Blood-brain barrier (BBB) is a unique feature of central nervous system (CNS) which isolates the brain from external environment and prevents an access of majority of circulating substances in the blood to the brain. This specialized barrier serves as an interface between circulating blood and brain interstitium and parenchyma, isolating the brain tissue from such blood constituents as ions, hormones and neurotransmitters. Thus the BBB maintains CNS homeostasis and prevents entry of substances that might alter neural function.

The BBB is composed of continuous layer of cerebrovascular endothelial cells connected by intercellular tight junctions. Another special feature of the BBB is a layer of astrocytic endfeet which represent an important part of BBB and secure brain involvement in mechanisms controlling access of external factors into the CNS. It has been suggested that astrocytic endfeet contribute to the structural integrity of the BBB. The restrictive features of the BBB capillaries are due primarily to a virtual absence of vascular pores, and to a lesser extent a discontinuous layer of phagocytic pericytes. All the areas of CNS do not contain a fully developed BBB. Selective non-BBB regions are present in the pitutary glands, median eminence, area postrema, pineal gland and most importantly in choroid plexus. BBB is considered to be a dynamic interface which has physiological functions for specific
and selective membrane transport of many compounds, as well as degradative enzyme activities. It has carrier-mediated transport mechanism for relatively small molecules, an absorptive-mediated endocytosis mechanism for positively charged peptides and a receptor-mediated endocytosis mechanism specific for certain other peptides. The development of unique properties of brain microvasculature is a consequence of tissue specific interactions between endothelial cells of extraneuronal origin and developing brain cells. There are three main phases of vascular development; vasculogenesis, angiogenesis and barriergenesis that lead to establishment of pattern and properties of the network and how they are coordinated with the developing nervous system. In vasculogenesis there is formation of the initial extraparenchymal plexuses, in angiogenesis the intraparenchyma sprouting occurs and barriergenesis is the final phase in which the special properties of the network and how they are coordinated with the developing nervous tissue develops. The functional maturation of BBB has been reported to develop in many respects from embryonic day (ED) 15 to the postnatal day (PND) 24 in case of rats. During the advanced stages of development of CNS microvasculature, cerebral endothelial cells have been shown to undergo a characteristic change from a non-barrier to barrier function following expression of alkaline phosphatase, butrylcholinesterases, transferrin receptors, stereospecific carriers for glucose, aminoacids, monovalent cations and other smaller molecules. Although the barrier function to large proteins e.g. albumin and macromolecular tracer horseradish peroxidase was found to be existing during embryonic stages, the complete functional maturation of smaller molecules and in other respect is achieved postnatally.

As the BBB represents a protective barrier and regulates the chemical environment of the brain, toxic injury to BBB may mediate direct neurotoxicity as well as disruption of specialized biochemical environment of the brain. There is a variety of pathological and experimental conditions in which barrier function is compromised leading to a loss of neuronal microenvironment regulation and consequently neuronal dysfunction. BBB dysfunction has been implicated in ischemia, infarction seizures, intracranial tumour, diabetes, hypertension, dementia and AIDS. A variety of toxins such as organic solvents, metals, polycations, herbicides, drugs are known to cause BBB damage, thereby causing CNS dysfunction. Some of these xenobiotics have been reported to alter cerebral glutathione levels
following their exposure which may be responsible for certain neurological dysfunctions. Glutathione (GSH), a non-protein thiol is known to have several important biological roles in a variety of cellular functions, including maintenance of membrane integrity and redox potential. It also plays a role in detoxification of reactive metabolites and reactive oxygen species generated in the cell. The presence of mental disturbances and neurological disorders have been reported in patients with inherited defects of GSH metabolism. Therefore, the present study was undertaken to investigate the possible role of GSH in the maintenance of BBB integrity and to evaluate the status of BBB in various pathological as well as under xenobiotic exposed conditions where cerebral GSH depletion is known to occur. Selected GSH depletors were used to achieve GSH depletion such as diethyl maleate (DEM), phorone, 2-cyclohexene-1-one (CHX) and acetaminophen so as to see the effect of GSH depletion on BBB permeability and its possible role in the maintenance of BBB integrity. Since developing individuals are more susceptible to the environmental and pathological challenges, developing BBB may also be the vulnerable target for xenobiotic exposure. We therefore studied the permeability function of BBB in GSH depleted rat fetuses and neonates. DEM and phorone decreased the brain and liver GSH levels in adult as well as in neonates of 7-, 14- and 21-days of postnatal ages. The levels of liver and placental GSH were depleted following exposure of DEM and phorone in fetuses at gestational day 18, but no significant change was observed in brain GSH levels and BBB permeability. A dose-dependent effect of GSH depletion on BBB permeability increase was observed in adult rats following DEM and phorone exposure. Brain GSH levels depleted to 24% to 52% and BBB permeability increased to 39% to 92% following exposure from 2 to 4 mmol/kg of DEM exposure. Phorone exposure from 2 to 3 mmol/kg resulted in decrease in brain GSH levels from 30% to 43% and increase in BBB permeability from 52% to 65%, whereas CHX exposure at the dose of 1 mmol/kg depleted the brain GSH levels by 62% and increased the BBB permeability to micromolecular tracer sodium fluorescein by 53% only. However, the increase in BBB permeability following GSH depletion was not as effective in neonates as in adults. The possible explanation for this may be that in neonates mechanism associated with GSH-dependent BBB integrity is not fully operative. In adults, effect of DEM was persistent even after 24 hr of exposure where as phorone-induced effects were normalized following 24 hr of exposure. DEM exposure at the dose of 4 mmol/kg repeatedly for 4 days also depleted the GSH levels in brain (47%)
and liver (48%), and increased the BBB permeability to sodium fluorescein (42%), significantly. DEM exposure did not have any effect on microvessel lipid peroxidation (LPO), whereas brain LPO increased marginally only at higher doses of DEM exposure. Acetaminophen, another GSH depletor decreased hepatic GSH levels, however, it did not affect the cerebral GSH levels as well as the BBB permeability. Thus, it shows that cerebral GSH is important for maintaining BBB integrity. Our studies also demonstrate that the increase in BBB permeability following GSH depletion was only for micromolecular tracers such as sodium fluorescein and 14C-sucrose and not for macromolecules namely horseradish peroxide (HRP) and Evans blue. Since GSH is known to play a crucial role in the maintenance of redox potential of the cell, studies were also extended to explore the protective effect of some antioxidants (e.g. α-tocopherol, ascorbic acid and turmeric) and sulfhydryl agents (e.g. methionine, N-acetyl cysteine and GSH) on DEM-induced BBB changes in adults. Antioxidants pretreatment did not have any protective effect on GSH levels and BBB permeability induced by DEM exposure. Sulfhydryl group containing agents namely methionine and N-acetyl cysteine treatment partially protected the increased BBB permeability resulted due to GSH depletion. Intravenously administered GSH in DEM treated rats did not recover the cerebral GSH levels, but was highly effective in recovering microvessels GSH levels and normalizing BBB permeability. This suggests that microvessel GSH plays a very crucial role in maintaining BBB integrity. Nitric oxide (NO), free radical as well as neuronal messenger molecule has a role in vasodilation so an attempt has also been made to see its involvement in BBB function. N-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor treatment did not protect the GSH levels as well as BBB permeability induced by DEM. Its rules out the possibility of involvement of NO in GSH depletion induced BBB disintegration. Thus cerebral GSH is important for the maintenance of BBB function and under various pathological and xenobiotic exposed conditions, depletion in GSH level may affect BBB integrity which may lead to CNS dysfunctions.

BBB may also be possible target of certain xenobiotic exposure, therefore we have conducted some studies on the effect of selected pesticides and heavy metals on BBB permeability during development as the developing CNS is more sensitive to toxicant induced damages due to presence of functionally and structurally immature BBB. Pesticides are potentially hazardous to nontargeted organisms leading
to a variety of pathological disorders including neurological disturbances and biochemical alterations. Our studies emphasize the risk of pesticides namely quinalphos (QP), cypermethrin (CP) and lindane (LD) belonging to structurally and functionally different groups of pesticides on BBB permeability to micromolecular tracer sodium fluorescein during development. The study was further extended to compare the pesticide-induced changes in developing versus adult animals and to evaluate the BBB permeability after the withdrawal of treatment in order to find out whether the changes are reversible and if so up to what extent. Quinalphos (QP), cypermethrin (CP), and lindane (LD) treatment at a dose of 1/50<sup>th</sup> of LD<sub>50</sub> significantly increased the BBB permeability to sodium fluorescein by 97%, 37% and 72% respectively, 2 hr after single exposure. This increase in BBB permeability was persistent even after 3 days of single exposure of QP and LD, but it was recovered fully in case of CP. When treatment was given daily from PND 10 to 30, the increase in permeability was found to be 43% at PND 13, 130% at PND 17 and 35% at PND 30 following QP exposure at the dose of 1/50<sup>th</sup> of LD<sub>50</sub>. In another study, the treatment was stopped following daily exposure from PND 10-17 and studies on the selected pesticides were conducted at different time intervals. The effects of QP exposure was fully recovered at PND 30 i.e. following withdrawal for 13 days (PND 18-30). CP exposure from PND 10 also significantly increased the permeability at PND 13 (71%), 17 (80%) and 30 (61%). The changes produced following CP exposure from PND 10-17 took 43 days of withdrawal (PND 18-60) before a complete recovery occurred. LD exposure from PND 10 affected the BBB permeability at PND 13 (35%) and 17 (50%), but no effect was observed at PND 30. LD induced changes at PND 17 also took 13 days of withdrawal (PND 18-30) for a complete recovery of BBB permeability. A further low dose of pesticide exposure (1/100<sup>th</sup> of LD<sub>50</sub>) from PND 10-17 also increased the BBB permeability to sodium fluorescein in case of QP and CP, but the magnitude of alterations were less as compared to 1/50<sup>th</sup> of LD<sub>50</sub> dose. LD did not affect the BBB permeability at this low dose. Single exposure of QP, CP and LD also increased the permeability of PND 15 at a dose of 1/50<sup>th</sup> of LD<sub>50</sub> by 48%, 15% and 20% 2 hr after exposure but the magnitude of the effects was less pronounced as compared to their effect in 10-day-old rats at the same dose. When adult rats were exposed to these pesticides (1/25<sup>th</sup> of LD<sub>50</sub>) alone or in combination with cytochrome P-450 inhibitor, piperonyl butoxide (PB) which enhances the toxic effect of pesticides, for 3 consecutive days no effect on BBB permeability to sodium fluorescein was observed. This shows
that well developed BBB is quite resistant to pesticide exposure. Thus, a low-level of pesticide exposure during critical period of development may interfere with the normal development and maturation of this barrier, that may lead to neurological dysfunctions immediately and at later life.

Heavy metal exposure to humans and a variety of animal species is of great concern due to their potential to produce multiorgan toxicity. Metals exposure is known to cause neurological and mental deficits in humans. It is of considerable concern that some of these effects produced during early period may persist even in adulthood. Our studies deal with the effect of heavy metal exposure [e.g. aluminium (Al), lead (Pb), cadmium (Cd) and triethyltin bromide (TET bromide)] on the functional integrity of BBB during development. The data revealed that Al exposure significantly increased the BBB permeability to sodium fluorescein at PND 30 when exposed daily from PND 5 at a dose of 10 mg Al/kg/d (39%) and 30 mg Al/kg/d (31%) as Al lactate. However, neonates exposed to Al from PND 10-17 did not show any effect on the BBB permeability. Our studies show that exposure of Pb as Pb acetate was more effective when given through intraperitoneal (i.p.) route as compared to oral intubation. Pb significantly increased the BBB permeability (62%) at 10 mg/Kg/d at PND 30 when exposed through i.p. route from PND 7-21. Whereas, the same dose of Pb exposure through oral intubation did not affect the BBB permeability. Cd exposure as Cd chloride at a dose of 10 ppm Cd, in drinking water through out gestational, lactational and postnatal period did not affect the BBB integrity to sodium fluorescein at PND 30. Another metal TET as TET bromide also did not affect the BBB integrity in adult mice following 10 and 20 ppm exposure through drinking water for 20 days. These studies emphasize the risk to metal exposure during development. Functional impairment of BBB due to metal exposure during early life may be associated with CNS damage.

Free radical mediated neurotoxicity is one of possible mechanisms of the xenobiotic exposure. Free radicals may be involved in the pathophysiology of tissue injury and also in some diseases including neurodegenerative ones. It has become apparent that many drugs, chemicals and physical induced toxicities may be evoked in the developing nervous system by the way of oxidative stress. Because the developing brain is sensitive to oxidative stress and lacks a functional BBB, expression of antioxidant defense system is important in cerebral microvessels. Therefore, we have studied the developmental profile of important enzymatic and
nonenzymatic antioxidants like superoxide dismutase (SOD), catalase, glutathione peroxide (GPx), reduced glutathione (GSH) as well as generation of reactive oxygen species namely hydrogen peroxide ($H_2O_2$) and superoxide anion in the cerebral microvessels of rat pups at 7-, 14- and 21-days of postnatal age and also adult rats. The activities of marker enzymes of brain microvessels namely $\gamma$-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) was also monitored in these animals of different age groups. The data obtained from this study showed a marked increase in superoxide anion (67%) and $H_2O_2$ production (200%) at PND 21. Superoxide anion production was significantly lower at PND 14 (24%) as compared to adults whereas at PND 7 it was almost comparable to adult values. However, $H_2O_2$ production at PND 7 and PND 14 was found to be almost comparable to its rate in adult microvessels. Lipid peroxidation (LPO) which was measured as thiobarbituric acid reactive substances (TBARS) was found to be significantly lower (18%) at PND 21 as compared to other time intervals. The level of antioxidant substance GSH was maximum at PND 7 in brain microvessels which declined subsequently. The activity of SOD increased with development up to PND 21. In adult brain, microvessels SOD activity was almost comparable to its level at PND 21. Catalase activity was found to decrease with development. Se-dependent GPx activity in brain microvessels increased with age up to PND 21 showing a marked increase from PND 14 to 21, however, in adults it declined. The activity of Se-independent GPx was found to be very low in cerebral microvessels. The developmental profile of GR activity showed considerable variation. The activity of marker enzyme, GGT increased with age, whereas, ALP showed a slight increase up to PND 14 and thereafter it declined. Thus developing brain microvessels contain high level of antioxidant enzymes which should be capable to overcome the oxidative stress produced due to xenobiotic exposure or under various pathological conditions.

Experimental finding of this dissertation implicate that developing BBB is highly vulnerable to xenobiotic exposure. The increase in BBB permeability after cerebral GSH depletion led to conclusion that GSH is important for the maintenance of BBB integrity. Our studies also implicate that sulfhydryl agents partially protect the increased BBB permeability due to GSH depletion. These studies should be helpful for those pathological and xenobiotic exposed conditions where cerebral GSH depletion is known to occur. Increased BBB permeability following pesticide exposure had led conclusion that a low-level pesticide exposure during critical
period of development may cause adverse neurological effect at later life. The observed persistent effect of these pesticides especially in case of CP further emphasizes the possible long term effect of such exposure at an early life. The adult animals were quite resistant to these pesticide exposure. The present study clearly demonstrated the risk of metal exposure during early life which may cause serious implications in adulthood. Developing brain microvessels contain high level of antioxidant substances and appears to capable to overcome the toxic challenges due to free radical injury or xenobiotic exposures.

It may therefore be concluded that cerebral GSH plays an important role in the maintenance of BBB function. The functional integrity of BBB may also be disturbed under xenobiotic exposure especially in neonates and growing animals which has potential to impair the proper development and maturation of brain. Pesticide induced dysfunction may persist for longer duration of time suggesting that an early exposure may produce some serious implications in later life.

The main conclusions drawn from the present investigation are as follows:

1. It has been demonstrated that GSH plays a major role in maintaining the functional integrity of the BBB. Thus it seems possible that under various pathological and xenobiotic exposed conditions where cerebral GSH depletion is known to occur BBB function is likely to be disturbed. It may be one of the possible mechanisms responsible for the altered CNS functions.

2. Xenobiotics exposure (pesticides and heavy metals) at an early life have potential to cause nervous system dysfunction by adversely affecting the BBB integrity. Pesticide-induced alterations in the functional integrity of BBB had persistent effects after single and repeated exposures especially in case of cypermethrin in which case changes in BBB permeability took longer period for complete recovery.

3. Developing brain microvessels contain high level of antioxidant substances and thus appears to overcome the toxic challenges produced by oxidative stress through removal of free radicals that are produced by xenobiotic exposure and pathological challenges.

Therefore, blood-brain barrier may be the possible target in certain pathological and xenobiotic exposed conditions for central nervous system dysfunction.
The blood-brain barrier constitutes a highly specialized feature of the central nervous system. The specialization is manifested both by morphologically and functionally. The BBB function is compromised under various pathological and diseased conditions, namely, hypertension, inflammation, brain edema, tumour, hypoxia, Alzheimer's disease, etc. The biochemical constituents of the endothelial cells e.g. proteins, lipids, receptors, transporters, antioxidant enzymes, etc. play an important role in maintaining the integrity of this barrier. An alteration in any of these important biomolecules may cause BBB aberration with damaging consequences. Among these cellular constituents, glutathione (GSH) is the most abundant non-protein thiol in the cell and is known to have several physiological roles in a variety of cell functions, including maintenance of membrane integrity, redox potential, aminoacid transport and detoxification reactions. To investigate whether GSH has any role to play in the maintenance of BBB function, studies were carried out to find out the possible role of GSH in BBB function. Our studies have shown that depletion of cerebral GSH by various depleting agents namely diethyl maleate, phorone and 2-cyclohexene-1-one resulted in the increased BBB permeability to micromolecular tracer sodium fluorescein. BBB permeability to another micromolecular tracer namely $^{14}$C-sucrose was also found to be increased following GSH depletion with DEM, whereas BBB permeability to macromolecular
tracer namely Evans blue and HRP was unaltered under depleted conditions. The studies were extended with acetaminophen, a known GSH depleting drug which does not alter the cerebral GSH because of inability to cross the BBB effectively. Our studies have shown that BBB permeability was not altered with acetaminophen, depicting that the cerebral GSH levels help in maintaining the functional integrity of the BBB. Studies of BBB permeability of neonates at the 7-, 14- and 21-days of age show that increase in BBB permeability following GSH depletion was not as effective in neonates as in adults which could probably be due to the absence of a fully operative mechanism associated with GSH in the maintenance of BBB integrity at an early age. Attempts were made to recover the changes occurred due to GSH depletion by pretreating the animals with a variety of antioxidants namely \( \alpha \) -tocopherol, ascorbic acid and a natural product tumeric. Pretreatment of these antioxidants could not recover either the depleted cerebral GSH levels or the increased BBB permeability. However, sulphhydryl agents namely, methionine and N-acetyl cysteine partially recovered the increased BBB permeability due to GSH depletion, whereas, intravenously administered GSH almost fully recovered DEM-induced increased BBB permeability and microvessel GSH. Thus, these studies indicate that GSH has an important role to play in the maintenance of functional integrity of blood-brain barrier.

Another important aspect studied was the development of the BBB. Since the development of the BBB starts during gestation and continues up to early period of postnatal life. The developmental period is very crucial in the sense that if any anomaly takes place during this time owing to one or the other reasons, could have deleterious effects in later life. The development of BBB is a complex process which occurs under three main phases i.e. vasculogenesis, angiogenesis and barrierogenesis. These three phases appears to be well coordinated with organogenesis and histogenesis of tissues of the CNS. Brain is reported to be vulnerable to agents that interfere with any of these processes involved in its development. Since, the BBB of neonates is structurally and functionally immature, therefore a xenobiotic challenge at this critical period of life may be highly injurious. Studies undertaken at this dissertation provides valuable information about the consequences of the xenobiotic exposure in the developing BBB. In addition, our studies also demonstrate the ontogenic profile of antioxidative defense mechanisms in cerebral microvessels during developmental stages in order to investigate whether the
developing microvessels are equipped with adequate antioxidative defenses and are able to cope with the oxidative challenges. It is clearly demonstrated from our studies that an exposure of ubiquitous environmental pollutant, namely pesticides and metals, at an early life have adverse effects on the developing BBB. Our studies with different pesticides namely quinalphos, cypermethrin and lindane show that the developing BBB is highly vulnerable following single and repeated exposure of these pesticides. Pesticide-induced alterations on the functional integrity of the BBB had persistent effects following exposure specially in the case of cypermethrin which took a longer period for complete recovery. Present investigation also emphasize the risk of certain metal exposure namely, lead, aluminium and cadmium during early life because developing BBB is sensitive to metal exposure which can cause toxic injury to CNS. Thus toxicants produce BBB dysfunction to a significant extent that may lead to permanent neurological impairment. Furthermore, studies regarding the development of enzymatic and non-enzymatic oxidants as well as the production of reactive oxygen species were also conducted. The data of our studies suggest that antioxidative defense machinery is well developed in brain microvessels at an early developmental period and therefore it seems that microvessels are equipped to cope up with the oxidant-mediated toxicity during early life.

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