYFSLWKGV
      Ne FTPAAEFYER GVLHLHESSG IHDIGLPQWQ LLLCLMVVVI VLYFSLWKGV
         * *  ** **  **    * *   * *   * *  ** *     **  ****
         301             M5                                 350
     Tau KSTGKVVYFT ATFPFAMLLV LLVRGLTLPG AGAGIKFYLY PDISRLEDPQ
    Bgt1 KTTGKVVYFT ATFPYLMLVI LLIRGITLPG AYQGVIYYLK PDLLRLKDPQ
    Gaba GWTGKVVYFS ATYPYIMLII LFFRGVTLPG AKEGILFYIT PNFRKLSDSE
   Sert1 KTSGKVVWVT ATFPYIVLSV LLVRGATLPG AWRGVVFYLK PNWQKLLETG
   Sert2 KTSGKVVWVT ATFPYIVLSV LLVRGATLPG AWRGVVFYLK PNWQKLLETG
 Dopabov KTSGKVVWIT ATMPYVVLFA LLLRGITLPG AVDAIRAYLS VDFHRLCEAS
 Doparat KTSGKVVWIT ATMPYVVLTA LLLRGVTLPG AMDGIRAYLS VDFYRLCEAS
      Ne KTSGKVVWIT ATLPYFVLFV LLVHGVTLPG ASNGINAYLH IDFYRLKEAT
         ** ****  * ** **  *   ** ** **** *  *   **       *
         351             M6                              M7 400
     Tau VWIDAGTQIF FSYAICLGAM TSLGSYNKYK YNSYRDCMLL GCLNSGTSFV
    Bgt1 VWMDAGTQIF FSFAICQGCL TALGSYNKYH NNCYRDSIAL CFLNSATSFA
    Gaba VWLDAATQIF FSYGLGLGSL IALGSYNSFH NNVYRDSIIV CCINSCTSMF
   Sert1 VWVDAAAQIF FSLGPGFGVL LAFASYNKFN NNCYQDALVT SVVNCMTSFV
   Sert2 VWVDAAAQIF FSLGPGFGVL LAFASYNKFN NNCYQDALVT SVVNCMTSFV
 Dopabov VWIDAAIQIC FSLGVGLGVL IAFSSYNKFT NNCYRDAIIT TSVNSLTSFS
 Doparat VWIDAATQVC FSLGVGFGVL IAFSSYNKFT NNCYRDAIIT TSINSLTSFS
      Ne VWIDAATQIF FSLGAGFGVL IAFASYNKFD NNCYRDALLT SSINCITSFV
         ** *** *** ** * * * *  *  *****  ******        *  ***
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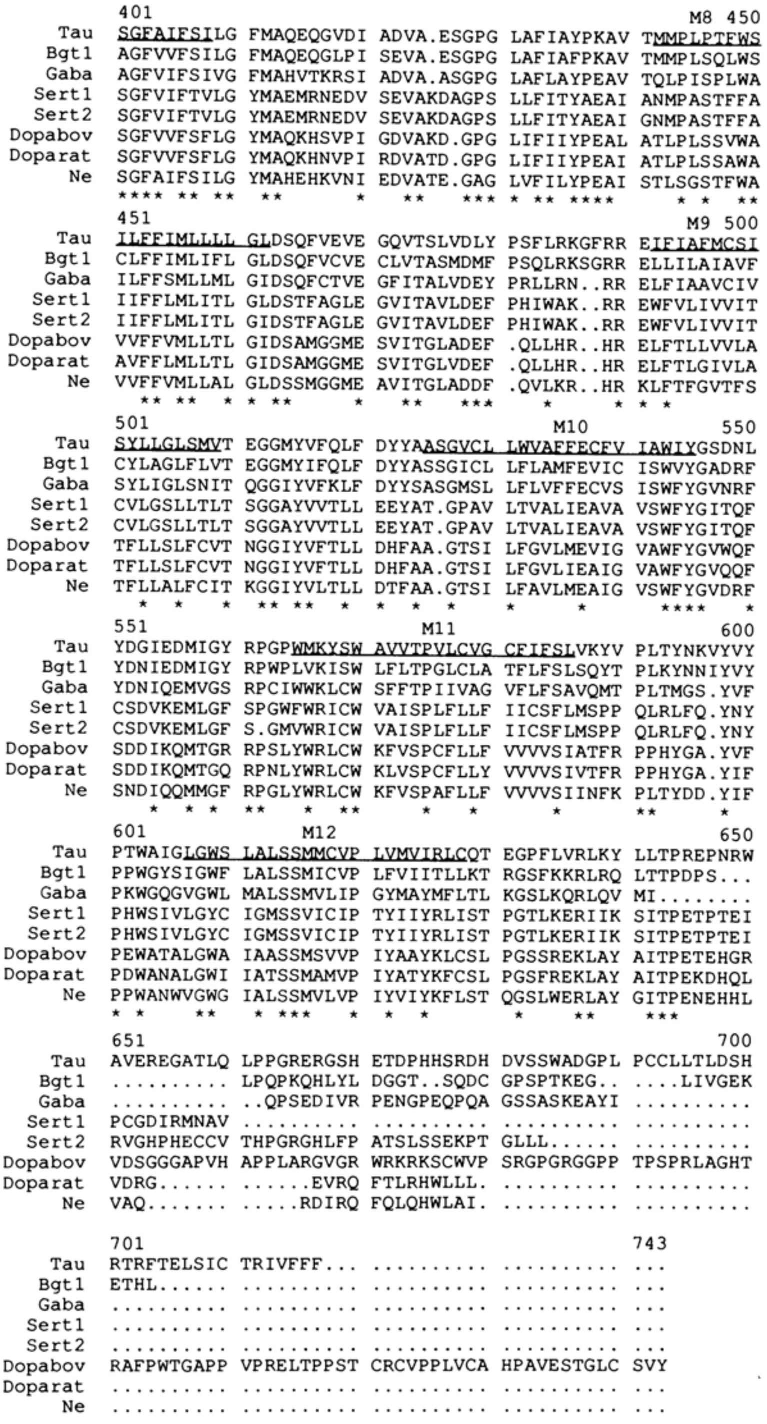
Table 2-7 (Cont.) Deduced Amino Acid Sequence Of MDCK Cell Taurine Transporter



```
         401                                             M8 450
     Tau SGFAIFSILG FMAQEQGVDI ADVA.ESGPG LAFIAYPKAV TMMPLPTFWS
    Bgt1 AGFVVFSILG FMAQEQGLPI SEVA.ESGPG LAFIAFPKAV TMMPLSQLWS
    Gaba AGFVIFSIVG FMAHVTKRSI ADVA.ASGPG LAFLAYPEAV TQLPISPLWA
   Sert1 SGFVIFTVLG YMAEMRNEDV SEVAKDAGPS LLFITYAEAI ANMPASTFFA
   Sert2 SGFVIFTVLG YMAEMRNEDV SEVAKDAGPS LLFITYAEAI GNMPASTFFA
 Dopabov SGFVVFSFLG YMAQKHSVPI GDVAKD.GPG LIFIIYPEAL ATLPLSSVWA
 Doparat SGFVVFSFLG YMAQKHNVPI RDVATD.GPG LIFIIYPEAI ATLPLSSAWA
      Ne SGFAIFSILG YMAHEHKVNI EDVATE.GAG LVFILYPEAI STLSGSTFWA
         **** ** **  **      *   **   *** * ** ****     * *  **
         451                                             M9 500
     Tau ILFFIMLLLL GLDSQFVEVE GQVTSLVDLY PSFLRKGFRR EIFIAFMCSI
    Bgt1 CLFFIMLIFL GLDSQFVCVE CLVTASMDMF PSQLRKSGRR ELLILAIAVF
    Gaba ILFFSMLLML GIDSQFCTVE GFITALVDEY PRLLRN..RR ELFIAAVCIV
   Sert1 IIFFLMLITL GLDSTFAGLE GVITAVLDEF PHIWAK..RR EWFVLIVVIT
   Sert2 IIFFLMLITL GIDSTFAGLE GVITAVLDEF PHIWAK..RR EWFVLIVVIT
 Dopabov VVFFVMLLTL GIDSAMGGME SVITGLADEF .QLLHR..HR ELFTLLVVLA
 Doparat AVFFLMLLTL GIDSAMGGME SVITGLVDEF .QLLHR..HR ELFTLGIVLA
      Ne VVFFVMLLAL GLDSSMGGME AVITGLADDF .QVLKR..HR KLFTFGVTFS
           ** **  * * **     *   **   ***    *     * * *
         501                                  M10           550
     Tau SYLLGLSMVT EGGMYVFQLF DYYAASGVCL LWVAFFECFV IAWIYGSDNL
    Bgt1 CYLAGLFLVT EGGMYIFQLF DYYASSGICL LFLAMFEVIC ISWVYGADRF
    Gaba SYLIGLSNIT QGGIYVFKLF DYYSASGMSL LFLVFFECVS ISWFYGVNRF
   Sert1 CVLGSLLTLT SGGAYVVTLL EEYAT.GPAV LTVALIEAVA VSWFYGITQF
   Sert2 CVLGSLLTLT SGGAYVVTLL EEYAT.GPAV LTVALIEAVA VSWFYGITQF
 Dopabov TFLLSLFCVT NGGIYVFTLL DHFAA.GTSI LFGVLMEVIG VAWFYGVWQF
 Doparat TFLLSLFCVT NGGIYVFTLL DHFAA.GTSI LFGVLIEAIG VAWFYGVQQF
      Ne TFLLALFCIT KGGIYVLTLL DTFAA.GTSI LFAVLMEAIG VSWFYGVDRF
           *  *   *  ** **  * *  *  *    *     *        ****   *
         551                       M11                      600
     Tau YDGIEDMIGY RPGPWMKYSW AVVTPVLCVG CFIFSLVKYV PLTYNKVYVY
    Bgt1 YDNIEDMIGY RPWPLVKISW LFLTPGLCLA TFLFSLSQYT PLKYNNIYVY
    Gaba YDNIQEMVGS RPCIWWKLCW SFFTPIIVAG VFLFSAVQMT PLTMGS.YVF
   Sert1 CSDVKEMLGF SPGWFWRICW VAISPLFLLF IICSFLMSPP QLRLFQ.YNY
   Sert2 CSDVKEMLGF S.GMVWRICW VAISPLFLLF IICSFLMSPP QLRLFQ.YNY
 Dopabov SDDIKQMTGR RPSLYWRLCW KFVSPCFLLF VVVVSIATFR PPHYGA.YVF
 Doparat SDDIKQMTGQ RPNLYWRLCW KLVSPCFLLY VVVVSIVTFR PPHYGA.YIF
      Ne SNDIQQMMGF RPGLYWRLCW KFVSPAFLLF VVVVSIINFK PLTYDD.YIF
            *  * *  **   *  **     *   *        *      **     *
         601             M12                                650
     Tau PTWAIGLGWS LALSSMMCVP LVMVIRLCQT EGPFLVRLKY LLTPREPNRW
    Bgt1 PPWGYSIGWF LALSSMICVP LFVIITLLKT RGSFKKRLRQ LTTPDPS...
    Gaba PKWGQGVGWL MALSSMVLIP GYMAYMFLTL KGSLKQRLQV MI........
   Sert1 PHWSIVLGYC IGMSSVICIP TYIIYRLIST PGTLKERIIK SITPETPTEI
   Sert2 PHWSIVLGYC IGMSSVICIP TYIIYRLIST PGTLKERIIK SITPETPTEI
 Dopabov PEWATALGWA IAASSMSVVP IYAAYKLCSL PGSSREKLAY AITPETEHGR
 Doparat PDWANALGWI IATSSMAMVP IYATYKFCSL PGSFREKLAY AITPEKDHQL
      Ne PPWANWVGWG IALSSMVLVP IYVIYKFLST QGSLWERLAY GITPENEHHL
         * *    **   * ***   *  *  *       *    **     ***
         651                                                700
     Tau AVEREGATLQ LPPGRERGSH ETDPHHSRDH DVSSWADGPL PCCLLTLDSH
    Bgt1 .......... LPQPKQHLYL DGGT..SQDC GPSPTKEG.. ....LIVGEK
    Gaba .......... ..QPSEDIVR PENGPEQPQA GSSASKEAYI ..........
   Sert1 PCGDIRMNAV .......... .......... .......... ..........
   Sert2 RVGHPHECCV THPGRGHLFP ATSLSSEKPT GLLL...... ..........
 Dopabov VDSGGGAPVH APPLARGVGR WRKRKSCWVP SRGPGRGGPP TPSPRLAGHT
 Doparat VDRG...... ......EVRQ FTLRHWLLL. .......... ..........
      Ne VAQ....... .....RDIRQ FQLQHWLAI. .......... ..........

         701                                         743
     Tau RTRFTELSIC TRIVFFF... .......... .......... ...
    Bgt1 ETHL...... .......... .......... .......... ...
    Gaba .......... .......... .......... .......... ...
   Sert1 .......... .......... .......... .......... ...
   Sert2 .......... .......... .......... .......... ...
 Dopabov RAFPWTGAPP VPRELTPPST CRCVPPLVCA HPAVESTGLC SVY
 Doparat .......... .......... .......... .......... ...
      Ne .......... .......... .......... .......... ...
```

Taurine Transporter Expression

Expression of TAUT genes is either down- or up-regulated depending on various factors. First, it was found that taurine itself down-regulates the gene expression of TAUT (Bitoun and Tappaz, 2000). Taurine transport appeared to be enhanced after low taurine diet but declined following the high taurine diet and these changes are found to be in the rates of taurine transport rather than the affinity for taurine after diet manipulations (For review, see: Tappaz, 2004). In vitro investigations have shown that such regulations are the results of decreased transcription rate of TAUT gene (Han et al., 1997). At molecular level, there found to be a region proposed to be responsible for taurine binding. The Cis-elements in the 5ˈ-flanking region of the TAUT gene (between -574 to -1532) exists the taurine responsive element. Though this proposed region has not yet been directly identified (Han et al., 2000). Additionally, taurine is thought to be the first signal for down-regulation of its transporters while intracellular calcium appears to be part of the signaling pathway because the same down-regulation is disrupted after calcium depletion (Han et al., 2000). Moreover, it was found recently (Han et al., 2000) that TAUT gene contains putative consensus binding sites for tumor suppressor protein p53. A study in renal cells by Han and coworkers in 2002 reported that taurine transport, TAUT protein, and TAUT mRNA levels were all decreased after endogenous p53 induction by a DNA-damaging drug in renal cell cultures. Additionally, a mutated form of p53 failed to down-regulate the transcription of TAUT gene. These findings suggest that TAUT gene may represent a downstream target gene for p53 which acts as a repressor of its transcription. However, further investigations on biological signaling cascades and messenger systems are necessary to understand the regulation of taurine transporter down-regulation.

TAUT gene expression is found to be up-regulated in various conditions. Firstly, many types of cells (renal cell lines, hepatoma cells, liver macrophages, liver endothelial cells, liver stellate cells, intestinal cell lines, aortic endothelial cells and astrocytes in primary cultures) exposed to hypertonic medium were found to increase in their maximal velocity for taurine transport and TAUT mRNA levels (For review, see: Tappaz, 2004). Similar to the down-regulation mechanisms, TAUT gene up-regulation is thought to be through a responsive element located on 5ˈ-flanking region of the TAUT gene. This specific region is thought to be sensitive to osmolarity and tonicity, thus called ORE: Osmotic Responsive Element. ORE has been identified in other well-established osmolyte transporters but has not been directly identified in TAUT gene. However, in the 5ˡ-flanking region of the TAUT gene, there exists on the minus strand 101 bases upstream the transcription start site a sequence that fits the functional consensus site as that of identified ORE (Han et al, 2000). It remains to be investigated whether this specific sequence is actually necessary for the tonicity-induced response in TAUT gene. Apart from exposure to hypertonicity, TAUT mRNA levels and taurine transport is found to be up-regulated by a tumor necrosis factor-α (TNF-α). The results indicate that the transcriptional activity of TAUT gene may be activated through this molecule as well. This up-regulation may be NFκB-mediated since TNF-α is known to activate the NFκB transcriptional activity by nuclear translocation. The 5ˡ-flanking region of TAUT gene is located with consensus binding sites for NFκB (Han et al., 2002). However, again there is no direct evidence showing that this region has been clearly identified.

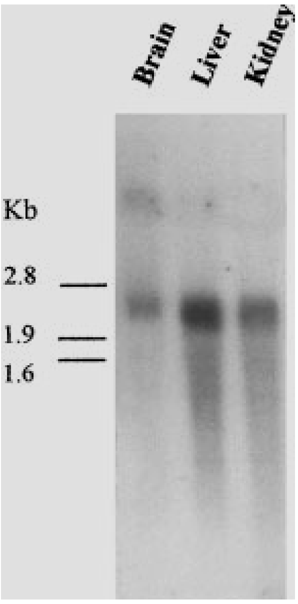
Taurine Biosynthetic Enzymes

Another source of intracellular taurine is its biosynthesis. There are two main enzymes in the pathway called cysteine sulfinate pathway, responsible for intracellular taurine biosynthesis. One is cysteine dioxygenase (CDO), oxidizing cysteine to cysteine sulfinate or cysteine sulfinic acid; and cysteine sulfinic acid decarboxylase (CSAD or CSD), responsible for decarboxylation of cysteine sulfinic acid into hypotaurine. Molecular identification of both enzymes has been achieved (For review, see: Tappaz, 2004). CDO genes encode a protein of 200 amino acids with molecular weight of 23 kDa. Mouse and rat CDOs show 92% amino acid sequence identity to human CDO. The CDO gene spans 15 kb and contains 5 exons. The 5ˡ-flanking region of the CDO gene contains several putative consensus Cis-acting regulatory sequences.

CSAD gene encodes 493 amino acids protein with molecular weight of 55.2 kDa. Rat and mouse possess 98% homology in the amino acid sequence while human CSAD has about 90% homology with those of rodents. The CSAD polypeptide chain contains the sequence NPHK which is a putative consensus site for pyroxidal phosphate binding that are also found in other amino acid decarboxylases. CSAD is found to have highest homology with glutamate decarboxylase isoforms GAD65 and GAD67.

Two forms of CSADs are found, brain form and liver form. These two forms are identical proteins on the basis of similar enzymatic properties, and identical molecular weight. On the other hand, the two forms are encoded by two distinct mRNAs which differ in their 5'-untranslated region and possibly are the result of alternative splicing.

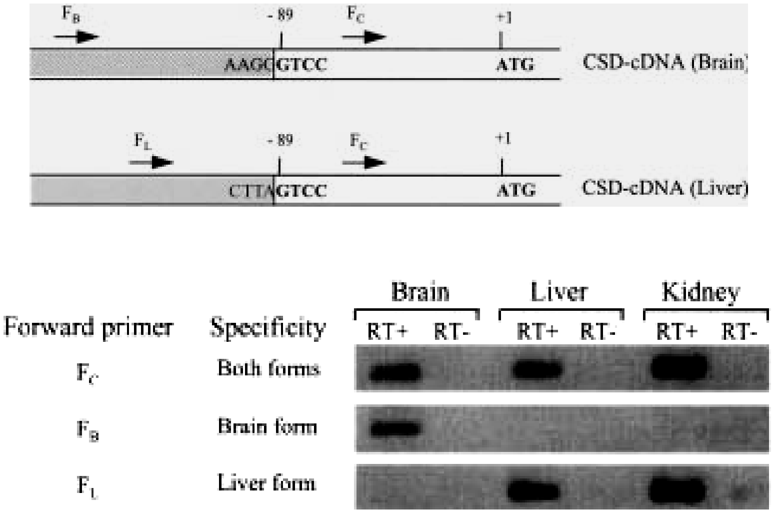
Figures 2-9 Northern Blot Of CSAD RNA From Brain, Liver And Kidney



Twenty micrograms of total RNA was hybridized with a random primed phospholabeled CSD cDNA probe. A single main band; 2.5 kb was detected in each organ.

Though the two forms of CSAD are identical proteins, their expression regulation may be different. The brain form of CSAD mRNA is found in brain only whereas the liver form is found both in liver and kidney (For review, see: Tappaz, 2004).

Figures 2-10 Tissue-Specific Expression Of Brain And Liver Forms Of CSAD-Mrna



Brain and liver CSAD are identical proteins encoded by two distinct mRNAs that differ in the 5′-untranslated region from nucleotide 89 upstream of the translation starting codon ATG (top panel). PCR forward primers positioned as indicated permitted to amplify through RT-PCR either form of CSAD-mRNA (FC) or specifically the brain form (FB) or liver form (FL) of CSD-mRNA (bottom panel). The brain form of CSAD-mRNA was detected in brain extracts only, whereas the liver form of CSD-mRNA was present in liver and kidney extracts. *Tappaz, 2004*

The cellular localization of taurine biosynthetic enzyme CSAD is demonstrated by immuno-stains in oligodendrocites, a few cells in the granular layer and cells around Purkinje cells within the cerebellum, presumably the Golgi epithelial cells. The enzymatic activity was detected in glial cell fractions enriched in oligodendrocites and astrocites (Liu et al, 1992)

The two taurine biosynthetic enzymes, CDO and CSAD are regulated by various factors. Such factors are, for instances, dietary proteins and sulfur amino acids, thyroid hormones, hypertonicity and taurine exposure (For review, see: Tappaz, 2004).

The most striking factor is that of hypertonicity-induced regulation. In astrocyte primary cultures exposed to hypertonic medium, gene expression of both enzymes were found to remain unchanged. Nevertheless, the CDO and CSAD mRNAs in kidneys are found to be either up or down-regulated when exposed to differences in osmolarity. These findings suggest that CDO and CSAD genes may be tonicity sensitive, however, this sensitivity is much less than that of TAUT gene. With much provision of the molecular identification and regulation of taurine transporters and its biosynthetic enzymes, there still miss an aspect of primary signals and transduction pathways leading to the transcription rates of the genes. Further investigations are necessary in understanding the mechanisms of regulation and candidates for intracellular signaling cascades.

2.2.2 Taurine Movements across Cell Membrane in the CNS

Influx / Transport

The structure-activity requirements of the taurine transport seem to be universal from bacteria to mammals and in all systems of mammalian tissues (Huxtable, 1989). The transport system is selective for β-amino acids. Taurine transport into the cells is against concentration gradients and is believed to be powered by the coupling with Na^+. An active transport of taurine is Na^+ dependent, regardless of cell types and sources. The ratio of Na^+ molecules to taurine molecules varies from 3:1 to 1:1 depending on the tissues. For instance, synaptosomes in hypothalamas transport one Na^+ per a taurine molecule while cultured neuroblastoma and cells transport 2 Na^+ (Holopainen et al, 1984; Hanretta and Lombardini, 1987;). In brain slices, at least 3 molecules of Na^+ is needed. Na^+ dependency in taurine transport is supported by the findings that monensin, a Na^+ ionophore, involving with Na^+ gradients, inhibits taurine transport and that ouabain, a Na^+ / K^+ ATPase inhibitor also inhibit such transport.

Taurine transport occurs both in neurons and glia. It was reported in 1976 by Schousboe and co-workers that glia have higher transport capacity than neurons. The idea supporting this is that biosynthesis of taurine takes place largely in neurons. Glial transport appears to clear taurine levels released into extracellular space by neurons, thus maintaining the level in the intercellular space low. On the other hand, taurine released into the synaptic cleft is cleared rather by diffusion than by re-uptake. Actually this characteristic may help to explain the longer duration of taurine actions in various systems.

As mentioned earlier, taurine level in the retina increases as development proceeds. Transport into the retina shows both low and high affinity components and both are temperature-dependent (Dawson and Neal, 1984). Taurine transport in the retina heavily takes place in neuronal amacrine cells, photoreceptors and pigment epithelium. In retina, both neurons and glia transport taurine with high affinity.
Taurine high affinity transporters have been identified as TAUTs. Recently (Pow et al., 2002) two isoforms of taurine transporters, TAUT1 and TAUT2 are found to localize in various tissues of the brain, namely, pituitary, cerebellum, hippocampus as well as in retina. The transporters are found both in neurons and glia, one being higher than the other depending on tissues examined . TAUT1 localization in mouse (Vinnakota et al., 1997) and rat (Smith et al., 1992) are correlated with taurine distribution, found in retina and cerebellum whereas TAUT2 is localized in cerebellum and hippocampus (Pow et al., 2002).

Such findings indicate that taurine transport is crucial in various functions within the central nervous system and may underlie some signaling functions and maintenance of important biological processes within the brain.

Efflux / Release

The movement of taurine from the inside into the extracellular space is less known than that of the transport into the cells. There are at least three main kinds of taurine release. First one, being the “basal release”, measures the membrane permeability to taurine, depending on lipid composition on the membrane and other factors. Second kind of release is the “reversal of active-transport system” via amino acid carriers. This type of release occurs through homo- or hetero-exchange of taurine. Homoexchange, being taurine molecule in exchange with itself in and out of cells, remains the same concentration. On the other hand, heteroexchange needs a different molecule having affinity for the transport system such as β-alanine to be transported into the cell in exchange with taurine released out. Third, “stimulated efflux”, taurine is released when the cell is depolarized (For review, see: Huxtable, 1989).

The efflux of taurine is found, by a number of researchers during 1980’s, to be stimulated by certain damaging conditions like ischemia, hypoxia, and volume-regulated phenomena, suggesting roles of taurine in protecting the cells from damages caused by such conditions.

2.3 Taurine Functions in the CNS

Taurine Functions: From Discovery to Current Knowledge

The very first function of taurine was proposed to be related to osmolarity and volume regulation. This primitive idea sprouts from the findings that taurine is found in high concentrations within marine animals while it is absent or found only in traces within the terrestrial and freshwater animals of the same species. Marine animals are exposed to changes in salinity throughout their lives and must possess a special mechanism allowing themselves to adapt to such changes.

From playing a vital role as an osmoregulator, taurine started to be found more as doing other functions from even the lowest species. In 1963, Danielson found that taurine not only possess properties suitable for osmoregulation, it also serves as conjugation of bile acid prior to bile excretion. Conjugation of bile acids with taurine occurs in most vertebrates above selachians (For review, see Jacobsen and Smith, 1968). However, the evidence attesting the antiquity and ubiquity of taurine in the biological systems suggests that it should function beyond that of its role in osmoregulation and bile acid conjugation. These functions are both metabolic and non-metabolic. The metabolic functions involve those stemming from products in the biosynthesis of taurine, including bile salt conjugation in the liver, general detoxification and anti oxidation while the non-metabolic ones involve modulation of ions such as Ca^{2+}, interactions with protein and phospholipids and osmoregulation.

The most interesting function of taurine that has captured much intention is its role in the central nervous system. As shown earlier that taurine is distributed through various important structures within the brain especially during development. The level during development exceeds those of other amino acids, thus must have something to do with the critical times of such young animals. As the animal grows, taurine level in many areas seems to decline but is still present in high amount and is considered to be the second most abundant free amino acid, being less than that of glutamate. Several decades ago, taurine was thought to be an inhibitory neurotransmitter in the central nervous system. As more and more research has developed, taurine function as a neurotransmitter becomes less relevant. Many researchers have dedicated their time one this particular sulfonated amino acid and have later debated whether taurine functions as a neurotransmitter within the CNS. The answer, up to date, is no. Taurine doesn’t fall into the criteria of being a neurotransmitter in various ways, or if it does so, very ambiguously. Distribution of taurine within the CNS doesn’t mimic that of other neurotransmitter amino acids. Concentration of GABA and glutamate in CSF, for example, is an order of magnitude lower than that found in plasma while non-neurotransmitter like glutamine is an order of magnitude higher. Concentration of taurine, on the other hand, falls within the middle range of these two types of substances: the CNS concentration of taurine is comparable to CSF but lower than plasma level. In addition, taurine action is much slower than those of true neurotransmitters and last much longer even after depolarization has ceased.

Though it may be difficult to summarize what taurine really does because it has been found throughout the animal kingdoms and throughout the bodies of each species, information on taurine actions must provide undoubtedly significant knowledge in accordance with its ubiquitous distribution. To explore the significant actions of taurine, some of its roles not related to the CNS in other systems will be exempted here. However, each known function from evolution of taurine which provide the basic understanding of its roles in the CNS will be discussed below.

Table 2-8 Summary Of General Taurine Functions *Adapted from Huxtable, 1992*

Some biological actions of Taurine	
Systems	**Actions**
Cardiovascular	Positive inotropy at low calcium
	Negative inotropy at high calcium
	Potentiation of digitalis inotropy
	Antagonism of calcium paradox
	Hypotensive (central and peripheral action)
	Retardation of lesion development in calcium overload cardiomyopathy
	Increased resistance of platelets to aggregation
Retina	Maintenance of structure and function of hotoreceptors,
	outer segments and tapetum lucidum
Liver	Bile salt synthesis
Reproductive	Sperm motility factor
Muscle	Muscle membrane stabilizer
Brain	Modulator of neuronal excitability
	Maintenance of cerebellar function
	Antinociceptive against chemical stimuli
	Thermoregulation
	Antiaggressive actions
	Central regulation of cardiorespiratory responses
	Alteration of sleeping duration
	Resistance to anoxia/hypoxia
	Altered learning
	Altered motor behavior
	Antitremor actions
	Suppression of drinking
	Suppression of eating
General	Modulation of neurotransmitter and hormone release
	Osmoregulation

Table 2-8(Cont.) Summary Of General Taurine Functions

General	Stimulation of glycolysis and glycogenesis
	Attenuation of hypercholesterolemia
	Cell proliferation and viability
	Antioxidation
	Regulation of phosphorylation
	Xenobiotic conjugation

2.3.1 Osmoregulation

Osmoregulation is the most ancient and experimentally best established function of taurine. High concentration of taurine is found in structures ranging from unicellular to mammalian cells. The evolution of cells arises from the requirement for osmoregulation. Membrane excitability derives from ionic imbalances across the membrane. If the osmotic imbalance consequences were left undone, the alteration of cell volume would result. And this would lead further to membrane rupture and disruption of biochemical processes sustaining viability. Most cells handle the osmotic stresses by adaptation. They do this by means of modification in cellular concentrations of water, inorganic ions and organic osmolytes. The osmotic pressure of a cell is determined by the total osmolarity of cytoplasmic solutes. The solutes are consisted of inorganic ions, low molecular weight compounds and macromolecules. Osmoregulation involves alterations in the concentrations of substances in the first two classes. Typically, in response to a hypo- or hyper-osmotic stress, changes in concentration occur in selection of both classes. In particular, K^+ or Cl^- accumulation or release is usually involved in osmoregulation. (For review, see: Huxtable, 1992). With inorganic ions, the requirement for osmoregulation and the regulation of membrane excitability are not co-incident, restricting the osmoregulatory role of inorganic ions. What should be an ideal osmoregulatory organic osmolytes? The cytosolic concentration must change in concert with the osmolarity of the cell exterior. Changes in concentration must not drastically alter cell membrane potential, enzyme activities or other biochemical processes. To satisfy these ideal properties, the osmotic change should be similar to electrogenically neutral. To adjust the concentration gradient across the cell membrane, a selective transport system in needed which is sensitive to osmotic changes. Taurine meets the requirements for an almost ideally perfect osmoregulator. Taurine is transported by a β-amino acid transport system and is responsive to ionic changes, thus is Na^+ dependent. High intra and extracellular concentration gradient of taurine can be maintained, as a result of its lipophobic properties. Basically saying, during hyper-osmolarity, taurine uptake into the cell is stimulated while in hypo-osmolarity, taurine is released. This function of taurine is conserved strongly from amoebas to mammals.

The involvement of taurine in osmoregulation and maintenance of osmotic balance was suggested in 1915 when Kossel and Edlbacher found an amazingly abundant level of taurine in echinoderm *Astropecten aurunticus* (Grey). Then the role of taurine in osmoregulation was proposed by the idea that taurine is exclusively detectable in all marine species but was not present or present in a very low amount in terrestrial and freshwater animals (For review, see: Jacobsen and Smith, 1968). The presence of taurine in marine habitat but not fresh water animals suggests the role of this sulfur-containing amino acid in osmoregulation. High intracellular concentrations of free amino acids including taurine are found to be one characteristic of many marine animals. In 1939, Krogh, noting the high concentration of taurine in mollusks, emphasized the role of taurine in the regulation of osmotic pressure in these animals when he was working with aquatic animals and their osmotic regulations. In 1952, Baldwin stated that “Conceivably taurine plays an important role in regulating the osmotic pressure of the contents of those cells, in which it is present in high amount”. Lange, in 1963 suggested that taurine concentration increased relatively more than any other amino acids with increasing salinity.

Effects of taurine in the regulation of osmotic pressure may not only be applied to marine invertebrates. The taurine level of muscle but not brain tissues from toads was shown to significantly increase when the animals were shifted to 40% sea water (Gordon, 1965; Baxter and Ortiz, 1966). The brain is particularly vulnerable to osmotic disturbances (For review, see: Huxtable, 1992). Cerebral edema is a serious condition, leading to seizures and other damages that are sometimes impossible to treat. Taurine has anticonvulsant actions in a wide variety of experimental seizure states (Huxtable, 1986). The mammalian brain alters cell amino acid concentrations in response to hypo- and hyper- osmotic conditions by decreasing and increasing them respectively (Baxter et al, 1986). During the regulation phase, astrocytes under hypoosmolar conditions show a marked and unique increase in taurine efflux that depletes cell taurine content by up to 64% (Pierson et al, 1985). Microdialysis of the brain with hypoosmotic solutions resulted in marked increases in extracellular taurine concentrations (Solis et al, 1988; Wade et al, 1988). Osmoregulatory actions of taurine in mammals have both an intracellular and an extracellular component. During an osmotic imbalance, intracellular taurine alters inversely with the extracellular. In biological systems, everything is connected to each other. Disturbance in osmoregulation is involved in the response of brain cells to excitotoxins and of photoreceptors to light. Excitotoxins are neuroexcitatory acidic amino acids like glutamate, kainite, NMDA and cysteate that kill neurons. These substances produce cellular depolarization by increasing Na^+ entry, followed by a passive entry of Cl^-. Water then enters to counteract the osmotic action of Cl^-, the cell swells and lysis may result. Conditions that lead to cell swelling generally stimulate the efflux of taurine including seizures, ischemia, hypoglycemia and excitotoxicity (Tossman et al, 1985). Taurine must perform its osmoregulatory function by modification in movements of other osmotically active substances such as water and ions.

2.3.2 Membrane Stabilization

There are now at least six lines of evidence suggesting that taurine plays an important role in the stabilization of membranes. ***1.*** The original work of Hayes et al (1975) demonstrated the disruption of photoreceptor cell membranes in taurine-deficient cats. This work has been extended to include effects on the tapetum lucidum of taurine-deficient cats (Wen et al, 1979) and on the cones of photoreceptor layer of taurine-deficient monkeys (Sturman et al, 1984). ***2.*** Taurine counteracts damage caused by external agents to skeletal muscle, intracellular membrane (Huxtable and Bressler, 1973), and heart muscle (Kramer et al, 1981). ***3.*** Taurine protects isolated rod outer segments from frog retina against structural damage induced by illumination and by oxidants (Cruz and Pasantes, 1983). ***4.*** Taurine protects cultured cells against retinol-induced and ferrous sulfate-induced damage and/or swelling (Pasantes et al, 1984; Pasantes and Cruz, 1985). ***5.*** Taurine has an antioxidant effect in rabbit spermatozoa (Alvares and Storey, 1984). This raises the possibility that the protective effects might be mediated through an ability to reduce lipid peroxidation. ***6.*** Taurine protects the hepatic cell against damage to the membrane by carbon-tetrachloride (Nakashima et al, 1983). The omission or replacement of major osmotic determinants in incubation medium could decrease cell injury. This suggests that damage to the membrane results in increased permeability and transfer of water. Thus, taurine protects the cells from damage caused by iron-ascorbate, but it does not do so through a reduction in the extent of lipid peroxidation. Rather, the protective effect seems to be related to an action on permeability to ions and water. These differences in taurine protective effects may be related to differences in the reduced oxygen molecules involved. The oxidative effects may be exerted on components other than polyunsaturated fatty acids. Such oxidative effects would not be detected by the thiobarbituric reaction. It is possible also that the peroxidative and non-peroxidative effects both result in permeability changes, which in turn, result in osmotic changes due to shifts in water and ions, with resulting cell swelling and damage. Whether or not the protective effects of taurine on membrane are direct or indirect remain to be determined. Other evidence also suggested that the protective effects of taurine are mediated through an action on membrane permeability and ion transport. Taurine modifies calcium fluxes in sarcolemmal, synaptosomal, and retinal subcellular fractions (Pasantes et al, 1979; Azari and Huxtable, 1980; Pasantes and Gamboa, 1980). It induces changes in transmembrane potentials consistent with modifications in potassium permeability. It has been related to changes in sodium gradients in cultured cells and to osmolar changes in marine invertebrates (For review, see: Wright et al, 1986). Therefore, taurine seems to counteract a variety of agents that result in leaky membranes. Taurine may have a dual role in this regard. First, it may react with these deleterious agents via its amino group to detoxify them. Second, it may also exert a direct protective effect in preventing the ionic and water shifts that result in cellular damage and death.

2.3.3 Anti-oxidation / Detoxification

Taurine ubiquity among a variety of animal species, abundance in the free amino acid pools, inability to be translationally incorporated into proteins, and efficient mechanisms of cellular uptake and tissue conservation all suggest that taurine may have other and possibly more fundamental biochemical functions. Recent in vitro experiments have demonstrated that taurine may be a necessary and metabolically active amino acid. In a cell culture system where taurine concentrations can be controlled, taurine enhances the proliferation of human lymphoblastoid cells in a concentration-dependent manner. This action of taurine could result from its counteracting the antiproliferative effects of oxidative and peroxidative products, which may cause extensive damage and subsequent cellular death. This interpretation is supported by the fact that many known antioxidants also enhance the proliferation of cultured cells in biochemically defined cell culture systems. Furthermore, taurine concentrations are high in cells and tissues that possess considerable potential to produce oxidants. For example, there is a high concentration of taurine in the retina, tissues where various oxidants are generated photolytically and enzymatically. There is an exceptionally high concentration of taurine in neutrophils, cells that enzymatically produce oxidants during the phagocytic process. Taurine is also a radioprotectant, and since radiation is a potent generator of oxidizing molecules, taurine may be interacting with these oxidants. Certainly, retino-taurine is an example of the amino group of taurine reacting with a potentially toxic compound. However, the anti-oxidation is not directly through taurine itself. Instead, it stems, more likely, from taurine-related substances like hypotaurine, cysteamine and chloramines. If taurine is to protect against such damages, the possibility may stem from the amino group contained in the structure which is able to detoxify such deleterious agents. An interesting antioxidative role taurine is stated recently in 2000 by Selva and co-workers. It is found that taurine exerts an antioxidant effect, protecting the damages within the brain caused by exposure of ozone. Ozone is known to be the major air pollutant which causes free radicals and consecutively, oxidative stress. It was that taurine blocks the peroxidation caused by ozone, thus preventing the memory deficits resulted from ozone exposure in young, mature and adult rats.

2.3.4 Taurine Involvement in Hormones

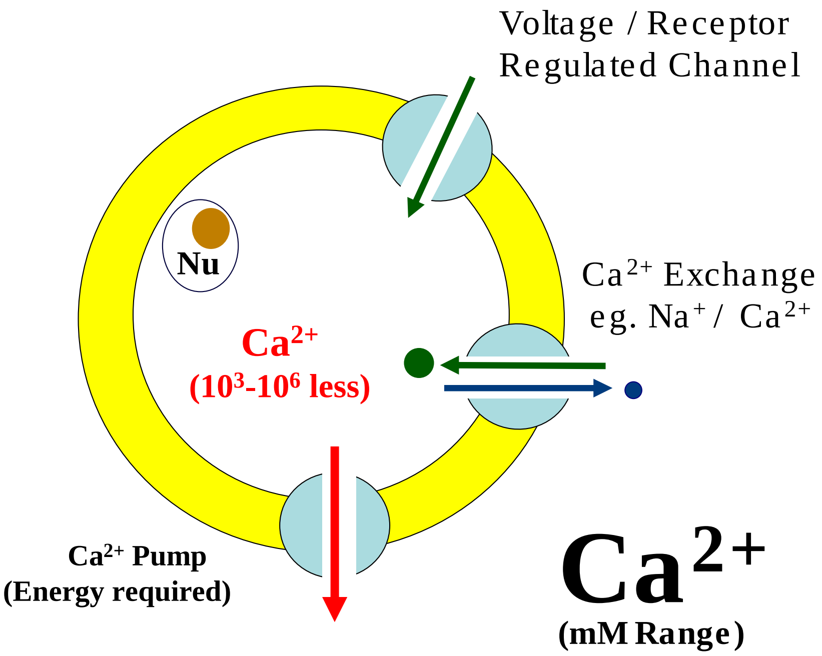
There are a couple of hormones found to be effected by taurine, namely, prolactin, somatostatin and growth hormone. One of the best studied is the level of prolactin increased by infusion of taurine into arcuate nucleus of the hypothalamus and anterior pituitary (Scheibel et al, 1980; Ikuyama et al, 1988). However, low level of taurine (0.25-1μmol) stimulates prolactin release while high level (4μmol) does not. Instead, at high level, taurine stimulates the release of somatosatin (at 4μmol, the release is enhanced while at 50μmol the release is marked in isolated median eminence; reviewed by Huxtable in 1989). However, none of the mechanisms in which taurine has an effect on hormones has been clarified.

The number of peptides with reported hormonal effects has skyrocketed. A taurine-containing hormone called glutataurine was discovered in the parathyroid gland of rats. Dr. L. Feuer and colleagues (1978) found that this peptide has highly selective action on adrenal hormones, which are involved in the body's response to stress and on rat brain neurotransmitters. The nutritional basis of glandular therapies may be in the ingested peptides. Glutataurine has vitamin A-like effects. It antagonizes cortisone and thyroxine and increases the development of thymus; increased levels of taurine have been found in hypothyroid patients. Dr. W. G. Lampson and his colleagues (1983) found that taurine increases some of the effects of insulin. Because insulin can have hypoglycemic effects, taurine should be given with caution to patients with blood sugar problems. Taurine can inhibit the release of adrenalin from the adrenal gland. Taurine and hypotaurine have physiochemical properties similar to the sperm motility factor. A role of taurine and hypotaurine has been demonstrated in preparing the sperm of experimental animals for fertilization. Conceivably, some problems of infertility may be related to taurine deficiency (The Healing Nutrients Within, Braverman). In mothers, taurine increases blood levels of the hormone prolactin, which triggers production and release of milk. Taurine is thus a useful supplement for nursing mothers, because it promotes lactation in mothers and a better development in the infants. In a study of three groups of mouse pups on high-, normal- and low-protein diets, taurine added to the mother's drinking water increased the pups' survival rate by increasing the supply of milk.

2.3.5 Taurine and Ca^{2+} Modulation

A number of works has reported an involvement of taurine on binding and transport of Ca^{2+} and on modification of Ca^{2+} dependent, Ca^{2+} stimulated processes. In general, Ca^{2+} concentration outside of cells is in mM range while it is about 10^3-10^6 times less inside of cells. Ca^{2+} entry into the cells proceeds down concentration gradient and does not require energy; it occurs through voltage or receptor-regulated channels or via an exchange processes e.g., Na^+ - Ca^{2+} exchange. On the other hand, Ca^{2+} removal proceeds against concentration gradient, requires energy and occurs through pumps on the cell membrane. A basic function of cell membrane is to maintain the vast differences of extra- and intracellular concentrations of Ca^{2+}. Adjustments on Ca^{2+} concentration lead to a reversible conformational changes which may be used as signaling devices or initiators of a number of processes including neurotransmission.

Figures 2-11 Calcium Concentration And Movement Across Cell Membrane



Taurine modulates numerous Ca^{2+} dependent processes in retina and brain tissues. The action of taurine on Ca^{2+} movement appears to be indirect (Irving 1982).

Taurine stimulates Ca^{2+} pump activity secondary to a stabilizing effect on the membrane, possibly through modification of phosphorylation process. It also modulates Ca^{2+} channel activity first by modifying Ca^{2+} delivery to the channel (secondary to an alteration in properties of Ca^{2+} binding sites on membrane acidic phospholipids). Secondly, taurine modulates Ca^{2+} channel activity by a direct effect on a hydrophilic site near or on the channels influenced by Ca^{2+} channel antagonists, thereby modifying the kinetics of channel opening or closing.

A number of central actions of taurine appears to be Ca^{2+} related. The excitotoxicity induces taurine release, thus, may protect cells from Ca^{2+} toxicity independent of an osmoregulatory functions. In the presence of a precipitating anion, taurine increases the Ca^{2+} storage capacity of the sarcoplasmic reticulum and possibly mitochondria and other intracellular organelles. Taurine stimulates the pumping rates of Ca^{2+} activated ATPase pumps, perhaps by increasing the turnover rate of the pump secondary to membrane modification. Taurine has the dual ability to increase Ca^{2+} availability under conditions of low Ca^{2+} but to protect against Ca^{2+} overload under conditions of high Ca^{2+} availability.

2.3.6 Taurine and Neurotransmission

Most aspects of the neurochemistry of taurine have been reviewed by Oja & Kontro and colleagues (1985) and a thorough review by Huxtable in 1989; followed by summary of its actions by Huxtable in 1992. A new development of great interest is here described. It was first proposed that taurine functions as an inhibitory neurotransmitter, though later was debated as not having enough actions to meet the requirements of a neurotransmitter. However, taurine has been found to involve in various processes within the central nervous system and the summary on findings is shown below.

Table2-9 Discovery On Central Actions Of Taurine

Discovery on central actions of taurine	
Year	**Functions**
1972, 1978, 1986, 1988	Blood pressure
1974, 1976, 1978, 1982, 1985	Motor behavior
1975-76	Suppression of drinking behavior
1975-76, 1978-79, 1981, 1983, 1985	Thermoregulation
1976	Suppression of eating behavior
1976, 1985-87	Sleep
1979	Learning
1981-83	Anticonvulsive actions
1981, 1985	Antiaggressive actions
1983-85	Cardiorespiratory effects
1984	Antitremor
1987-88	Resistance to anoxia/hypoxia
1996	Neuromodulation

Adapted (with additional data) from, Huxtable, 1989

It was found that a fairly large number of arthropods in crustaceans class characteristically contain large amount of taurine in muscles and nerve tissues. The probable role of taurine within the nervous system was proposed as an inhibitor of nerve impulses since mid 20th century (For review, see: Jacobsen and Smith, 1968; Huxtable, 1989). There were several reports emphasizing the role of taurine as an inhibitory neurotransmitter, starting from the work of Curtis and Watkins in 1960 when they found that taurine caused an inhibition in spinal neurons. Taurine was found in 1973 by Haas and Hosli to depress medullar and bulbar reticular neurons, to increase in conductance and hyperpolarize neuronal membranes. It was also inhibitory in the cerebral cortex, cerebellum and spinal cord (For review, see: Huxtable, 1992). However, though inhibitory, taurine actions show a slower onset of actions than those of other neurotransmitters like GABA. These findings raise a question to whether taurine is indeed a neurotransmitter.

In vertebrates, taurine is abundantly found in active tissues like the CNS. Retina is the area found to contain highest concentration of taurine of any regions derived from CNS. In rats and rabbits, exceptionally high amount (50mM) of taurine is found in the retina (Sturman, 1985). Taurine may be supplied to retina by vitreous humor or circulating plasma or synthesized in situ. Taurine transport into retina epithelium is through temperature sensitive, Na^{2+} dependent, energy dependent and high affinity transport (For review, see: Wright et al, 1986). Taurine synthesizing enzyme has been localized in inner nuclear layer and ganglion cell layer of rabbit retina. Most likely function of taurine in retina is the possible osmotic pressure regulator and regulation of Ca^{2+} homeostasis (For review, see: Wright et al, 1986).

In the past few decades, there were several lines of evidence in which taurine are thought to involve with other neurotransmitters. Taurine actions are closely related to those of GABA. However, in cortical evoked potential, GABA shows a fast action, within 30 sec of application while for taurine is much slower, within 3 minutes (Rossi and Bernadi, 1985). In addition, taurine is thought to be acting on the sites of GABA receptor complex separated from that of GABA itself. Taurine conceivably interacts with the complex either as GABA-like agonist, binding on the GABA site of the receptor; or as GABA modulator, binding other sites of the complex to modify the response to GABA in the transmission. (For review, see: Huxtable, 1989) Taurine hyperpolarizes excitable membrane by increasing Cl^- conductance, thus exhibits an inhibitory effects in the neuronal firings. However, as mentioned earlier, though actions are similar between taurine and GABA, their action sites are different. As a result, taurine and GABA is functionally related, but bio-chemically, clearly separated. In the brain, levels of taurine and acidic amino acids such as glutamate and aspartate are highly correlated (Van Gelder, 1981, 1982; Huxtable and Lippincott, 1982; Rassin et al, 1982). Taurine may stabilize cell membrane and cell elements of the ones containing these excitotoxic substances. The decreased level of taurine inside these cells may increase the release of these substances into the intercellular spaces.

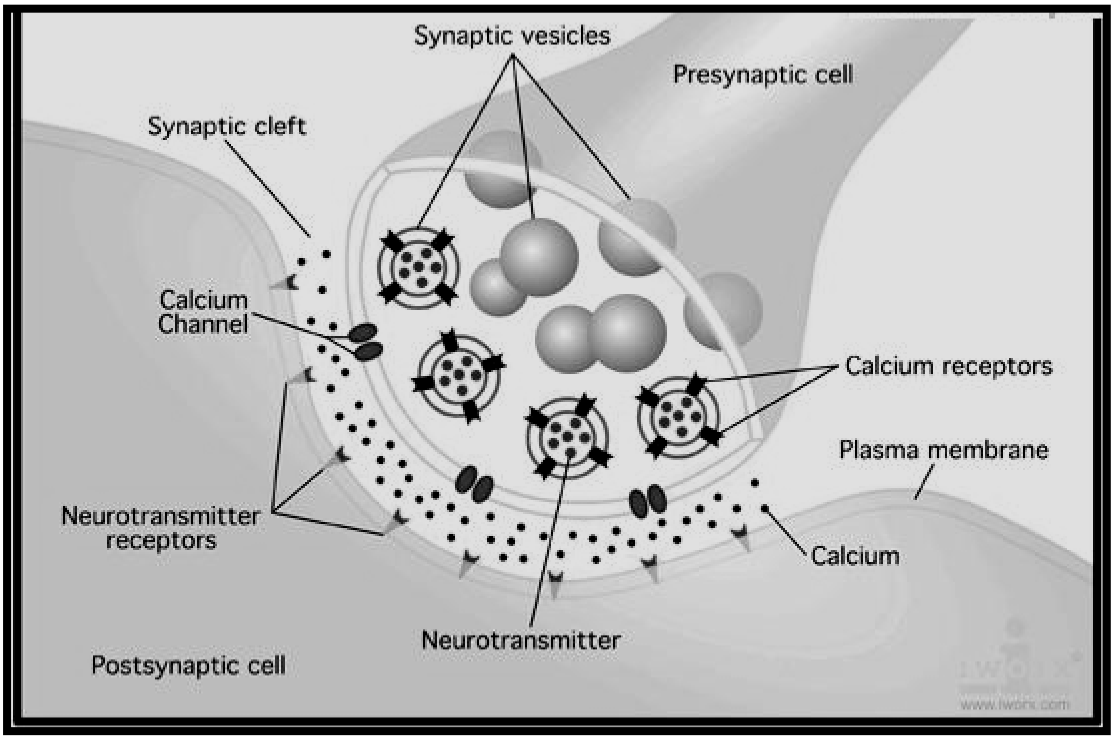
The mechanism by which taurine uses to modulate central neurotransmission may be related to its ability to regulate membrane stability, for instance, to increase membrane conductance and to regulate the membrane permeability to specific ions as well as its actions in osmoregulation to adjust the cells to environmental changes.

2.3.7 Taurine as a Neurotransmitter?

As described above that taurine has been found to be inhibitory in many systems. Researchers have tried to propose a role of taurine as an inhibitory neurotransmitter in the central nervous system. Whether or not taurine is a neurotransmitter, its actions must follow the criteria set by which other well known neurotransmitters have met. Over the years, there are 5 criteria to demonstrate whether a molecule acts as a neurotransmitter or not.

1. The presence of that substance within the presynaptic nerve terminal.
2. The release of the substance upon response to presynaptic depolarization must be Ca^{2+} dependent.
3. The presence of specific receptors to the substance on postsynaptic membrane.
4. Postsynaptic actions, mediating membrane potentials.
5. Removal mechanisms of the substance from synaptic cleft.

Figures 2-12 Neurotransmission



To be considered as a functional neurotransmitter, taurine must meet the five requirements. But does it do so?

1. Yes. Taurine is present in synaptosomes and synaptic vesicles in high amount. However, it is also present in glia and approximately only 25 % of total brain taurine is localized in neurons.
2. Partially Yes. The Ca^{2+} dependent mechanism of release can be detected under some conditions. However, the time course is slower in onset, lesser in magnitude and longer duration than that of other inhibitory neurotransmitters such as GABA.
3. Ambiguous. Taurine does not seem to possess a specific type of receptor on the postsynaptic membrane. Rather, it functions through other sites of other neurotransmitters like GABA and glycine receptors. In addition, sites at which taurine exerts its effects are similar, yet un-identical to the sites at which other inhibitory amino acids act. Moreover, no taurine receptors have yet been identified.
4. Yes. Taurine exerts its actions on membrane potential under pharmacological conditions. However, it is still not enough of the evidence since it only shows effects at pharmacological concentrations.
5. Yes. Removal mechanisms of taurine are through diffusion and re-uptake.

It can be concluded at this point that taurine is *NOT* a neurotransmitter. On the other hand, taurine seems to be very crucial in neurotransmission. In doing so, taurine may exert its actions on the neurotransmission by other means, indirectly via its functions on membrane stabilization, osmoregulation and Ca^{2+} modulation.

2.3.8 Taurine in Learning and Memory

As reviewed earlier, there is a highly ubiquitous distribution of taurine, both in the body and brain of a number of species. Taurine functions also vary greatly, for instances, being involved in bile acid conjugation in liver, excitability in cardiac muscles, neuroprotection and neuromodulation in the CNS, and osmoregulation, antioxidation, membrane stabilization in general. The antiquity and ubiquity of this sulfur-containing free amino acids are well correlated with its variety of protective functions throughout the whole animal especially electrically active tissues.

Although taurine functions have caught much attention from researchers since 1915 (Reviewed above, page 18) when it was found to be involved with osmoregulation, not much understandings of its roles in the central nervous system is clearly defined. In order for a role of taurine in specific systems to be illustrated, its present as well as mechanisms of actions must first be clearly understood. Taurine is virtually distributed throughout the central nervous system, especially highly concentrated in important structures as cerebrum, cerebellum, and hippocampus. Functions of taurine in each system, as previously stated, may stem from more than one action. In 1960, taurine was found to be inhibitory to neurotransmission first in spinal neurons and then continued to exert the same actions in various systems within the CNS. Extracellular taurine inhibits neuronal discharges (Huxtable, 1989). Its postsynaptic inhibitory effects are attributed to activation of a Cl^- conductance via $GABA_A$ and glycine receptors (Haas & Hosli, 1973; Hussy et al., 1997; Sergeeva & Haas, 2001). However, a novel function of role as neuromudulator, instead of neuroinhibitor, was proposed in 1996 by Galarreta and co-workers, then followed by other works from Olmo and co-workers in 2000. From those research works, it has been shown that, apart from inhibition, external taurine is able to induce an NMDA receptor-independent, long-lasting potentiation of synaptic transmission in the CA1 area of the hippocampus (Galarreta et al., 1996; Olmo et al., 2000).

Hippocampus is well defined area of the nervous system, known to be responsible for processes of learning and memory. As specific as the structure itself, there is an evidence that taurine is found in a high concentration (4-10mM) within CA1, CA3 and dentate gyrus of the hippocampus (Huxtable, 1989; Olmo et al, 2003). In addition, identified taurine transporters, TAUT1 and TAUT2 are also localized in these regions (Smith et al, 1992; Pow et al, 2002), thus raising a question of taurine functions in learning and memory.

There are reports on the effects of taurine in increasing concentration, attention and cognitive performances. In 1978, Rodger and co-workers stated that children fed with taurine-free formula scored less well in attainment tests than those fed with taurine supplements. In additions, there are reports on taurine-containing substances such as energy drinks (in combination with caffeine) by which consumers are found to improve in their cognitive performance and mood (Seidl et al, 2000). Similar reports were done in 2001, showing that caffeinated taurine drink improves memory and information processing in human subjects (Alford et al, 2001; David et al, 2001). Although taurine effects on memory and information processes cannot be solely concluded without the combination of caffeine, the mechanisms of action of these two substances seem to be separated, caffeine exerting its action in A_{2A} adenosine receptors while taurine does so elsewhere.

Additionally, reports on taurine and memory have skyrocketed. In 2000, Selva and coworkers stated effects of taurine in prevention of memory deficits induced by exposure to ozone. In the same year, Olmo and coworkers also reported the taurine action to induce a long lasting potentiation of excitatory synaptic potentials due to an enhancement of synaptic efficacy and axon excitability in CA1 region of the hippocampus. It was also noted in the work that the long lasting potentiation induced by taurine is similar to that of the late phase of LTP. Again, in 2003, Olmo and coworkers have clarified the similarities of LTP and the long lasting potentiation induced by taurine that they are mechanistically related. They found that taurine application enhances CREB phosphorylation and that taurine application somehow recruits the molecular pathway formed by cAMP, PKA, CREB phosphorylation and protein synthesis which are nothing than the characteristics of LTP.

There are actually two main possibilities that taurine may play a role in the learning and memory and the mechanisms underlying the process. First, LTP and long lasting potentiation induced by taurine, both require the present of Ca^{2+}. Taurine is known to play a role in Ca^{2+} modulation, involving Ca^{2+} movements in both directions across the cell membrane. Therefore, it may be likely that taurine provides intracellular Ca^{2+} to the cells. In addition, there is evidence that taurine accumulation inside the cell is also necessary for the maintenance of the long lasting potentiation in the hippocampus (Sergeeva et al, 2003). However, postsynaptic intracellular accumulation of taurine may function through an increase in release of Ca^{2+} from the intracellular store from the endoplasmic reticulum. The Ca^{2+} provision by taurine seems to be indirect. It may interact with phospholipids cell membrane at the Ca^{2+} binding sites, or by modulating Ca^{2+} channels' kinetics of the opening/closing on the postsynaptic membrane. Secondly, several lines of evidence suggest the possibility of taurine transport in the induction taurine-induced long lasting synaptic potentiation such as the Na^{2+} and temperature dependent of such potentiation (Galarreta et al, 1996; Olmo et al, 2000). The characteristic of amino acid transport which is electrogenic may induce the membrane depolarization and activate voltage-gated Ca^{2+} channels. As a result, taurine transport may serve as another possibility to induce such changes in dendritic depolarization and activation of Ca^{2+} influx through voltage-gated channels.

A work done by Sergeeva and coworkers in 2003 has stressed the idea that taurine transport is involved in the taurine-induced long lasting synaptic potentiation in mice. However, their work, using taurine transporter knockout mice, reported a role of taurine transport in cortico-striatal transmission, but a less pronounced effect in the CA1 region of the hippocampus. Moreover, they have concluded that the long lasting potentiation occurring in the hippocampus may be more likely to result from the intracellular accumulation of taurine rather than its transport. Nevertheless, Olmo and coworkers continued to debate on the role of taurine transport in the induction of long term synaptic potentiation in the CA1 region. The recent work done by this group of researchers indicate that taurine induces potentiation through the activation of a system transporting taurine and that taurine uptake is required for the induction of synaptic plasticity such as that of the late phase of LTP (Olmo et al, 2004).

2.3.9 Taurine and Neurodevelopment

2.3.9.1 Taurine-Deficient Studies and Developmental Deficits

The effects of taurine depletion during development have been best understood in deficient studies in cats. Taurine depletion in cats interferes with development of the animals and results in abortion, still-birth and those kittens survived are low in birth weight and show poor growth rates compared to taurine supplemented groups. In 1985, Sturman and his coworkers had shown that in full or almost complete taurine deficiency, achieved in kittens by deprivation of taurine both pre- and postnatally, physical and morphological dysfunctions occur, including cellular aberrations in the brain. Kittens born to deficient mothers and nursed on such mothers' taurine-limited milk are smaller than normal, at 8 weeks (weaning) being 60% of normal weight. Similar kittens given oral supplements of taurine reach full size, demonstrating directly that taurine is necessary for growth. The deficient kittens display other abnormalities as well: abnormal development of the hind legs, a peculiar gait with excessive abduction and paresis, and thoracic kyphosis. These symptoms are indicative of cerebellar dysfunction.

Study of the cerebellum then disclosed an alteration in its morphology. It was found that at 8 weeks after birth, the taurine deprived kittens' neuronal development is impaired. The cerebellar external granular layer is still present. Normally, cells in this layer started to migrate inward in the postnatal period. Mitotic figures in many cells of the deficient cerebellar external granule cell layer indicated that cell division was still going on, although this process normally is completed by about 3 weeks of age. No mitotic figures were seen in the normal tissue. The lack of taurine interferes with normal pattern of cerebellar development when the neurons undergo morphological and biochemical changes. Differentiation, proliferation, migration, synaptogenesis and myelination all occur in this period. Supplementation of deficient kittens with taurine resulted in an increase in the cerebellar taurine content to normal and the occurrence of normal migration of cells. The delay in cell migration in the absence of adequate taurine must upset the strict timetable of cerebellar development, resulting in a failure to form many synaptic connections. The lack of taurine is presented as tetarogenic, indicating that taurine plays a vital role in cerebellar ontogeny. In human infants fed with synthetic formula free of taurine score less well on attainment tests during childhood than those fed with taurine-containing compound (Rodgers, 1978).

It was found that the visual cortex of deficient kittens also was affected by the lack of taurine (Palackal et al, 1985). At birth, neuroblasts at both the ventricular and pial zones have failed to complete their differentiation and have not migrated into the molecular layer; subsequent arborization is poor and much organization must be lost.

Taurine is also involved in the maintenance of photoreceptor integrity and function. Low level of taurine has been shown to result in dysfunction and loss of structural integrity of the retina in cats, rats, and monkeys (For review, see:Huxtable, 1989). In 1984, Sturman demonstrated the importance of taurine in the retina by raising rhesus monkeys from birth to 26 weeks on a low taurine human infant milk formula, Nutramigen, and it was found that these animals showed retinal degeneration.

Monkeys maintained on the formula showed marked degeneration of retinal cone receptors and loss of visual acuity (Neuringer et al, 1987; Neuringer and Sturman, 1988).

In addition to the retinal degeneration in taurine-deficient cats, it was found earlier (Wen et al, 1979) that, there is disorganization and disruption of the cells in tapetum lucidum, a reflective multilayer located behind the retina of cats which serve to reflect light escaped from absorption to provide the retina a second chance to trap it. The structure is crucial for night vision. In taurine deficient cats, the layer is disorganized and degenerated. In 1984, Sturman and his colleagues had clarified the possible mechanisms of tapetum lucidum disorganization caused by taurine deficiency in cats. In normal animals, a zinc-enriched membrane surrounds the tapetal rod. Taurine has been known with its function in zinc retention, and its deficiency resulted in loss of zinc from the tapetal membrane. Ablation of the membrane resulted in disruption of the tapetum lucidum. It can be concluded here that the dysfunction and degeneration of retina caused by taurine deficiency is due to its secondary depletion, eg, loss of other agents vital in the development as a result of lack of taurine.

2.3.9.2 Taurine Distribution during Development

It was found that even in arthropods, taurine levels increase at metamorphosis, presumably due to proteolysis occurring during this stage of development (Jacobsen and Smith, 1968). A review by Jacobsen and Smith, 1968, stated that the chicken embryos are able to synthesize taurine and its concentration increases from 0.25 to 10 μmoles / 100 mg dry protein in the first 4 days, after which the level remains constantly stable. The taurine content in muscles of embryos is higher than that of 2-3 weeks old chicken. A relationship between taurine content and muscle activity was suggested by Bascheri and Fromageot who found that in Perdix perdix not yet capable of flight, the taurine content in wings is only 10% of that in the muscles of feet. Roberts and co-workers found, in 1950, that taurine level was higher in fetal mouse brain than adult brain but later, in 1957, found that the level is similar in heart tissues from embryo, newborn and adult mice.

In man, taurine levels are found to be higher of most areas in fetus than in adults. For instance, taurine is higher in fetal brain than in that of children and adult brains. Analyses of different sections of human brain also demonstrate that highest concentrations of taurine can be found in frontal and occipital lobes. (Okumura et al., 1960). In 1966, Ryan and Carver also found that taurine content is higher in human liver than in that of adults. It was found that the neonate receives its 59% of the total taurine contents from mother, and 41% is biosynthesized during fetus gestation (Sturman and Hayes, 1980). Taurine is needed very early in life, that it can be readily biosynthesized since fetal developmental period. As a result, it can be stated that taurine play a vital role in the early development of certain species. However, adult rats get 20% of total taurine contents from dietary sources and the rest 80% is biosynthesized within its body.

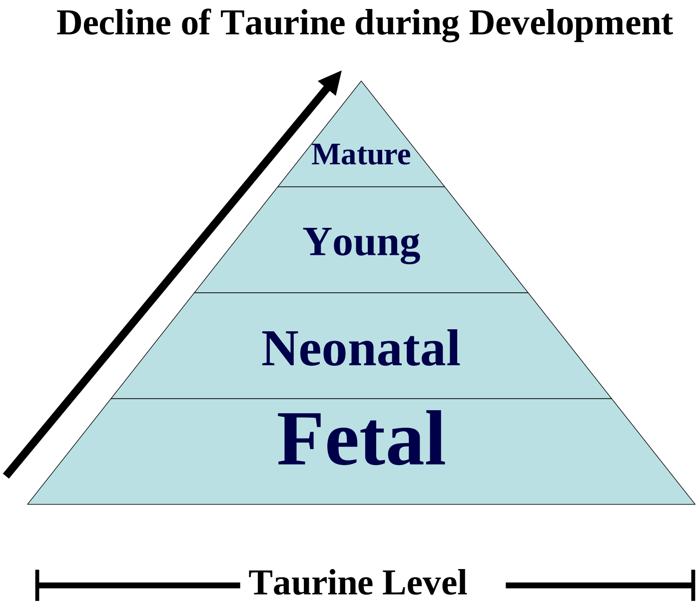
In a review by Jacobsen and Smith in 1968, it was stated that taurine level changes with age. In rats, taurine in the brain is much higher in newborn rats than in adults and the content is shown to decline with advancing age. While for other organs such as muscles, heart, and spleen, taurine level was shown to increase with age.

For development of the nervous system, taurine is one of the most important substances involving many structures as was evident in many research mentioned above of its high concentration in the brain during development. The highest concentration of taurine occurs in developing brain while other amino acids are found to be low (Davis and Himwich, 1973). With development, taurine level falls, in adults, being only 1/3 that of the newborns. This pattern of taurine concentration is observed in human, monkey, rat, rabbit and mouse (For review, see: Huxtable, 1989). In 1982, Parmer and coworkers reported a reversed pattern within the retina. It was found that developmental changes increase the level of taurine in correspondence with the formation of photoreceptor layer and the maintenance of retina structure and functions.

Lu and coworkers illustrated the localization of taurine during development of feline cerebellum using immunohistochemistry. It was found that taurine-like immunoreactivity reached a peak at 4 weeks after birth. Purkinje cells are all taurine-positive in cerebellum from neonatal but seem to show negative results when mature, with virtually no taurine (Lu et al., 1993); contrasting with observations reported with rodent cerebellum (Magnusson, 1994). The visualization of taurine by Lu and team indicated that taurine-like immunoreactivity is localized in dendrites, axons and glial processes. Granule cells migrating through this region also show prominent taurine-like immunoreactivity (Lu et al., 1993)

In 1994, Magnusson had shown the changes in localization of taurine-like immunoreactivity during development and regeneration in the rat brain. It was found that taurine is present in certain types of cells such as the cerebellar Purkinje cells and hippocampal pyramidal cells throughout development until adulthood. There were also certain time of the development that taurine was found to be not confined to the dendrites but rather found ubiquitously in the molecular layers of the two brain regions. This suggested that taurine may be present in significantly higher concentrations in certain cell types or subcellular structures during development than in adult rats. Moreover, taurine appeared to reverse its developmental decline in concentration in the presence of regeneration, suggesting as well the role of taurine in axonal sprouting and/or synapse formation (Magnusson, 1994). Taurine and glutamate are found to be developmentally related in such a way that their distributions coincide during development of the hippocampus and cerebellum as well as during plasticity of the dentate gyrus. Such distributions during early development suggested that taurine and glutamate are involved in dendritic outgrowth and synapse formation (Magnusson, 1996).

Figures 2-13 Decline Of Taurine During Development

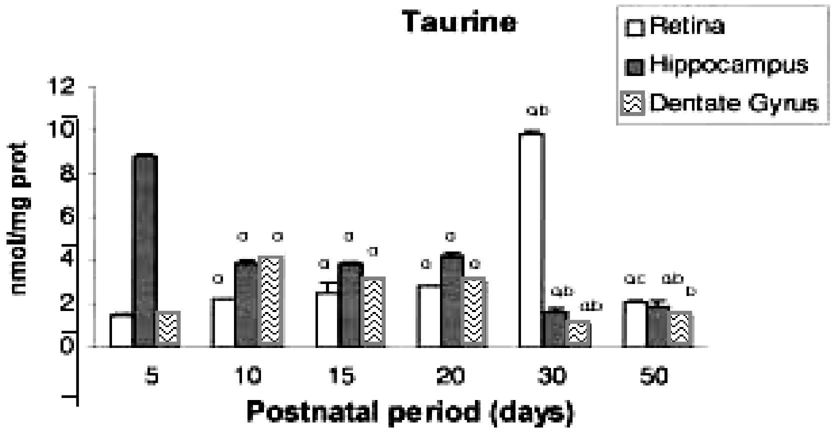


Apart from immunoreactivity of taurine itself, there are some studies indicating the development of taurine biosynthesizing system in cerebral cortical neurons and in primary cultures (Ohkuma et al., 1986). It was found, using phase contrast and scanning electron micrography and immunohistochemistry, that the contents of taurine metabolic intermediates in its biosynthesis (cysteine sulfinic acid and cysteic acid) in primary cultures showed decrease as the development advances. On the other hand, the activities of cysteine sulfinic acid decarboxylase (CSAD) and cysteine dioxygenase (CDO) were found to increase progressively both in primary cultures and cerebral cortices in vivo during growth. The immunohistochemical study using antitaurine antibody clearly demonstrated taurine is localized in cell bodies and the processes of neurons, and the immunoreactivity showed reduction as time proceeds.

Another interesting finding by Lake in 1992 suggested that taurine is localized mostly in glia during the development of rat optic nerve using immunocytochemistry to embryonic day 20 to postnatal 28 adult rat nerves. It was found at younger age, taurine immunoreactivity is intense and localized specifically in optic nerve axons but after postnatal day 15 and thereafter, the predominant localization was in macroglia. Some of these glia are astrocytes as indicated by a specific marker, GFAP (Lake 1992).

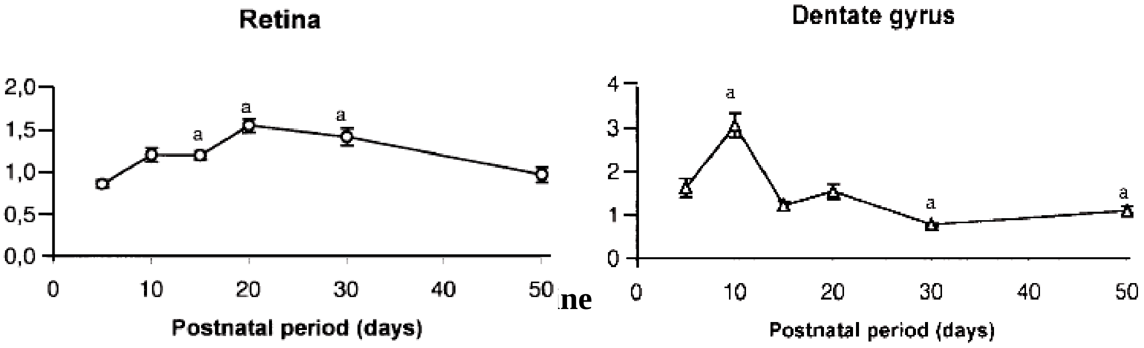
A study done by Lima and coworkers (Lima et al., 2004), using HPLC for taurine analysis, found that taurine levels increase with development in the retina while it showed a progressive decline in hipppcampus, and a slight increase followed by a decrease in dentate gyrus during development.

Figure 2-14 Taurine Levels *Adapted from Lima et al., 2004*



Figures below illustrate the ratio of taurine concentration expressed as M/mg of protein and taurine content expressed as nanomoles per region in retina, dentate gyrus, and hippocampus. As shown in the graphs, taurine level in the retina shows increase during development and starts to decline, with no statistical significance (Lima et al., 2004), but still remains at the same level at postnatal day 5. In dentate gyrus, there shows an initial increase during postnatal day 10 and declines afterwards. In contrast, in the hippocampus, taurine level is highest in the newborn, at postnatal day 5 and declines progressively as the development proceeds.

Figures 2-15 The Ratio Of Taurine Concentration Expressed



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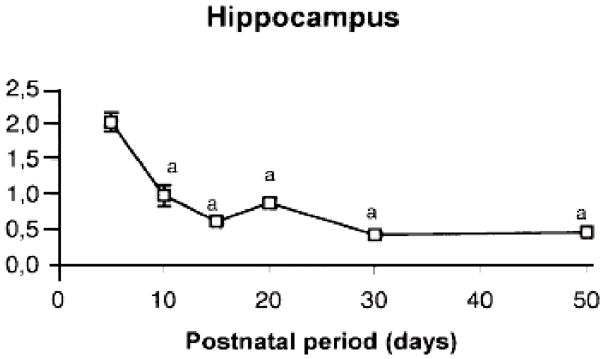


Adapted from Lima et al., 2004

In conclusion, taurine functions are accordingly enormous as its ubiquitous distributions. The functions of taurine in various systems are found to be either or not related to each of its properties. Basically saying, the actions of taurine within one single phenomenon may rise from more than one of its properties to regulate such phenomenon. As mentioned earlier, taurine acts as an osmoregulator possibly through its various actions. It may help regulate the osmotic imbalances by first altering the membrane stability, interacting with phospholipids on the membrane or by altering the sites near or on the ion channels, changing opening and closing of those channels to allow movements of ions. As the research advances, taurine is found to be more and more protective within various animal tissues. And as novel findings take place, ubiquitous actions of taurine may one day be thoroughly clarified, in the light that its supplements may be beneficial, ensuring optimal growth and development as well as in treatments of certain diseases. To summarize functions of taurine, and evolution on taurine proposed actions is shown below.

Table 2-10 Evolution In The Knowledge About Taurine

Evolution in the Knowledge about Taurine		
Year	**Scientists**	**Findings**
1827	Tiedmann&Gmelin	Discovery of taurine
1915	Kossel &Edlbacher	Role of taurine in osmoregulation
1930’s	N/A	Continuation of osmoregulation found by others
1960	Curtis and Watkins	Neuroinhibitory action in spinal neurons
1963	Danielson	Role of taurine in bile conjugation
1975	Raiha et al.	Nutritional significance of taurine in development
1980’s	N/A	✓ Damaging conditions in the brain found to induce taurine release ✓ Role in CNS development ✓ Focused on Roles in CNS
1986	Taber et al.	Taurine, proposed as an inhibitory neurotransmitter
1990’s	N/A	Debates on taurine as an inhibitory neurotransmitter
1996	Galarreta et al.	New role as a neuromodulator, enhancing long lasting increase of synaptic potentials
Current	N/A	✓ Taurine does not meet the requirements of being neurotransmitter ✓ Continuous findings on taurine functions

2.4 Interactions of Taurine and Alcohol in the CNS

Both taurine and ethanol exert positive allosteric modulatory effects on neuronal ligand-gated chloride channels ($GABA_A$ and glycine receptors) as well as inhibitory effects on other ligand- and voltage-gated cation channels (NMDA and Ca^{2+} channels). Behavioral evidence suggests that taurine can alter the locomotor stimulatory, sedating, and motivational effects of ethanol in a strongly dose-dependent manner. Microdialysis studies have revealed that ethanol elevates extracellular levels of taurine in numerous brain regions, although the functional consequences of this phenomenon are currently unknown. Finally, taurine and several related molecules including the homotaurine derivative acamprosate (calcium acetylhomotaurinate) can reduce ethanol self-administration and relapse to drinking in both animals and humans. Taken together, these data suggest that the endogenous taurine system may be an important modulator of ethanol effects on the nervous system, and may represent a novel therapeutic avenue for the development of medications to treat alcohol abuse and alcoholism (For review, see: Olive, 2002). Similar to taurine, ethanol also exerts a wide range of physiological effects on the nervous system, including neuromodulation, inhibition of neurotransmission, and alterations in Ca^{2+} homeostasis.

2.4.1 Similarities in the Neurochemical Actions of Taurine and Alcohol

Taurine is the most abundant neuroactive amino acid in the brain extracellular fluid. Microdialysis studies have demonstrated extracellular taurine levels to be in the low to mid-micromolar range in various brain regions, while extracellular concentrations of gamma-aminobutyric acid (GABA) and glutamate are approximately an order of magnitude lower (Huxtable, 1989). However, because microdialysis sampling is an indirect measure of synaptic overflow, effective taurine concentrations at the synaptic cleft are likely to be in the low millimolar range. Interestingly, low millimolar concentrations of ethanol are also necessary to produce physiological effects in the nervous system. Thus, taurine and ethanol appear to be relatively unique in requiring high effective concentrations in the nervous system as compared to other endogenous and exogenous neuroactive substances. Taurine is structurally similar to GABA. Not surprisingly, taurine can mimic the effects of GABA in enhancing chloride ion flux across neuronal membranes from numerous brain regions. These effects can be reversed by the classical $GABA_A$ receptor antagonists bicuculline or picrotoxin, indicating an action of taurine on the $GABA_A$ receptor chloride ionophore complex. Further evidence that taurine exerts some of its effects through $GABA_A$ receptors comes from binding studies in which taurine was shown to displace the binding of both direct and allosteric agonists of the $GABA_A$ receptor complex. Taurine has also been shown to bind to the $GABA_B$ receptor, although no functional consequences of this binding have been observed (Olmo et al., 2000). Numerous studies have demonstrated that the inhibitory effects of taurine on neurons are also blocked by the glycine receptor antagonist strychnine, indicating that taurine can also potentiate glycine receptor function. A handful of studies have also demonstrated that taurine can inhibit neuronal calcium channel function and responses to the excitatory amino acid N-methyl-D-aspartate (NMDA). Thus, taurine appears to exert its inhibitory effects on neuronal activity and neurotransmission by potentiating $GABA_A$ and glycine receptor function and inhibiting excitatory amino acid receptor and Ca^{2+} channel function. Interestingly, the most well characterized pharmacological effects of ethanol on the nervous system are strikingly similar to that of taurine: potentiation of ligand-gated chloride channel function and inhibition of excitatory amino acid receptor and calcium channel functions.

2.4.2 Alcohol-Induced Modulation of Extracellular Taurine Levels in the Brain

An early study demonstrated that chronic, but not acute, administration of ethanol to rats decreased whole brain taurine concentrations, which returned to basal level following ethanol withdrawal. However, the subsequent development of microdialysis technique has allowed researchers to examine the effects of acute and chronic ethanol administration and consumption on extracellular levels of taurine in discrete brain regions. Acute administration of ethanol (1–2 g/kg i.p.) has been shown to elicit a 50–100% increased extracellular levels of taurine in the nucleus accumbens of rats (Dahchour et al., 1996). These findings have also been replicated in the nucleus accumbens after acute administration of the ethanol metabolite acetaldehyde (20–100 mg/kg i.p.). Ethanol does not alter extracellular levels of glutamate or GABA in this region, and rats do not appear to develop tolerance to this effect after repeated treatment with 1–2 g/kg i.p. ethanol. Elevations in extracellular taurine levels by acute ethanol administration (1 and 3 g/kg i.p.) have also been observed in the amygdala, hippocampus and frontal cortex (Dahchour and De Witte, 2000).

Microdialysis experiments have also revealed a role for taurine in the neurochemical changes that occur during ethanol withdrawal. During acute withdrawal from chronic alcohol intoxication, CNS neurons become hyperexcitable and the organism becomes prone to convulsions. This phenomenon is believed to be mediated, at least in part, by an up-regulation of NMDA receptor number and function and increased glutamate release. In a study by Dahchour and colleagues (Dahchour et al., 2000), rats were made dependent on ethanol by vapor inhalation for 4 weeks and subsequently underwent microdialysis monitoring for 12 hours during the acute withdrawal phase. The investigators found that taurine treatment (45 mg/kg i.p.) 5 hours into the withdrawal phase completely suppressed withdrawal induced increases in extracellular glutamate levels in the nucleus accumbens. Along these lines, it suggests that increasing extracellular taurine levels in the nucleus accumbens suppresses basal glutamate release as well (Olive et al., 2000). Thus, taurine appears to have an inhibitory effect on excitatory amino acid neurotransmission, which may counteract the neuronal hyperexcitability observed during ethanol withdrawal.

The precise neurobiological significance and mechanisms underlying ethanol-induced increases in CNS extracellular taurine levels are currently unknown. Some investigators have speculated that taurine may be released in response to ethanol to compensate for osmotic changes caused by ethanol in the brain extracellular fluid (Dahchour et al., 1996). However, increases in extracellular taurine levels in the amygdala have also been reported to occur after acute saline treatment in combination with the presentation of a conditioned ethanol-associated olfactory stimulus. Thus, ethanol-induced increases in extracellular taurine may not be solely related to osmoregulatory processes, but may play a role in the environmental adaptations to ethanol exposure.

It is also possible that ethanol-induced taurine release may mediate several of the physiological effects of ethanol on the nervous system. As outlined above, both taurine and ethanol potentiate inhibitory amino acid receptor function and inhibit excitatory amino acid receptors and Ca^{2+} channel function. Thus, ethanol-induced release of taurine from intracellular stores may, in fact, potentiates the effects of ethanol itself on neuronal excitability. Since the release of taurine into the extracellular environment results in decreased intracellular taurine levels, this phenomenon may also have profound effects on cellular functioning. For example, both ethanol and taurine are known to modulate intracellular calcium homeostasis and protein phosphorylation (Huxtable, 1992). Thus, the lowering of intracellular taurine levels by ethanol may mediate some of the effects of ethanol on various intracellular signaling mechanisms. Finally, both ethanol and taurine are known to interact with lipid membranes (Huxtable, 1992), and it is possible that ethanol-induced increases in extracellular taurine may mediate some of the effects of ethanol on neuronal membrane composition, fluidity and stability.

2.4.3 Effects of Taurine on Ethanol-Induced Locomotor Activity

Several studies over the past two decades have demonstrated a significant interaction between taurine and acute behavioral actions of ethanol in rodents. Aragon and colleagues demonstrated that acute administration of taurine (30–45 mg/kg i.p.) inhibited the locomotor activity produced by a 1 g/kg dose of ethanol, whereas taurine administration (30–60 mg/kg i.p.) enhanced the locomotor stimulant effect of a higher (2 g/kg i.p.) dose of ethanol (Aragon et al., 1992). Thus, taurine appears to modulate ethanol-stimulated locomotor activity in a manner that is dependent on the dose of ethanol administered. The inhibitory effects of taurine on ethanol-stimulated locomotor activity may be a result of taurine acting on central GABAergic systems.

CHAPTER III

OFFSPRING BODY GROWTH

3.1 Background

It is well known that alcohol brings various parameters of detrimentally abnormal and retarded development of the growing life (Rasmussen and Christensen 1980; Gohlke, Griffith and Faustman 2005). Nevertheless, doses of alcohol which cause such damages vary and this depends not solely on the alcohol itself but rather with many involving factors such as genetics, age of the mother, maternal nutritional status, and combination of other drugs of abuse (Charles, Kristin and Feng, 2005). As a result, it is definitely of ethical questions to perform such studies in humans. Accordingly, this research utilized laboratory animals and mice were chosen because of the close genetic make ups, an easy maintenance, and a short gestational period. This section of the research measured how different levels of alcohol affected the offspring body growth and whether taurine supplementation provided protective effects against alcohol damages (Karin et al, 2007; Yang et al, 2009).

3.2 Materials and Methods

In order to avoid ethical questions on human studies and to be able to control doses of maternal alcohol given along with other sources of calories, adult ICR outbred mice were used in the study. Eight weeks old adult ICR outbred mice were mated and pregnant mice (Supplied by The National Laboratory Animal Center) at gestational day-GD 4 were housed in pairs to prevent stress from isolation in a clear plastic cage for general behavioral observations and were maintained in 12-hour light/dark cycles controlled area, with temperatures ranged between 23°- 25°C. The mice were allowed for a 3-day environmental adaptation and given water and food ad libitum during this period with additional liquid diet without alcohol given on the second day after arrival to familiarize the animals with this type of diet, until treatments began.

3.2.1 Animal Treatments

Treatment began on GD 7 when the mice were randomly assigned to receive different treatments, namely, *normal control*—water and food ad libitum without any treatments, *isocaloric control*—isocaloric sucrose liquid diet, *taurine*—1g/kg body weight in drinking water, and *alcohol*—various doses of alcohol in a liquid diet (5%, 10%, 20% and 30% v/v—from food grade 95% alcohol, supplied by The Government Pharmaceutical Organization). The alcohol and isocaloric sucrose treatments were prepared fresh daily in a nutritionally balanced liquid diet (Ensure, Abbott Laboratories) at the final volume of 25ml per animal. Each treatment group also received 25% of daily solid food (Rat chow) requirements to prevent unnecessary stress from whole liquid diet as mice are rodents and as well with water ad libitum. Numbers of each treatment group (n) ranged from 4 to 12 mothers per group.

3.2.2 Alcohol Dose-Effect Study

Eight weeks old adult ICR outbred mice were mated and pregnant mice (Supplied by The National Laboratory Animal Center) at gestational day-GD 4 were housed in pairs to prevent stress from isolation in a clear plastic cage for general behavioral observations and were maintained in 12-hour light/dark cycles controlled area, and temperatures ranged between 23°- 25°C. The mice from each group (n=12) were allowed for a 3-day environmental adaptation and given water and food ad libitum during this period with additional liquid diet without alcohol given on the second day after arrival to familiarize the animals with this type of diet. The same method was used for the isocaloric control group using sucrose as a measure for calories controls, receiving no alcohol content in the liquid diet to ensure that all groups of animals obtained the same method of feeding. Liquid diet was given at the beginning of the dark cycle when mice were fully awake and alert. Daily liquid diet intake was monitored to measure levels of alcohol consumed by the mothers during pregnancy and lactation. Pregnant mice were weighed every other day to reassure the pregnancy. The pairs were separated early morning of GD 17 to prepare the mothers of their delivery. From this day onwards, pregnant mice were checked every few hours for appearance of pups. As the pups arrived on a particular day, it was defined as postnatal day 0 (P0). After delivery, pregnancy outcomes, namely, miscarriage, perinatal death and postnatal mortality, length of gestational days and litter size were recorded. Within 5 hours after delivery, pups were measured for their birth weight and their successive body weight were then monitored weekly along with treatments continuing throughout lactation until the pups reached 21 days (P21--weaning) and again at adult age (P56). The pups were weighed with careful handling techniques to prevent unfamiliar odor changes in pups. The techniques were practiced where the researchers wore laboratory gloves, hands washed and thin pieces of tissue paper were used to pick up each pup for weighing and changed for every pup. Pieces of white clean paper were also laid on top of the scale for each pup and changed consistently. At weaning, they were separated from the mother and housed with other pups of the same gender from the same litter until adult-P56, given solid food and water ad libitum, with alcohol abstinence since weaning.

3.2.3 Taurine Involvement in Alcohol Teratology

After finishing the first session to compare and select the relevant dosage, 10% alcohol was used to investigate whether taurine supplementation provided protective effects against alcohol on body growth and development of the offspring. The 10% alcohol was chosen because of a number of rationales. Firstly, there was maximal number of pups left per litter as compared to other alcohol doses. Secondly, the successive body weight observed in this group was of reasonable pattern. Thirdly, there were a certain number of offspring deaths observed as compared to other groups of lower or no alcohol given at all. Fourthly the dose is comparable to human alcohol intake, being the content in most alcoholic drinks. And finally, the learning capacity was yet obtainable using this dose—if it were of higher doses, the learning and memory processes might not be acquired after all. The same methodology was implemented where mice from each group (n=3 to 6) were allowed for a 3-day environmental adaptation and given water and food ad libitum during this period with additional liquid diet without alcohol (except normal control group with only water and rat chow ad libitum) given on the second day to familiarize the animals with this type of diet. The same method of feeding and handling was performed as the previous session. An additional treatment was taurine given at the dose of 1g/kg body weight mixed into drinking water (Yang, 2007). The methods used to ensure that the mice took the given taurine was to provide first 10ml of distilled water mixed with taurine at the beginning of the dark cycle, and then refilled the bottle with plain tap water throughout the rest of the cycles. The same methods were used for other groups, the only difference being that the 10ml distilled water given initially contained no taurine.

After having completed the first two sessions, the offspring from the latter session, where mothers were fed 10% alcohol, 10% alcohol + taurine, isocaloric pair-feds and normal non-treated mothers were separated from their mothers at weaning (P21) and housed with pups from the same litter, separated from the opposite gender to prevent mating. Taurine was given to offspring from alcohol-treated mothers (Receiving no taurine during pregnancy and lactation) and the isocaloric pair-fed mothers (Receiving no taurine during pregnancy and lactation) to investigate whether taurine also exerts its protective effects post-weaning in the alcohol affected offspring. Again, the same method of feeding was performed for taurine supplementation, only that the weaned offspring were given solid foods and water ad libitum without any liquid diet. In other words, alcohol abstinence started since weaning.

3.3 Results

Alcohol Dose-Effect Study: All 4 groups of pregnant mice receiving different doses of alcohol displayed a comparable length of pregnancy (Ranged from 17 to 19 gestation days), similar litter size (From 14 to 17 pups per litter) and none of the groups had experienced miscarriage, when compared to control group—receiving 0% alcohol (Table 3-1).

There found to be perinatal death in 10% and 20% alcohol treated groups. The highest number of perinatal death occurred in offspring from 20% alcohol treated mothers while 5% alcohol group showed no offspring mortality throughout the life of the offspring until they were sacrificed.

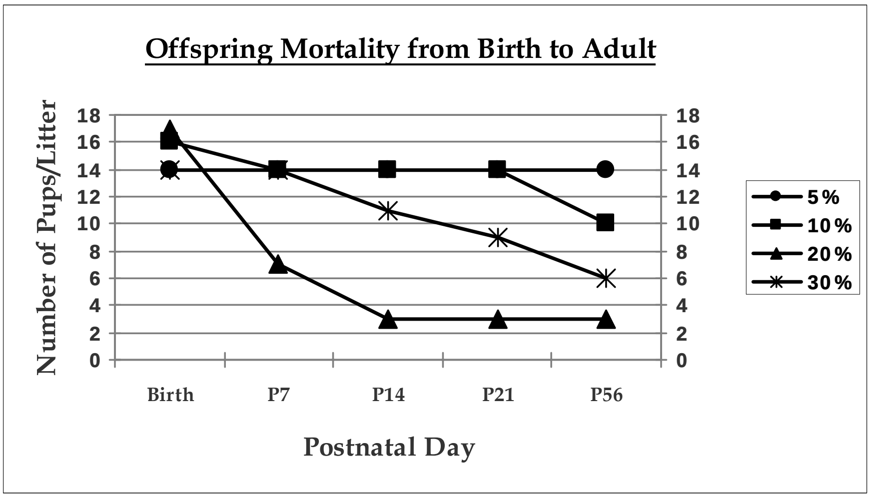
Table 3-1 Pregnancy Outcomes:

Outcome variables	Different doses of alcohol				
	0%	5%	10%	20%	30%
Maternal death	No	No	No	No	No
Spontaneous abortion	No	No	No	No	No
Perinatal death	No	No	✓	✓	No
Gestation length (Days)	19	18	17	18	18
Litter size (Pups)	17	14	16	17	14

(Representation of one mother from each study group)

Moreover, pups from 10%, 20% and 30% alcohol treated mothers died continuously throughout their lifespan (Figure 3-1).

Figure 3-1 Offspring Mortality:



Percentages of survived pups from the whole litter at adult age (P56) were only 62.5%, 17.6%, and 42.9% respectively (Figure 3-2). For normal control groups, it was found to be no offspring death throughout the study, thus resulting in 100% survival rate from birth to adult age (Data no shown). With no offspring death observed from lower level of alcohol contained in the diet (5% alcohol), it was found to be comparable with normal controls where no treatments were give until the end of the study when all mice were sacrificed.

Figure 3-2 demonstrated bar graphs depicting the number of pups left per little at birth (Solid bars) and again at adult age (Striped bars). It is obvious from these graphs that the rate of survival reduced as the alcohol increases. However, the lowest level of survival was observed in 20% alcohol treated groups while that of 30% alcohol demonstrated a higher rate of survival.

Figure 3-2 Offspring Survival Rate:

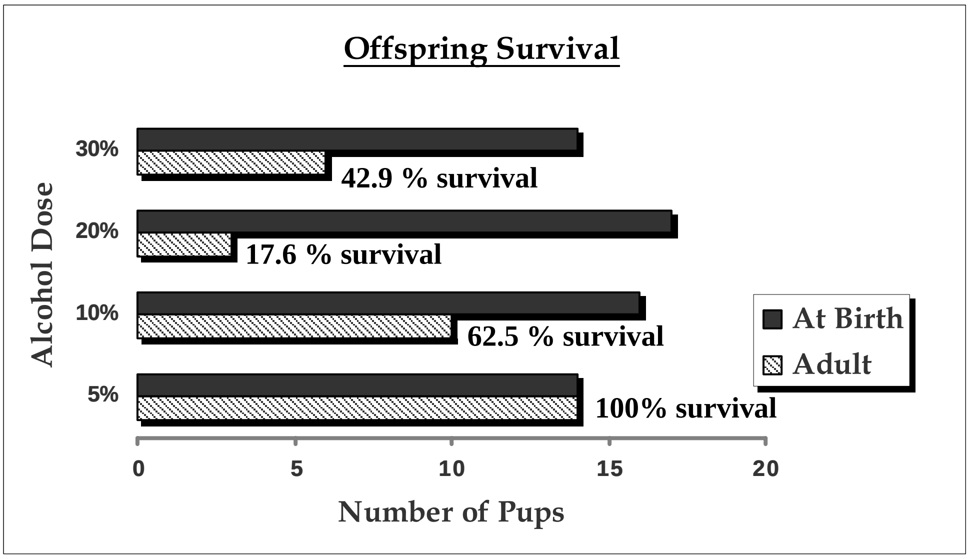


Table 3-2 Demonstration Of Offspring Body Weight From Birth To Adult

Weight in grams; P = Postnatal day; SD = Standard deviation

Alcohol	0%		5%		10%		20%		30%	
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P0	1.53 ± 0.19		1.63 ± 0.14		1.53 ± 0.17		1.59 ± 0.13		1.89 ± 0.14	
P7	4.15 ± 0.61		4.52 ± 0.31		3.47 ± 0.26		3.67 ± 0.34		3.88 ± 0.73	
P14	6.26 ± 0.26		5.91 ± 0.26		4.42 ± 0.29		8.80 ± 0.53		5.52 ± 0.44	
P21(Wean)	8.37 ± 0.37		7.06 ± 0.61		4.24 ± 0.19		11.17 ± 1.24		5.03 ± 0.60	
P56 (Adult)										
Male	37.50 ± 1.87		33.13 ± 2.59		33.00 ± 2.74		40.00 ± 0.00		37.00 ± 2.74	
Female	27.50 ± 1.87		27.50 ± 2.74		27.00 ± 2.74		30.00 ± 0.00		25.00 ± 0.00	

Offspring from control groups demonstrated a continuous weight gain pattern from birth until weaning. Similarly, the same pattern of weight gain was observed in offspring from 5% alcohol treated mothers but offspring from 20% alcohol treated mothers maintained a slightly higher weight gain than normal. On the other hand, those of 10% and 30% groups showed delayed weight gain from P7 onwards and at P21, the mean body weight of these groups was reduced when compared to the weight at P14 (Table 3-2; Notice the reduction in body weight as development proceeded from P14 to P21 in 10% and 30% alcohol treated groups) and offspring from these groups demonstrated abnormal gait movement as early as 2 weeks of age with writhing behaviors (Data not shown). On the opposite, from Figure 3-3, offspring from 20% alcohol treated mothers maintained a slightly higher weight gain starting from P14 than normal growing, untreated mice (Mice profile--The National Laboratory Animal Center—Data not shown). At adult age (P56), mean body weight of most female offspring in all groups was normal when compared to control while that of male was slightly below average except those from 20% alcohol mothers by which their weight was noticeably higher than any other groups examined.

In addition, statistical analysis was performed using ANOVA test. It was shown that offspring weights from birth to weaning (P21) were statistically significant at P=0.05. However, adult weights among the groups were not significantly different with the P-value = 0.197.

Figure 3-3 Graphs Plotted for Offspring Successive Body Weight

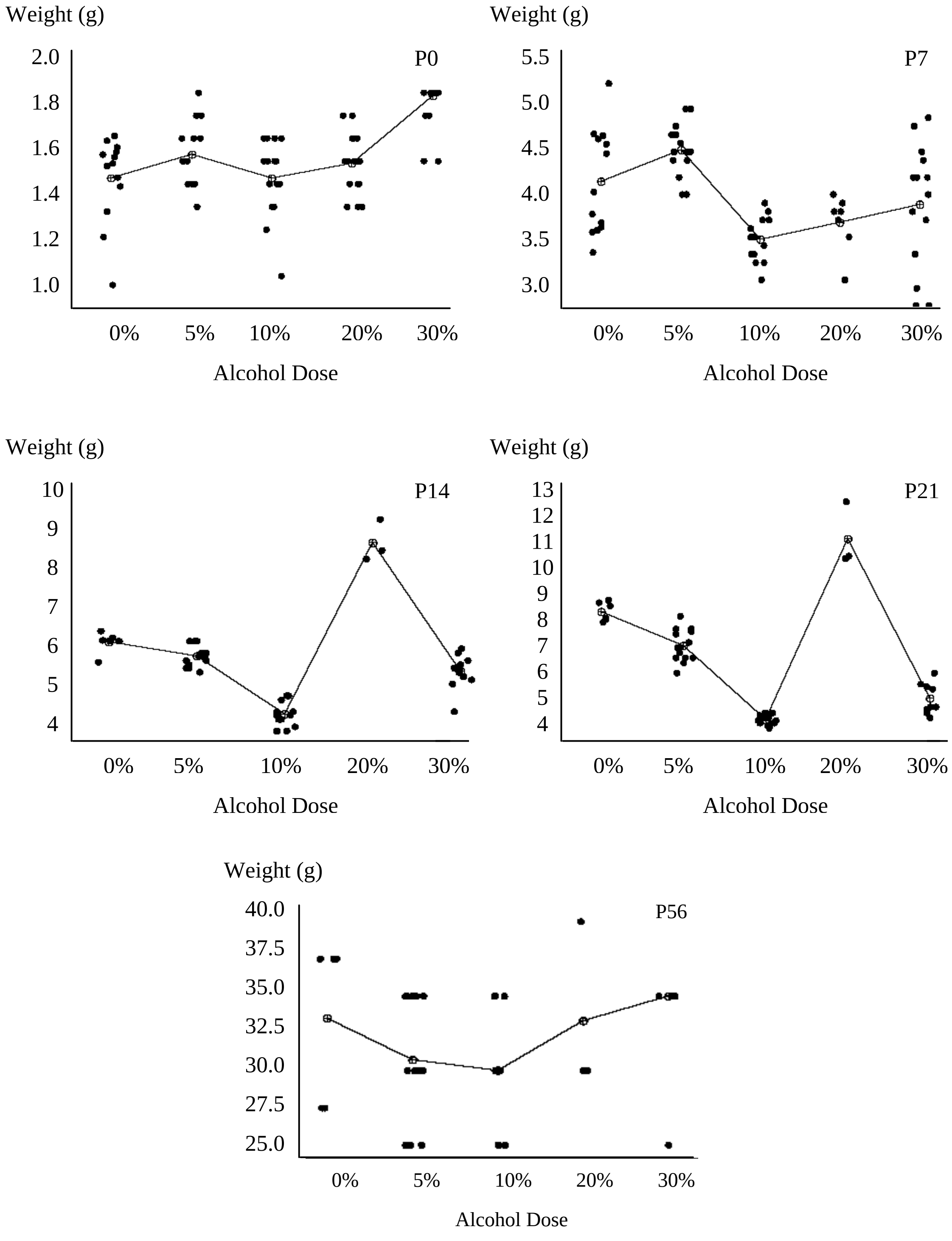


Figure 3-3 demonstrates offspring body weight from birth (P0) to adult (P56) plotted from median values of the scattered data. Notice the abnormally high offspring weights in 20% alcohol from P14 onwards.

Involvement of Taurine in Alcohol Teratology: Pregnancy outcomes were monitored from each of the treatment groups. It was observed that there were no maternal death or spontaneous miscarriage from any groups investigated, and all groups carried similar length of gestation (19-21 days) and the litter sizes ranged from 12 to 18 pups per litter. However, in alcohol-treated mother, it was found that perinatal death took place. Pups died from a mother of alcohol-treated alone and from a mother having both alcohol and taurine (Table 3-3).

Table 3-3 Pregnancy Outcomes-Pair-Fed

Treatments Outcome Variables	NM	Alc	Suc	AlcTr	SucTr
Maternal death	No	No	No	No	No
Spontaneous miscarriage	No	No	No	No	No
Perinatal death	No	✓	No	✓	No
Gestation length (Days)	19	21	20	21	20
Litter size (Pups)	16	16	14	12	18

Records of offspring successive body weight from birth (P0) to weaning (P21) when compared to normal, non-treated mice demonstrated an underdevelopment at certain stages (Table 3-4). At birth, it was found that isocaloric pair-fed groups either with or without taurine, the offspring weights were comparable to normal pups while offspring from alcohol-treated mothers weighed less than control. At P7, the weights gained among isocaloric pair-fed were comparable to normal mice while pups from alcohol and alcohol + taurine showed weights below normal, and the same pattern of delayed weight gain was also observed at 14 days of age. All pups reaching weaning were observed to gain weight to the normal levels.

Table 3-4 Mean Offspring Successive Body Weight (n= mothers)

	P0	P7	P14	P21
Normal Control (n=2)	1.76	3.74	6.89	9.50
Alcohol (n=6)	1.40	2.69	6.07	10.33
Sucrose (n=6)	1.44	3.15	6.02	9.33
Alcohol + Taurine (n=4)	1.36	1.85	4.73	8.28
Sucrose + Taurine (n=3)	1.48	3.84	5.86	8.42

ANOVA test was performed for this session of the research. It was found that survival rates of the groups examined were significantly different at $p < 0.0001$ when compared the groups: alcohol and sucrose, alcohol and normal control, alcohol +taurine and sucrose, alcohol + taurine and normal control, alcohol + taurine and sucrose + taurine; and at $p = 0.0001$ when compared the groups of alcohol and sucrose + taurine. Figures 3-5, 3-6, 3-7 and 3-8 depicts interaction bar plots for offspring body weight from birth (P0), P7, P14 and P21, respectively.

Figure 3-4 Offspring Mortality: Pair-Feds

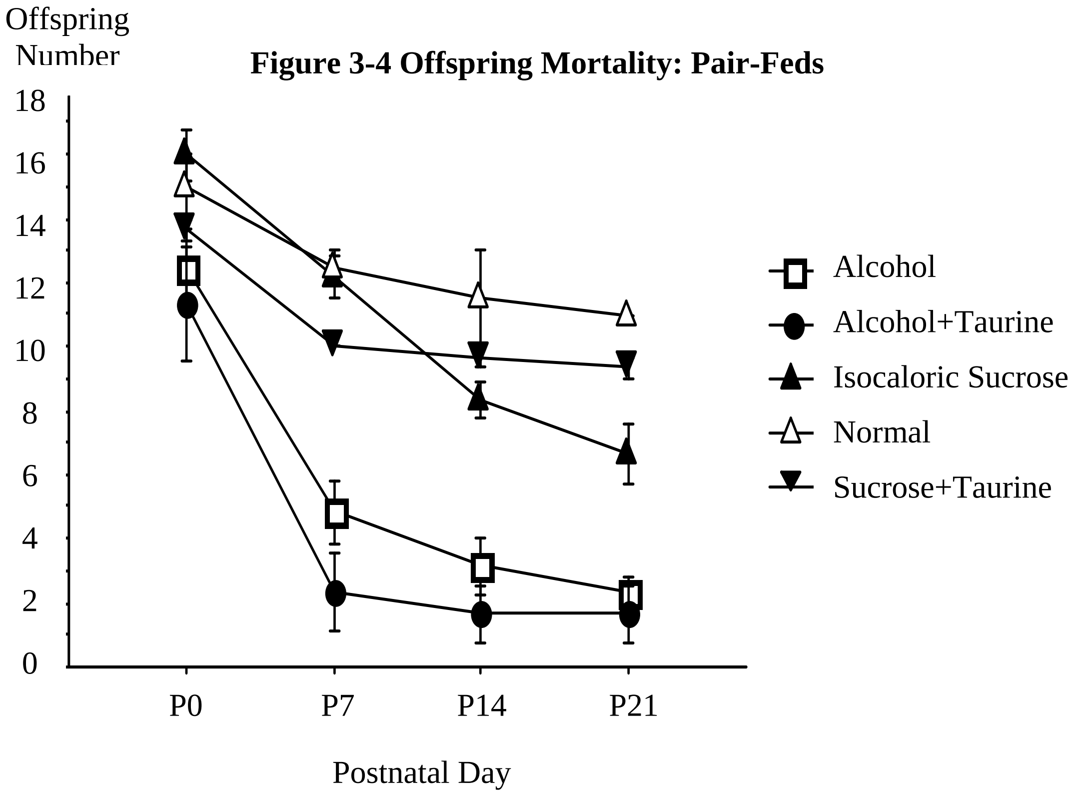


Figure 3-5 Offspring Birth Weight

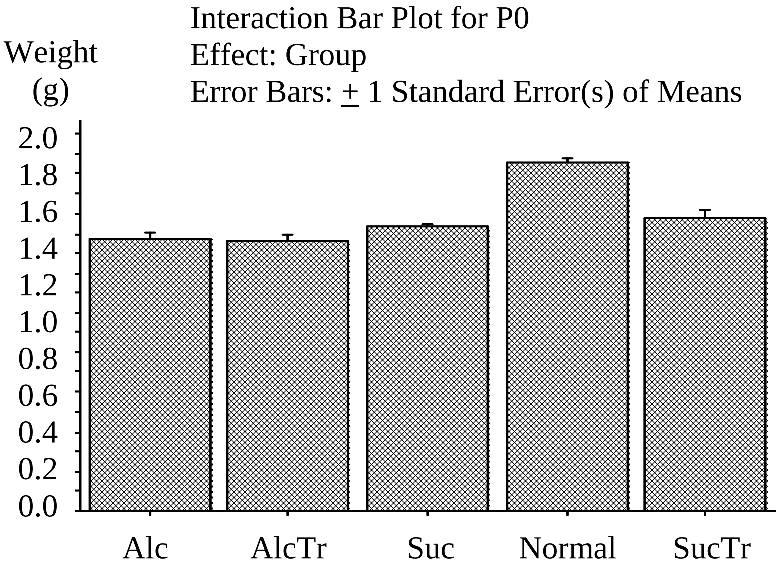


Figure 3-6 Offspring Body Weight At P7

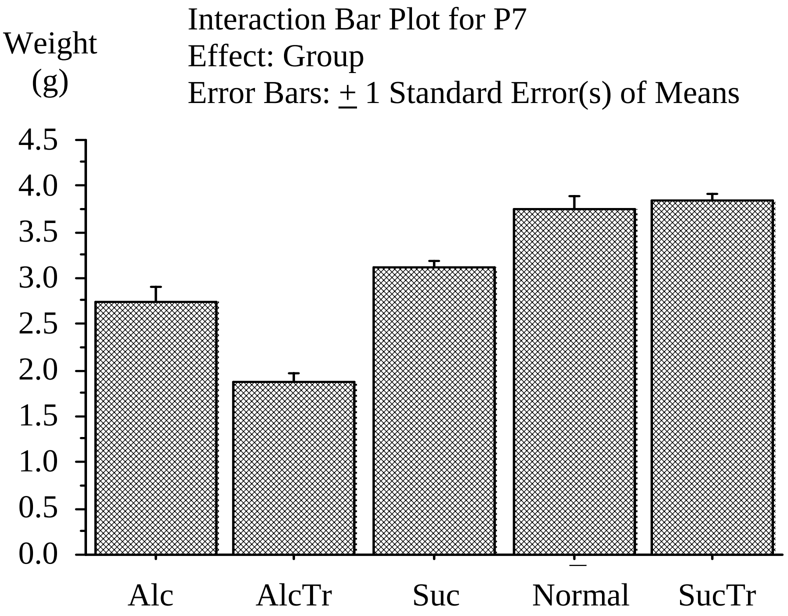


Figure 3-7 Offspring Body Weight At P14

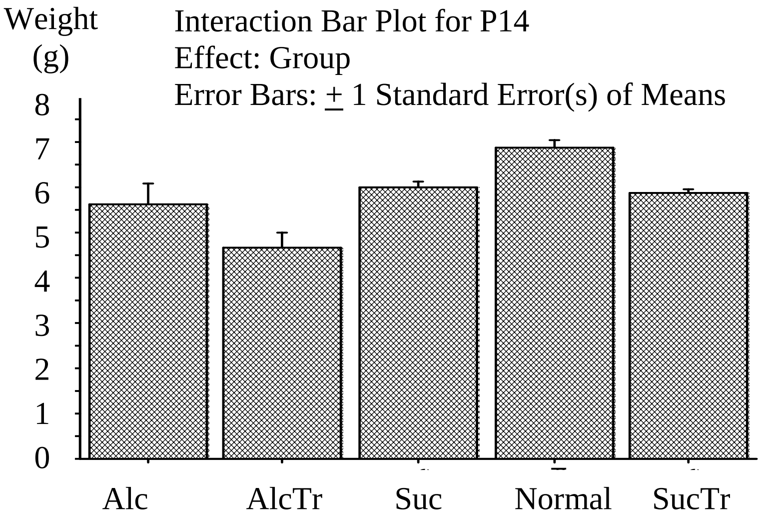
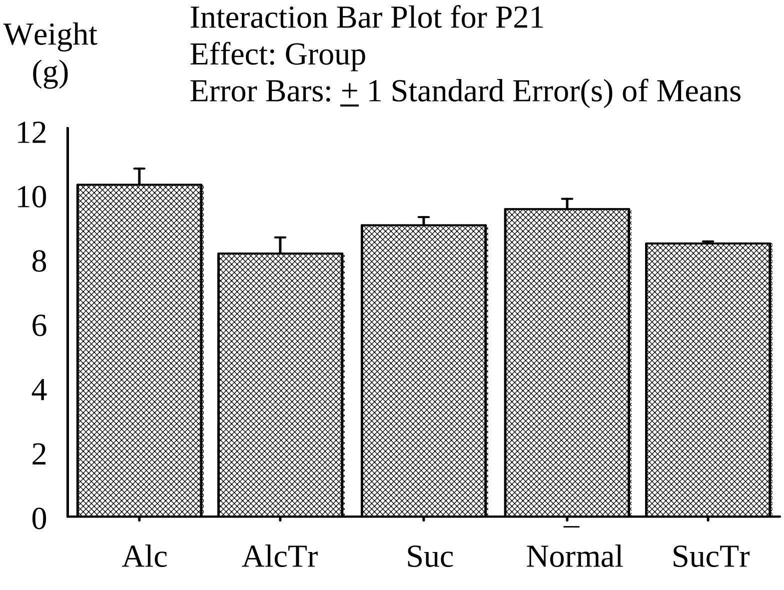


Figure 3-8 Offspring Body Weight At P21



When the pups reached adulthood (P56), their weights became comparable to normal non-treated mice, in both male and female investigated (Table 3-5).

Table 3-5 Mean Body Weight of Adult Offspring

Males	NM	Alc	Suc	Alc+Tr	Suc+Tr
Means	35.29	36.17	34.81	35.10	35.13
Females	NM	Alc	Suc	AlcTr	SucTr
Means	25.04	24.79	28.36	24.50	28.11

Among all groups examined, it was demonstrated that the highest percentage (90%) of survival was observed of pups from normal, non-treated control which survived throughout the experiment while the lowest (31%) being pups of mothers receiving alcohol and taurine (Data not shown).

Statistical Scheffe was used to analyze survival rate and at significant level of 5%, the group interactions investigated for survival rates demonstrated a significant difference at $p < 0.0001$ for alcohol and sucrose, alcohol and normal control, alcohol + taurine and sucrose, alcohol + taurine and normal control, alcohol + taurine and sucrose + taurine and at $p = 0.0001$ for alcohol and sucrose + taurine. Other groups compared were not found to be significantly different at $p = 0.4171$ for alcohol and alcohol + taurine, at $p = 0.4033$ for sucrose and normal control, at $p = 0.999$ sucrose and sucrose + taurine and at $p = 0.4426$ for normal control and sucrose + taurine (Table 3-6).

Table 3-6 Scheffe For Survival Rate (Group)
Significant Level: 5%

	Mean Diff.	Crit.Diff	p-Value	Significance
Alc, AlcTr	1.458	2.494	0.4171	
Alc, Suc	-5.083	2.037	<0.0001	S
Alc, NM	-6.792	2.880	<0.0001	S
Alc, SucTr	-4.958	2.494	0.0001	S
AlcTr, Suc	-6.542	2.494	<0.0001	S
AlcTr, NM	-8.250	3.220	<0.0001	S
AlcTr, SucTr	-6.417	2.880	<0.0001	S
Suc, NM	-1.708	2.880	0.4033	
Suc, SucTr	0.125	2.494	0.9999	
NM, SucTr	1.833	3.220	0.4426	

When compared among groups at certain ages, it was found at significant level of 5% that there was a significant difference at $p < 0.0001$ for P0 and P7, P0 and P14, P0 and P21 and at $p = 0.0228$ for P7 and P14 and at $p = 0.5564$ for P7 and P21 (Table 3-7).

Table 3-7 Scheffe For Survival Rate (Postnatal Day--P)
Significant Level: 5%

	Mean Diff.	Crit. Diff	p-Value	Significance
P0, P7	5.600	1.702	<.0001	S
P0, P14	7.500	1.702	<.0001	S
P0, P21	8.350	1.702	<.0001	S
P7, P14	1.900	1.702	.0228	S
P7, P21	2.750	1.702	.0004	S
P14, P21	.850	1.702	.5564	

The one way ANOVA (Table 3-8) revealed a significant effect among the treatment groups at $p < 0.0001$, from birth to weaning at $p < 0.0001$ and of the interaction between groups investigated and offspring age at $p = 0.0076$.

Table 3-8 ANOVA Table For Survival Rate

	DF	Sum of Squares	Mean Square	F-Value	p-Value	Lambda	Power
grp	4	713.854	178.464	43.825	<.0001	175.298	1.000
Subject	15	61.083	4.072				
Category for survival rate	3	578.241	192.747	56.110	<.0001	168.329	1.000
Category for survival rate * grp	12	112.029	9.336	2.718	.0076	32.612	.956
Category for survival rate * subject	45	154.583	3.435				

3.4 Discussion

The results suggested that maternal alcohol consumption affects pregnancy outcomes and offspring development (Alcohol Research and Health, 2004-2005; Charles, Kristin and Feng, 2005) in a dose-dependent manner and is teratogenic since early development of the affected offspring. Nonetheless, the higher weight gain observed in offspring from 20% alcohol treated mothers may possibly be the result of the least number of offspring left per litter due to high percentage of perinatal death, thus, there was less competition for milk from the mother than any other groups where the numbers of offspring survived were higher per litter. Although low level of alcohol, at 5%, showed no changes when compared to normal, it cannot be concluded at this point that it is harmless (Mukherjee et al, 2005) because detrimental effects from this level of alcohol can possibly be located within certain tissues and structures of the body and the nervous system, holding compensatory mechanisms and exerting its effects later in the life of the affected offspring. In addition, the effects may be disclosed through other measures involving intellectual performances such as learning and memory (Riley, Mcgee and Sowell, 2004). In addition, the comparable results from alcohol effects of 10% and 30% alcohol treated groups may be explained by the incidence of lower liquid diet intake found in 30% alcohol treated mothers throughout the treatment, partially as a result of pungent flavor and vile odor of higher levels of alcohol contained in the diet, thus summed up into less overall alcohol consumed.

Delayed weight gain observed in the two groups of 10% and 30% alcohol can partially be the results of a drunken mother refused to feed both herself and her pups during pregnancy and lactation, resulting in a mal-nutritional state in the offspring as is similarly observed in human cases as was as well proposed by a research suggesting low body weight gain in alcoholic mothers (Rasmussen and Christensen, 1980). One recent study showed that the amount and form of alcohol consumed affect the overall intake and the general growth and development of the offspring up until adolescence (Luciana, Francisca and Cybelle, 2009). The abnormal gait movement seen in offspring from 2 weeks of age is likely affected either by abnormal or delayed development of certain brain areas responsible for movement, such as cerebellum which develops extensively during the first 3 weeks of life in mice through such damaging mechanisms as cerebellar volume reduction (Edward, Christie and Elizabeth, 2004) and cell loss, and other extensive tissue damages (Carrie and Jape, 1979) such as disruption in neuronal development and growth factor signaling, interference of molecular functions, and neuronal oxidative stress and death (Gohlke, Griffith and Faustman, 2005) and the reduction in cranial vault and callosal displacement (Carrie and Jape, 1979). Possibly more than one of these disrupted developmental processes took place and resulted in the overall disturbed physical balance and abnormal gait movements seen in the affected offspring.

With taurine supplementation, it was observed that offspring development post-weaning demonstrated a catch-up in weights. The offspring was able to gain their body weight to the similar level when compared to normal and pair-fed controls. This may be explained by the ubiquitous functions of taurine that this conditionally essential amino acid can help at the level of liver detoxification (Shanmugam et al, 2009) to assist alcohol break-down and could only be helpful during acute stages of alcohol toxicity. For chronic alcohol consumption, the liver may become too exhausted and that blood alcohol concentrations being stably high, taurine is less likely to be protective possibly both at the level of detoxification in the mother and for the development of the offspring. In addition, though the alcohol received through lactating mothers from birth to weaning is excruciatingly detrimental (Rasmussen and Christensen, 1980; Charles, Kristin and Feng, 2005), taurine available within the offspring themselves or those obtained from treatments after weaning may exert its protective effects (Seda et al, 2009) in unknown mechanisms not investigated in this session during times at which alcohol was no longer delivered to the offspring from post-weaning onwards. Moreover, along with the alcohol received through the mother’s milk, taurine may get a chance to exert its protective roles throughout the rest of the lives of the affected offspring well long after alcohol becomes absent since weaning, again, in mechanisms not investigated in this session. However, it cannot be concluded at this point that taurine is certainly not protective against alcohol during development because the effects observed from growth and body weight may result from the maternal alcohol-treated behaviors of not feeding and nesting the offspring well enough, thus, resulting in retarded growth and development solely independent of the alcohol effects.

CHAPTER IV

MATERNAL BEHAVIORS

4.1 Background

Maternal care is universal across species especially among mammals. Rodents are known to elicit maternal behaviors such as feeding, licking, sleeping near her pups, carrying the pups around with her mouth for a better cozy spots, nesting burrowing and grooming. Alcohol and other drugs of abuse deviates the pattern of maternal care (and parental care in general) to a certain extent as is also evident in humans (Francesca et al, 2009). In humans, when a mother gets herself chronically drunk, the appetite for foods goes down and she refuses to feed herself despite the fact that she has got a baby to care for (The 2nd National Alcohol Conference, 2006). In addition, prenatal alcohol consumption affects mother and child interaction to a certain extent (Mary, Marian and Connie, 1992). This session of the research aimed to investigate how alcohol intake affected the mother's behaviors during pregnancy and lactation and how taurine may play a role in this phenomenon.

4.2 Materials and Methods

All treatment groups studied in CHAPTER 3 were also monitored for digression of maternal behaviors from feeding herself to nursing her pups (Ewart and Cutler, 1979; Ladyman and Woodside, 2009).

4.2.1 Food Intake

The pattern of food intake in non-pregnant mice was monitored first to investigate how alcohol affected general non-maternal behaviors (Luciana, Francisca and Cybelle, 2009). Pattern of food intake were compared between alcohol and alcohol + taurine treated pregnant mice along with isocaloric pair-feds. The food consumed was also calculated to yield daily caloric intakes. Measurements of food intake were performed using measuring bottles (Figure 4-1) where liquid diet was given at 25ml per animal each day; the amount left per day was monitored and calculated. For solid food, the rat chow was given at 3 pellets per animal; those leftover were weighed to monitor the amount of grams eaten. Overall food intakes were calculated into calories for each of the days treated. Figure 4-2 demonstrates how treatments were given to the mice.

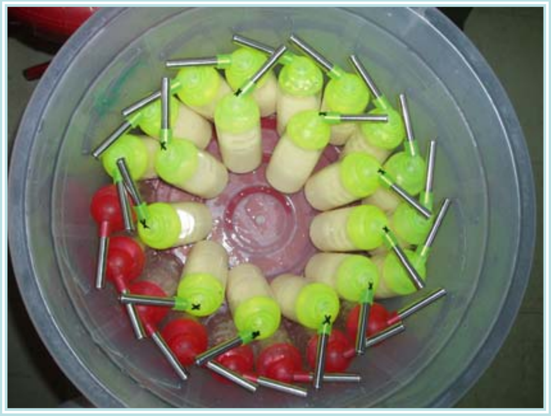


Figure 4-1 Feeding Bottles

Figure 4-2 Treatments (Alcohol or sucrose in liquid diet feeding bottle, rat chow and water ad libitum**)**

4.2.2 Maternal Weight Gain

Pregnant mice were weighed every other day to reassure the pregnancy and to compare weight gain among all groups as well as to augment the food intake patterns of how the mother fed herself and the resulting weight gain to support the progression of pregnancy (Black, 2009).

4.2.3 Behavior Monitoring

The last method performed in this session was to observe general behavioral changes compared to normal controls using video cameras. The time used in this task was 24 hours (monitoring day and night). Animals were observed in paired groups placing the two plastic clear cages next to each other with a white, clean cardboard separating the two cages in the middle. There were a total of 3 cameras used in this session. One camera was set above the cage, and the other 2 ones were set at the one side of each cage. Labels were place both above and beside the cage. Behaviors investigated were agitation, sleeping, feeding and nursing for pups (Ladyman and Woodside, 2009). The frequency of these behaviors were monitored within a specific timeframe and compared among the groups investigated. Figure 4-3 illustrates camera setting.

Figure 4-3 Cage Setting With Video Cameras

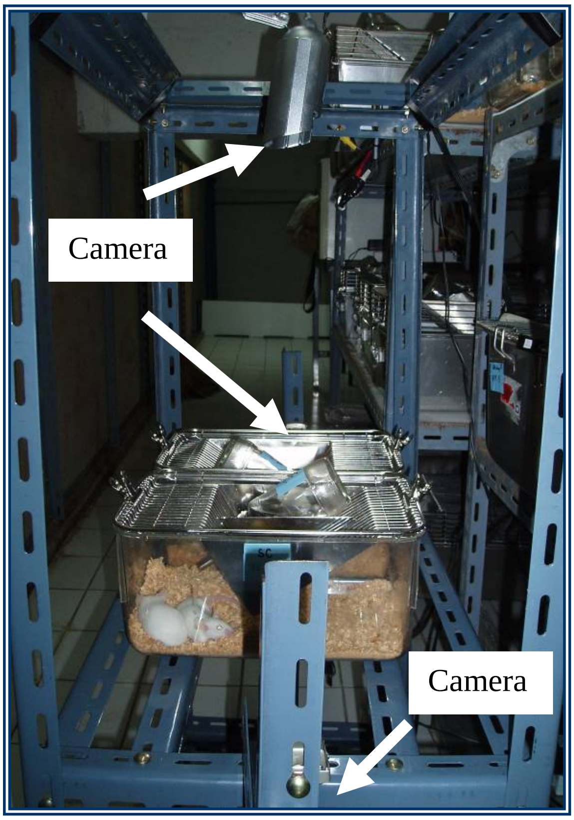


Figure 4-4 Top And Side Views From Video Camera With Labels

4.3 Results

Food Intake: Records from non-pregnant mice of liquid diet intakes between pair-fed groups demonstrated an unstable food intake among alcohol-treated mothers regardless of taurine supplementation. When combined the two types of foods given: solid chow and liquid diet, it was found that the pattern of food intake, both liquid and solid in non pregnant mice was different in the levels eaten each day for the alcohol treated mice. In isocaloric sucrose pair fed mothers, the intake was quite constant from day 1 to day 10, with calories ranged from 40.16kcal to 52.72kcal for two mice living in the one cage. The caloric intakes from alcohol treated mothers were observed to be less throughout the ten days, ranging from 32.44kcal to 40.22kcal (Table 4-1). (Average food intake for mouse—20-30kcal; with approximately 3-6 grams of solid chow for normal mouse and 20% higher in lactating mouse)

Table 4-1 Comparison Of Food Intake In Non-Pregnant Mice

Days	Intake	Suc	Alc
Day 1	Chow (g)	3	3
	Liquid (ml)	25	20
	Calories (kcal)	48.12	40.22
Day 2	Chow (g)	3	2.5
	Liquid (ml)	20	16
	Calories (kcal)	40.22	32.56
Day 3	Chow (g)	3.5	4
	Liquid (ml)	20	13
	Calories (kcal)	41.74	32.44
Day 4	Chow (g)	4	4
	Liquid (ml)	18	13
	Calories (kcal)	40.24	32.44
Day 5	Chow (g)	4	4
	Liquid (ml)	21	14
	Calories (kcal)	44.92	34
Day 6	Chow (g)	5	4
	Liquid (ml)	16	14
	Calories (kcal)	40.16	34
Day 7	Chow (g)	4	4
	Liquid (ml)	26	14
	Calories (kcal)	52.72	34
Day 8	Chow (g)	4	4
	Liquid (ml)	26	14
	Calories (kcal)	52.72	34
Day 9	Chow (g)	4	4
	Liquid (ml)	24	16
	Calories (kcal)	49.6	37.12
Day 10	Chow (g)	4	4
	Liquid (ml)	24	17
	Calories (kcal)	49.6	38.68

In pregnant mice, the pattern observed was similar to non-pregnant animals; alcohol-treated mothers ate less than controls. When compared the group of mice receiving taurine supplementation together with alcohol, it was found that food intake during the first 3 days of treatments demonstrated a comparable level of intake to isocaloric controls where later into the treatment progression, the intake became less as the mothers became consistently drunk (Table 4-2).

Table 4-2 Comparison Of Food Intake In Pregnant Mice

Gestation Day	Intake	Suc	Alc	AlcTr
GD7	Chow (g)	8	6	0
	Liquid (ml)	35	16	48
	Calories (kcal)	78.92	43.2	74.88
GD8	Chow (g)	6	6	6
	Liquid (ml)	30	25	30
	Calories (kcal)	65.04	57.24	65.04
GD9	Chow (g)	6	6	4
	Liquid (ml)	20	20	25
	Calories (kcal)	49.34	49.34	51.16
GD10	Chow (g)	6	6	2
	Liquid (ml)	45	37	26
	Calories (kcal)	88.44	75.96	46.64
GD11	Chow (g)	6	6	3
	Liquid (ml)	45	26	25
	Calories (kcal)	88.44	58.8	48.12
GD12	Chow (g)	6	6	2
	Liquid (ml)	30	30	12
	Calories (kcal)	65.04	65.04	24.8
GD13	Chow (g)	6	6	3
	Liquid (ml)	45	29	38
	Calories (kcal)	88.6	63.48	68.4
GD14	Chow (g)	6	6	4
	Liquid (ml)	45	35	18
	Calories (kcal)	88.44	72.84	40.24
GD15	Chow (g)	6	6	1
	Liquid (ml)	48	19	17
	Calories (kcal)	93.12	47.88	29.56
GD16	Chow (g)	6	6	2
	Liquid (ml)	30	14	12
	Calories (kcal)	65.04	40.08	24.8
GD17	Chow (g)	6	6	2
	Liquid (ml)	45	14	19
	Calories (kcal)	88.44	40.08	35.72

A standard deviation of alcohol and alcohol + taurine revealed an irregular food intake pattern when compared to isocaloric controls (Table 4-3).

Table 4-3 Pattern Of Liquid Diet Intakes (In Milliliters)

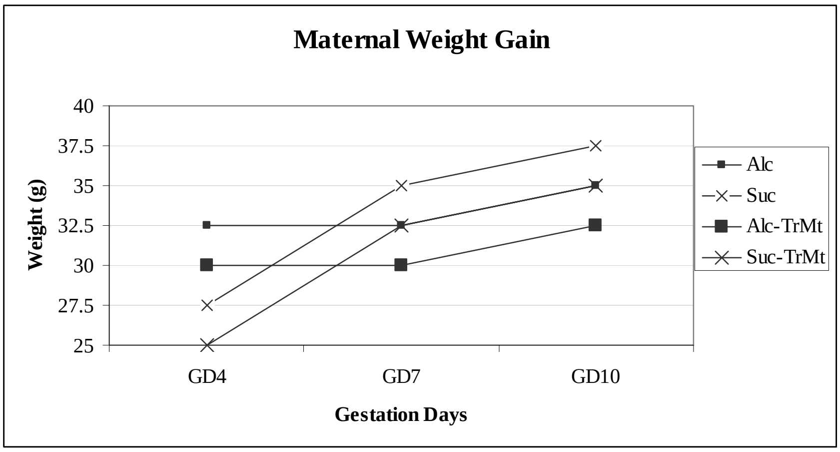
Treatments Gestation Day	Alc	Suc	AlcTr	SucTr
GD7	20	20	23	40
GD8	21	38	17	36
GD9	40	40	40	40
GD10	30	40	40	32
GD11	40	40	21	34
GD12	40	40	40	40
GD13	12	36	40	31
GD14	40	40	40	30
GD15	40	40	30	40
GD16	16	40	35	35
GD17	26	40	25	30
Standard Deviation	11.02	5.99	8.99	4.20

Maternal Weight Gain: When weight gain during pregnancy was monitored, it was found that there was a rise of weight observed in isocaloric controls throughout pregnancy while mothers given alcohol demonstrated a slightly abnormal and inconsistent weight gain. This supports what was observed in the pattern of food intakes depicted in Table 4-3. Graphs depicting rises of weight during the first week of pregnancy was also shown in Figure 4-4.

Table 4-4 Maternal Weight Gain (In Grams)

Treatments Gestational Day	Alc	Suc	AlcTrMt	SucTrMt
GD1	32.5	27.5	30.0	25.0
GD3	32.5	35.0	30.0	32.5
GD7	35.0	37.5	32.5	35.0

Figure 4-5 Maternal Weight Gain



Maternal Behaviors: It was observed that isocaloric-sucrose mothers illustrated similar maternal behaviors when compared to normal controls. However, any groups of mothers receiving alcohol, both alone and with taurine, demonstrated a digression of normal maternal behaviors, namely, hyperactivity, refusal of feeding, ignorance and infancidal behaviors. Ignorant and infancidal behaviors were also revealed with the offspring being left dead within the first few days of birth (Figure 4-6, left column) and was eaten alive from the back by its own mother (figure 4-6, right column). The mothers ignored her own pups to the level that the pups were left undernourished and were much smaller than controls as observed from pups at the age of 21 days shown below (Figure 4-7--SC-sucrose, NM-normal control, LQ-alcohol).

Figure 4-6 Offspring Death

Figure 4-7 Offspring Size At Postnatal Day 21

Maternal behaviors from isocaloric groups were found to be subjectively comparable to normal controls where the mothers elicited nursing, feeding, nesting and burrowing behaviors for care for her pups. In contrast, it was found that the mothers drinking alcohol spent most of their time wandering around and were observed to be abnormally agitated (Jumping, exploring--data not shown). During this agitation, the mother-pup interactions were reduced to the level that the mother ignored her pups and this may explain the outcome of pups being left dead during the first few days of life shown in the above paragraph. The maternal sleeping behaviors were not affected from video camera monitoring. However, it was seen that instead of nesting the burrow for her pups and nursing them with her, the alcohol affected mothers spent her sleep alone and left her pups unattended (Data not shown).

4.4 Discussion

The results indicate that the behavioral changes seen in normal females drinking alcohol regarding food intakes were not different from child-bearing females. Whether or not the animals were pregnant, their food consumption seemed to reduce as they continued drinking alcohol and became consistently drunk. Once they became pregnant, food consumption was still low possibly as a result of reduced appetite (The

2nd National Alcohol Conference, 2006) and the maternal instinct of caring for the developing life within herself diminished as they refused to feed themselves despite the fact that they were carrying their own brood within the womb. This was obvious in delayed weight gain observed in alcohol treated mothers. The pattern of offspring body weight observed in CHAPTER 3 also supports this finding that from pregnancy through lactation, mothers were not caring for their pups as they supposed to and this might indirectly acted as one factor contributing to delay body growth of the affected offspring as a result of too low of a weight gain to support healthy baby (Black, 2009). In addition, the pattern of delayed weight gain observed in all groups receiving alcohol supported the deviated food consumption behaviors. However, it was shown that at the beginning of the treatments, mothers of taurine treated groups consumed comparable amount of foods to controls. This could indicate that taurine may help reduce the effects of alcohol in the mother acutely drinking alcohol; such protective mechanism of taurine may lay with its well-known detoxifying properties in the liver to help rid alcohol faster (Jacobsen and Smith, 1968; Huxtable, 1992). Nonetheless, as the treatments proceeded and the mothers became consistently drunk, the pattern of reduced food consumption surfaced (Kesse et al, 2001). This can be explained that the drunken mothers lack the awareness to seek care for her own brood as often observed as well in humans . The irregular food intake is also similar to human behaviors. When drinking alcohol, people tend to eat less or not eating at all as their appetite reduces. With the mothers refused to care for her own pups and to hurt her own brood, it may be an indication of a disruption and disturbance process within the nervous system responsible for maternal instinctive behaviors.

Infancidal behaviors observed in mothers drinking alcohol can be the effects of alcohol itself that the mother forgot her own brood or it can be that the mothers were very stressed out that she ate her pups. Research on stress studies (Edward and Cutler, 1979; Mary, Marian and Connie, 1992) also demonstrated a digression of maternal behaviors as seen in alcohol-affected animals.

With taurine supplementation, it was observed that both maternal behaviors and offspring development postweaning where they demonstrated a catch-up in weights, it may be explained that taurine may help at the level of liver detoxification (Yang et al, 2009; Seda et al, 2009; Shanmugam et al, 2009) to assist alcohol break-down and may only be protective (Oja et al, 1985) against alcohol damages during acute stages of alcohol toxicity. For chronic alcohol consumption, the liver may become exhausted and that blood alcohol concentrations being stably high, taurine may be less likely protective. In addition, taurine available within the offspring themselves or those obtained from treatments after postweaning may get a chance to exert its protective effects throughout the rest of the lives of the affected offspring where alcohol is absent from weaning onwards and from which mechanism on investigated and observed in this research. However, it cannot be concluded at this point that taurine is certainly not protective against alcohol during pregnancy and lactation because as discussed, it is more likely to be protective at the level of live detoxification than when alcohol has been chronically consumed and the mothers were consistently drunk. Nonetheless, alcohol has its chance of destroying behaviors and other biological mechanisms; abstinence before during pregnancy and lactation is the safest approach (Mukherjee et al, 2005).

CHAPTER V

HIPPOCAMPAL-DEPENDENT LEARNING

5.1 Background

Morris water maze, a form of battery of task, is used to test reference, place learning and memory (Vorhees and Williams, 2006). Rodents are natural swimmers, thus, making it effective selecting this battery of task performance to test hippocampal-dependent learning and memory (Morris, 2008). The swimming pool is a delicate balance of motivation provided by stress/reward and a difficult task (Malleret et al, 1999). The two main advantages of using water maze over other types of mazes are first the mice want to get out, it will start searching for the escape immediately, and second, there are no local cues such as scent trail inside the water as often found using other mazes. Accordingly, questions of performance as a result of traces from other animals prior to them within the maze can simply be disregarded.

5.2 Materials and Methods

The pool consisted of a round tank of water, made opaque with nontoxic white paint. The apparatus used was 150 cm in diameter, with 30cm high wall (Fill ‘n Fun Portable Pool). The pool was place in the middle of a set-up room surrounded by white walls. The walls were made of white canvas and placed 1.5 meters away from each side of the pool. Different shapes of cues, namely, stripes, semicircle (fan shape) and a round clock, were placed upon each wall. During the task, the tank was filled with water made opaque with non-toxic colloidal white paint (Nippon Odorless) at a height of 20cm from the bottom of the pool. The water inside in the pool was kept at 20°C during task performance. Inside the pool was an escape circular platform with a diameter of 12cm. The platform was placed 0.5cm below surface of the water (Malleret et al, 1999).

Figure 5-1 Applied Morris Water Maze—Real Setting

Figure 5-2 Non-Slippery Platform For Escape

The following paragraph describes experimental protocol adapted from Malleret and colleagues (Malleret et al, 1999).

On the first day, each mouse received a first pre-training session consisting of placing the animal on the platform where it had to stay at least 15 seconds, followed by a 30 seconds swimming period, and ended by several trials of climbing onto the platform until each subject was able to climb without help. During the task performance, each animal was subjected to a daily four-trial session. Before the first trial, the mouse was placed for 15 seconds on the platform. Each trial consisted of releasing the mouse into the water facing the outer edge of the pool at one of the quadrants (except the quadrant where the platform was located) and letting the animal escape to the platform before 90 seconds elapsed. A trial ended when the animal reached the platform, where it was allowed to remain for 15 seconds. The animals that failed to find the platform within the time limit was placed onto the platform and stayed there for 15 seconds before being removed and placed back in a cage for a 15 minutes break interval. At the end of the last daily sessions, the mice were placed under heat lamp to allow warmth and complete dryness of themselves and to prevent stress from cold water before being placed back to their home cage. The animals were tested in this water maze for a period of 6 days and one day pre-training session. The releasing point differed at each trial (for example, east, west, south, and east if the platform was in the north quadrant) and different sequences of releasing points were used from day to day. Each animal was performed with a visually guided orientation session, a series of four trials for which the submerged platform had a visible black and white striped cylinder on the top. The performance of the animals was recorded using video camera. This allowed for the calculation of the escape latency (time required to find the platform, in seconds), percentage of escape per day per group, and learning curves were drawn to illustrate how the animals learn from the first to the last day of the experiment.

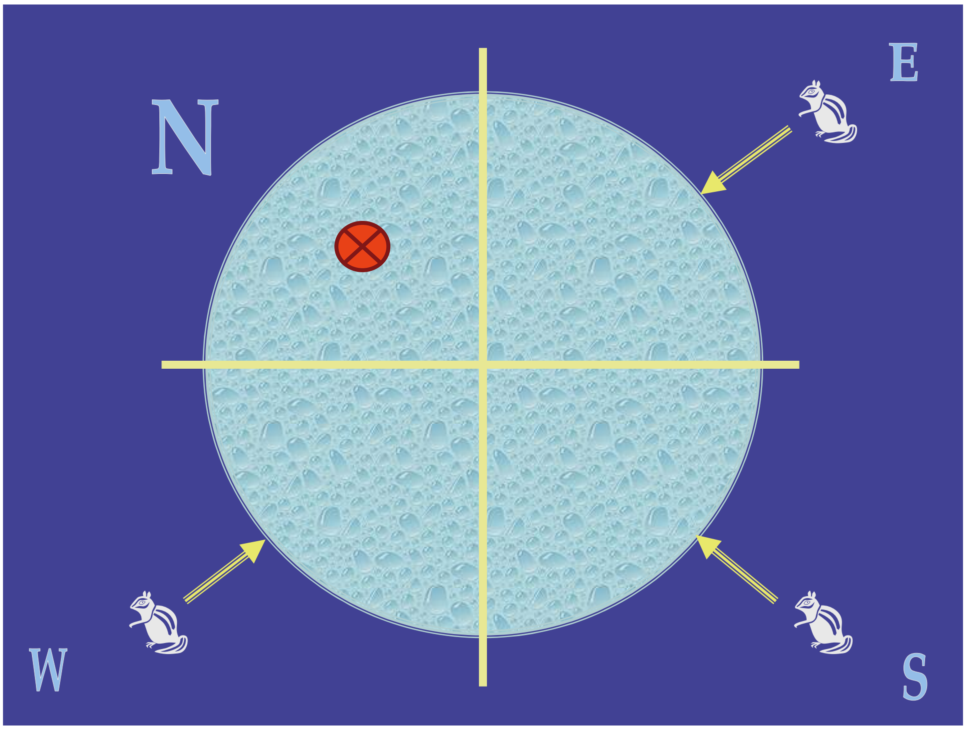


Figure 5-3 Points Of Maze Release (Platform is in the north quadrant**)**

5.3 Results:

Table 5-1 Escape Latency: consecutive days (seconds), n = 3 per group

Days	1	2	3	4	5	6
NM	41.67	44.58	35.58	29.58	25.5	18.83
Suc	58.17	46.75	46.42	45.927	39.67	16.58
Alc	69.58	51.83	53.25	33.67	52.75	42.25
SucTr	71.25	49.50	47.17	39.58	33.92	30.33
AlcTr	81.75	60.50	39.67	26.33	38.00	28.50
SucTrPW	58.92	43.83	41.33	29.50	26.67	9.17
AlcTrPW	68.92	67.25	56.33	63.58	51.75	32.25

This table illustrates escape latency of offspring from each group from day 1 to day 6 of the experiment. It was shown that normal controls tended to learn the maze task and were able to escape within less time as the experiment proceeded. The same pattern of learning was also observed in iso-caloric control groups. The offspring from mothers receiving taurine also demonstrated the tendency to escape requiring less time as the experiment proceeded as compared to normal and iso-caloric controls. In contrast, the alcohol-affected offspring that received taurine from postweaning did not show a reduction in escape latency as compared to other groups (Table 5-1)

Table 5-2 Means: Escape Latency (Seconds)

Groups	Overall means
NM	30.13
Suc	40.17
Alc	48.84
SucTr	43.35
AlcTr	43.87
SucTrPW	32.51
AlcTrPW	55.23

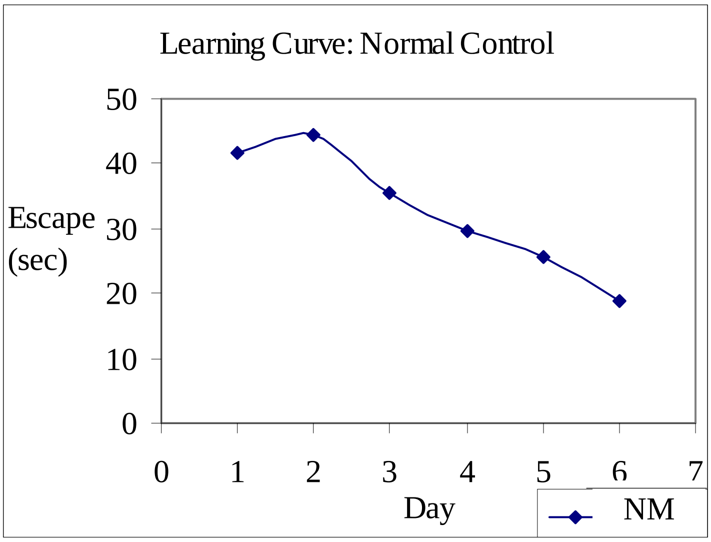
Table 5-2 illustrates an overall mean of escape latency among all groups investigated. It was found that normal control groups were able to escape within the shortest time

(30.13 sec). When compared among groups of pair-feds, it was observed that offspring from the groups of mother receiving alcohol showed longer time spent to escape than isocaloric sucrose (In seconds: 40.17, 48.84 for sucrose and alcohol respectively). Offspring from mothers receiving taurine during pregnancy and lactation demonstrated similar time spent (In seconds: 43.35, 43.87 for sucrose + taurine and alcohol + taurine respectively). However, those offspring which received taurine from postweaning showed much longer time spent to escape (In seconds: 32.51, 55.23 for sucrose + taurine post-wean and alcohol + taurine post-wean respectively).

Learning curves were plotted from the first until the last day of the experiment to illustrate how the offspring from each group learn the water maze task. Curves are depicted below.

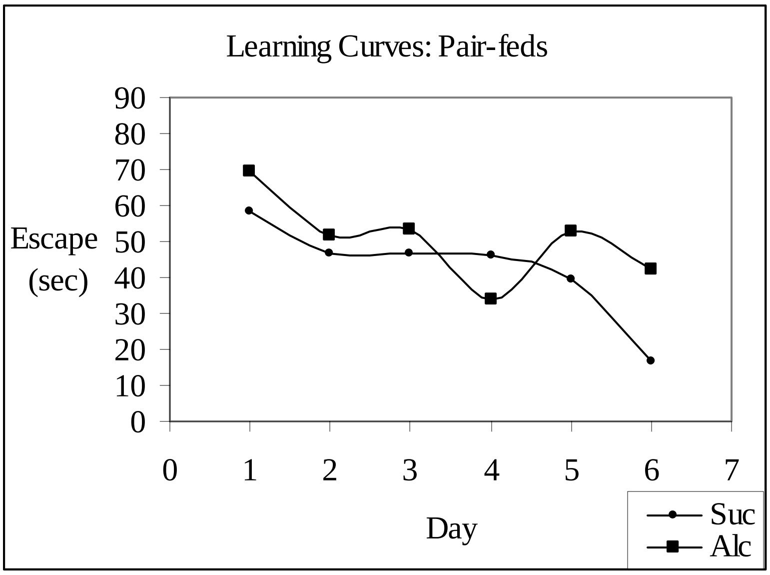
It was found that mice from normal control tended to learn how to escape from the water to the hidden platform within a shorter period of time, progressively from day 1 to day 6 (Figure 5-4).

Figure 5-4 Learning Curve: Normal Control



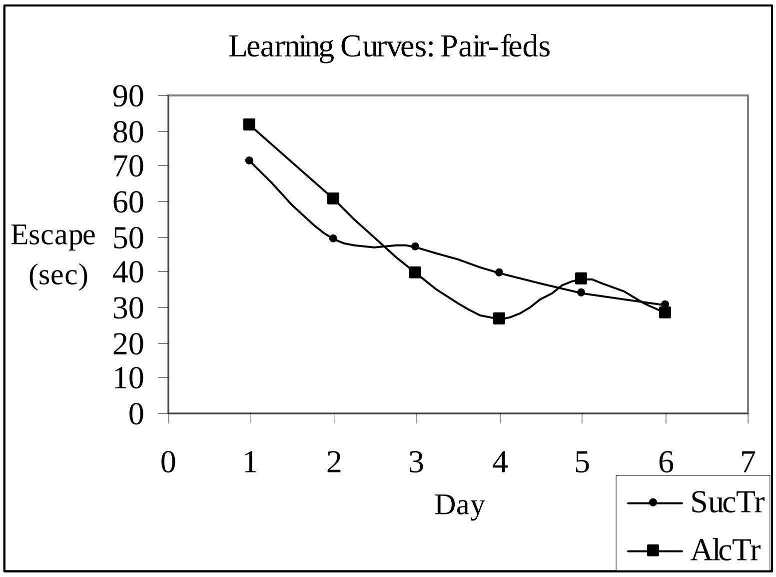
When compared between pair-fed groups, it was observed as well that mice from iso-caloric sucrose required less time to escape to the hidden platform as the experiment proceeded while alcohol were lost within the pool from time to time and did not perform the task with a hint of intact learning and memory processes (Figure 5-5).

Figure 5-5 Learning Curves: Pair-Feds Comparison Of Alcohol VS Sucrose



When taurine was given during pregnancy and lactation, it was found that alcohol affected offspring required less time to escape as the experiment proceeded, indicating a somewhat conserved learning and memory processes when compared to its pair-fed counterpart (Figure 5-6).

Figure 5-6 Learning Curves: Pair-Feds Comparison Of Alcohol VS Sucrose With Taurine During Pregnancy And Lactation



In contrast, when taurine was given to the offspring themselves after weaning, although the graph of alcohol affected offspring depict a glide downward, indicating a slight reduction in time required to reach the platform, the overall seconds required were still longer than its pair-fed counterpart (Figure 5-7).

Figure 5-7 Learning Curves: Pair-Feds Comparison Of Alcohol VS Sucrose With Taurine To Offspring Postweaning

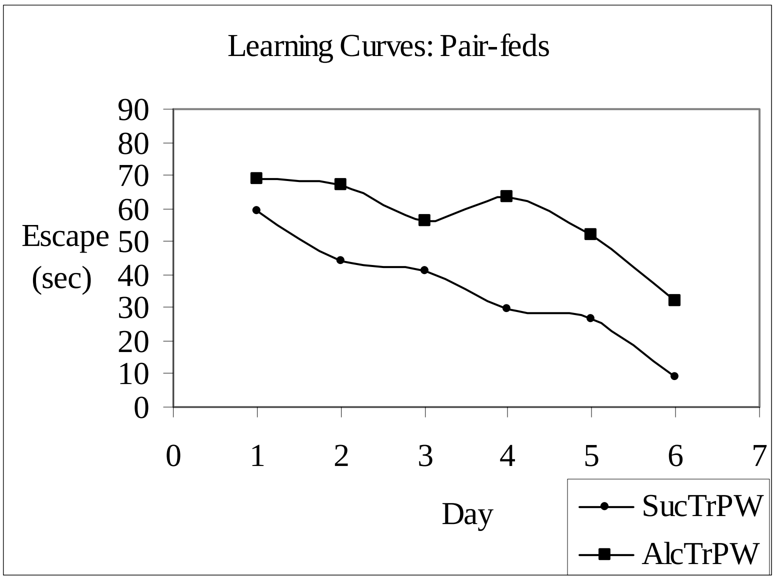
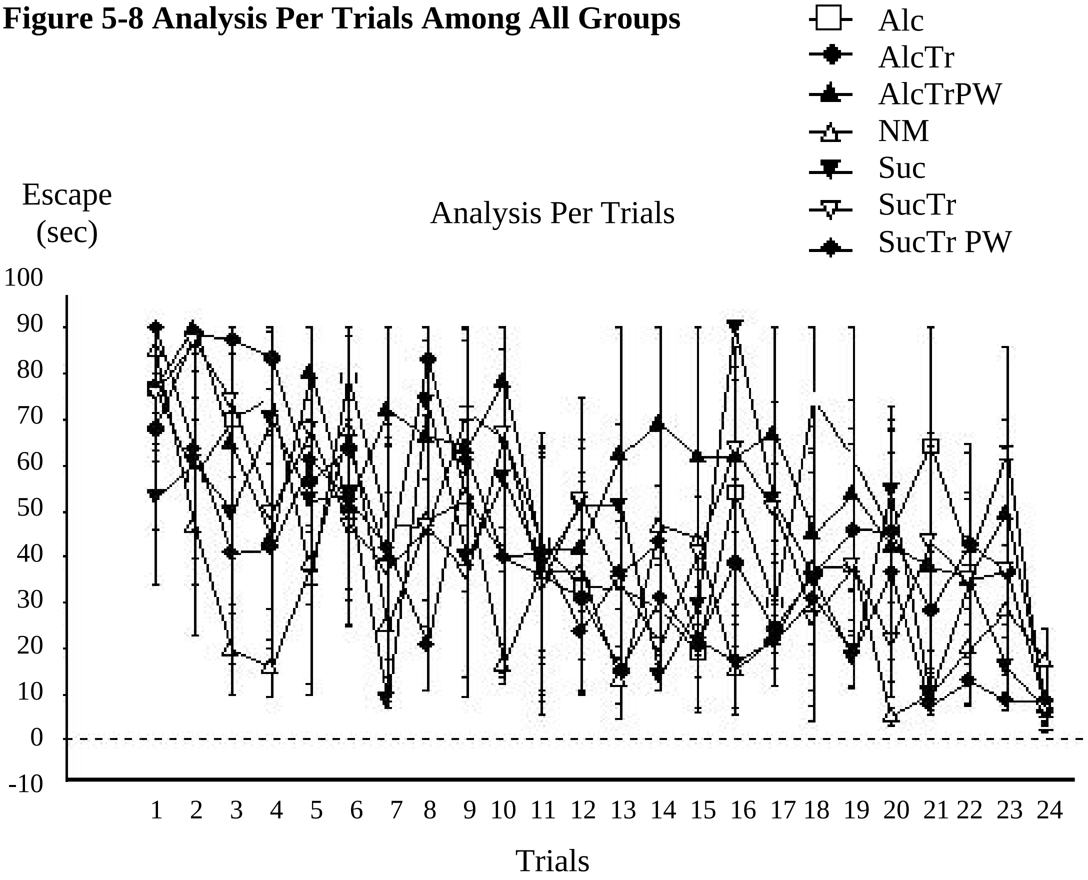


Table 5-3 demonstrated means of the percentage of escape among all groups investigated. It was also found that normal controls showed the highest percentage of escape in this type of task performance of 86.11%. Offspring from alcohol treated mothers showed low percentage of the ability to escape the water (66.67%, compared to its sucrose pair-fed: 70.83%) and taurine given to offspring after weaning did not seem to assist the learning process as it was only 54.17% escape (Compared to its pair-fed sucrose + taurine postwean: 84.72%). In contrast, it was observed that when given taurine to mothers during pregnancy and lactation, offspring from mothers given alcohol showed comparable percentage of escape to its pair-fed counterpart (76.39% and 77.78% for alcohol + taurine and sucrose + taurine respectively).

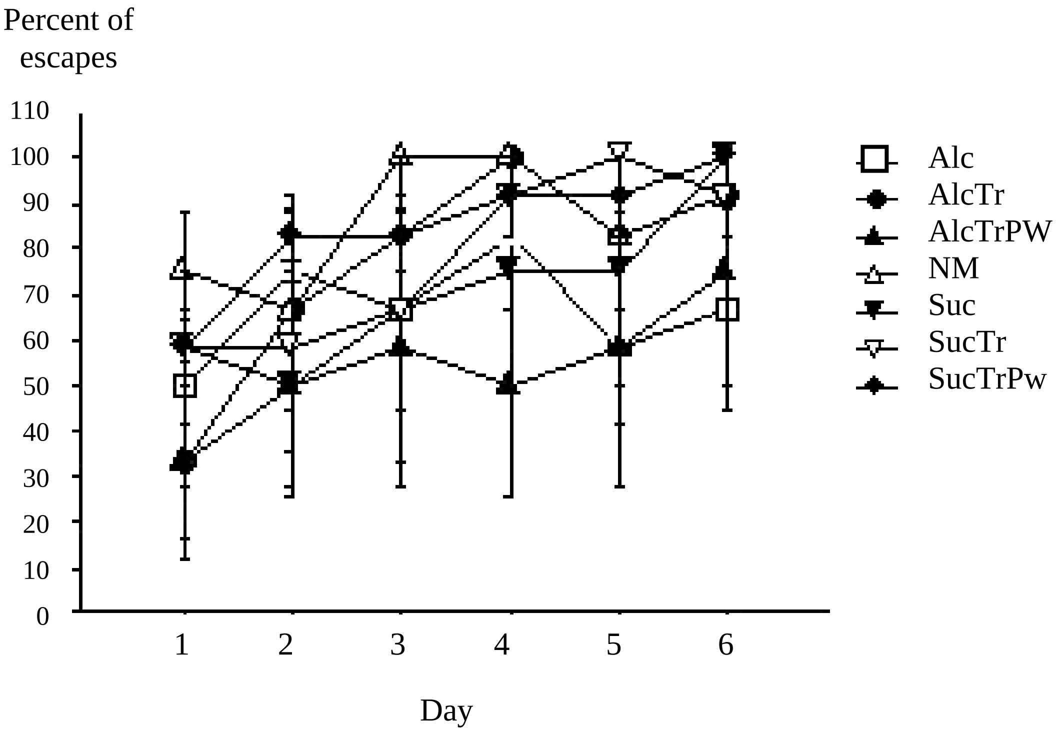
Table 5-3 Means: Percentage Of Escape (%)	
NM	86.11%
Suc	70.83%
Alc	66.67%
SucTr	77.78%
AlcTr	76.39%
SucTrPW	84.72%
AlcTrPW	54.17%

Figure 5-8 Analysis Per Trials Among All Groups



It was found that when analyzed using ANOVA analysis per trials, there is a level of significant difference among trials at p = 0.0001.

Figure 5-9 Analysis of Percentage of Escapes



When analyzed the percentage of escapes among all groups investigated, it was found that they were significantly different at p = 0.0001.

5.4 Discussion

It is obvious that alcohol offers no benefit to the learning and memory processes of the developing brain and the affected adult offspring elucidated deviated pattern of intellectual performance (Hall, Church and Berman, 1994; Clement et al, 2005). On the other hand, the results suggested that taurine may play a protective role against damages of alcohol to certain brain structures involving learning and memory (Aragon and Trudeau, 1992) and that it has to be given at early stages of development of the nervous system as it was shown to elucidate normal pattern of learning when compared to normal control and iso-caloric pair-feds when taurine was given to mothers during pregnancy and lactation. However, as the development proceeded and taurine was given later in life to the offspring themselves after weaning, it is less likely to be protective against alcohol damages to the learning and memory mechanisms.

It may be stipulated that taurine may exert its protection through the marvelous various functions such as osmoregulation and protection against excitotoxicity as alcohol is known to cause excitotoxic damages as well as disruption of cell membrane osmolarity (Baxter and Ortiz, 1966; Dahchour, Quertemont and De Witte, 1996). However, taurine functions in liver detoxicification is not likely for this leanring and memory processes of the offspring as the alcohol was given to the mothers during pregnancy and lactation and that it would have already reached and destructed the nervous tissues of the growing offspring that liver detoxifying processes were way too long gone and ineffective.

The results also indicated that taurine is likely to be protective when the damages were acutely taken place and that when the damages had been done; taurine cannot be of any assistance.

CHAPTER VI

DISCUSSION AND CONCLUSION

First of all, this research was aspired to perform scientific research in a behavioral approach which mimicked human behaviors to a certain extent. Accordingly, the research is not an analytical piece of work and does not involve molecular drudgery but is putative, allegedly natural and instinctive. Human alcohol consumption varies to a great extent and cannot be simply predicted and determined of the exact alcohol levels consumed (Javad and Miles, 2008; Scott et al, 2009). Moreover, alcohol consumption varies from culture to culture and the effects the alcohol does to the body may vary according to genetic make-ups, individual tolerances and enzymatic availability, timing of consumption, nutritional status and involvement of other drugs of abuse (Scott et al, 2009). It is even harder to predict alcohol consumption during pregnancy as this has been an ethical issue since alcohol was found to be teratogenic. In some cultures and beliefs, a little alcohol each day is said to be healthy and the claims range from high anti-oxidant content found in wines to sedative effects alcohol offers as a mean to help the body de-stress; and in some cultures, it is even used among labors to assist pain relief and to ease their hard-working days (The 2nd National Alcohol Conference, 2006). Maternal alcohol intake has become controversial and there is no specific research stating the ‘safe’ level of alcohol recommended to be consumed per day (Mukherjee et al, 2005). In most western countries, drinking beer and wine with meals seem to be as common as or even more commonly practiced than drinking water and it is even believed to increase digestion and regulate appetite (Michael, Anne-Marie and Paul, 2008). In contrast, other cultures especially in Asia and Africa, alcohol consumption is not as widely acceptable and drinking among females is even less prevalent. In Thailand alone, alcohol drinkers are widespread in male population but in females, it tends to be more common far out in the country and remote areas of Thailand and these are where fetal alcohol spectrum disorders are mostly found especially in northern and north eastern parts of Thailand (The 2nd National Alcohol Conference, 2006; Javad and Miles, 2008). Drinking behaviors among pregnant women seems unpredictable as they may drink out of the blue, drink during times of stress and after hard work, drink addictively, and some women may only be a social drinker. Thus the effect alcohol has on the developing life varies not only because of drinking behaviors, but also of influential factors described above. Accordingly, the research provided setting for alcohol consumption in a purportedly natural approach where alcohol was provided along with normal solid foods and water and that no precise alcohol quantity was put into practice. No force feeding or inhalation methods were implemented to ensure that the animals drink alcohol under their own preferences to a great extent.

During the research, no blood alcohol concentration measurement was performed. This is because of two main reasons. Firstly, it is due to technical difficulty as the laboratory animals used in this research was ICR outbred mice which are very small. The amount of blood needed for blood alcohol concentration using analysis machine is too vast compared to the size of the animals and this would involve too many lives only to measure the blood alcohol level. Secondly, as mentioned above, the animals were kept in as a natural state of life as feasible to ensure they received the least stress from experimental procedures.

In latter session of the research following alcohol dose-effect study, 10% alcohol was chosen because of various considerations as follow. First of all, the alcohol effects on offspring successive body weight was not linear as expected with 20% alcohol demonstrating somewhat compromised mechanisms that the weight of the affected offspring seemed to be comparable or even higher than normal controls and that with 30% alcohol, there tended to be less alcohol consumed and that with both 20% and 30% alcohol the data shown were too scattered to be discussed and explained of appropriately as there were many factors involved such as vile taste and odor of the alcohol and other compromised mechanisms. For that reason, higher doses could not be selected and that 5% alcohol also elucidated normal data comparable to control which might make it harder, with taurine supplementation, to compare and analyze using the low dose. Secondly, the rate of offspring survival was highest from 10% alcohol so that there were maximal numbers of pups left for continuation of the research. Thirdly, the mean data of body weight at adult age (P56) were in the middle and that the successive body weights from birth to adult were reasonable compared among all groups investigated. Fourthly, 10% alcohol is the closest to human alcohol consumption, being the content in most alcoholic drinks. And finally, with 10%

alcohol consumed, the learning and memory processes occurred in offspring could still assumingly take place and could be examined. With higher doses of maternal alcohol intakes, the affected offspring might not be able to learn at all and the questions asked in this research would not be possible to be investigated after all.

By means of taurine supplementation, the method of feeding was of natural approach as well. Taurine was given through drinking water and not through other precise feeding methods such as force-feeding or injection. Accordingly, taurine reaching the body was though enteric route passing all metabolic processes within the body before reaching the nervous tissues (Huxtable and Lippincott, 1982) . As a result, the amount of taurine given which was able to cross the blood-brain barrier could not be stated precisely. Moreover, taurine provides ubiquitous benefits within various tissues of the body and that it could travel to any tissues necessary for its functions during times of development and other conditionally essential circumstances (Jacobsen and Smith, 1968; Chapman and Greenwood, 1988; Huxtable and Peterson, 1988; Kendler, 1989). Nevertheless, this is how human consumption is like and this is how the research was intended to mimic.

It can be concluded from this research that alcohol is teratognic since an early stage of development and affects the development in a dose-dependent manner. Although the lowest level of alcohol given (5%) provided normal results when compared to controls, it cannot be stated that this level of alcohol is safe (Mukherjee et al, 2005) because the damages might be concealed under certain tissues of the body and may be disclosed later in life and some forms of intellectual functions not tested in this research. For instances, prenatal alcohol consumption was found to affect the cell-cell adhesion during development (Charness, Safran and Perides, 1994; Charles, Kristin and Feng, 2005). Moreover, other teratogenic effects alcohol brings to the developing nervous system are neuroapoptosis within the cerebellum and brain stem (Dikranian et al, 2005); alcohol also alters the interaction of neuron and glia when taken chronically at a low dose (Evard et al, 2003). Maternal alcohol consumption is considered toxic even at a low dose (Evard et al, 2003), it is neurotoxic (Gohlke, Griffith and Faustman, 2005) and that it also interferes with the hormonal regulation of the development at endocrine level (Zhang, Sliwowska and Weinberg, 2005).

Furthermore, taurine given to pregnancy and lactation does not seem to protect the digression of maternal behaviors and the offspring from alcohol detrimental influences on the body growth during development. However, learning and memory processes of the affected offspring seem to be preserved and protected as observed from hippocampal dependent water maze learning when taurine was supplemented during pregnancy and lactation. It was found that both alcohol and taurine play a role in in the hippocampus; while alcohol offers a detrimental effect by interfering with short-term memory through reduction of hippocampal c-Fos expression (Clements et al, 2005) and alteration of hippocampal phospholipids profile (Wen and Kim, 2004), taurine provides a protective measure by induction of synaptic potentiation (Olmo et al, 2000; Olmo et al, 2003; Olmo et al, 2004). Nonetheless, taurine given to offspring themselves after weaning did not seem to safeguard the learning and memory mechanisms of the offspring as it was obvious that their task performance was not comparable to their pair-fed counterparts. Accordingly, taurine may be protective against alcohol damages only during times at which alcohol exerts its effects and during an early development of the nervous system (Sturman et al, 1985; Chapman and Greenwood, 1988; Gohlke, Griffith and Faustman, 2005). Once the damage has been done, taurine is not likely to provide any protection and the damages done could no be liberated.

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