Chapter-1

Review of Literature

Neural transplantation: an overview

Lead and its associated toxicity

Iron-deficiency

Iron-deficiency & lead exposure
Neural Transplantation: An overview:

Recovery from neural injury in the central nervous system of adult mammals is typically limited. Under ordinary circumstances few neurons replicate the events of normal development and cell division does not replace lost neurons. Thus, once some areas of the brain get damaged as a result of exposure to drugs, environmental toxin or with age, neural degeneration sets in and functional deficits follow. Neural circuitry is of particular interest because of its critical role in higher cognitive functions such as learning and memory and its vulnerability to degeneration by various diseases and to a lesser extent during the course of ageing. Neural transplantation in mammals is just about 100 years old. In 1890, Thompson published his article on successful brain grafting, which he ended with the often-quoted sentence:

"I think the main fact of this experiment - namely, that brain tissue has sufficient vitality to survive for 7 weeks the operation of transplantation without wholly losing its identity as brain substance suggests an interesting field for further research, and I have no doubt that other experimenters will be awarded by investigating it."

[Gilman Thompson, 1890]
The issue of the vitality of the grafted neural tissue, which was Thompson's principal interest was pursued by others, but initially with very mixed results and little impact. Ranson (1903) and Saltykow (1905) took neural tissue from the cerebral cortex of rats and transplanted it to the neocortex of adult animals. The transplant survived but could not integrate well with the host brain. It were Del Conte (1907) and Altobell (1914) who for the first time attempted to graft embryonic neural tissue into adult host animals but long term survival of the graft was not achieved.

Partial survival of the brain tissue transplants in rats was first reported by Dunn (1917) followed by Tidd (1932) and Le Gros Clark (1940, 1942, 1943). Glees (1955) transplanted different parts of embryonic brain and spinal cord and showed that transplants survived and exhibited growth characteristics similar in normal cortex.

Neural transplantation research then went through a quiescent interval of 15 to 20 years after which renewed interest and real enthusiasm in its applicability was stimulated, to study the embryogenesis of the central nervous system, during the seventies. The present era of neural transplantation research started with a series of studies published by Gopal Das and co-workers and followed by Andres Bjorklund and co-workers. The two groups working independently gave a comprehensive analysis of newer and better techniques, their applicability and significance in evaluating growth potential and functional viability of the neural grafts. Das and Altman (1971) made encouraging observations on the viability of grafted neural tissue when they transplanted [3H]Thymidine-labelled neuroblasts into neonatal brain. Bjorklund and Stenevi (1971) reported stimulation of CNS regeneration when they observed growth of central catecholaminergic neurons into iris neurons. Several studies were conducted to study the integration of the graft into the host and the establishment of host-graft connections. Lund and Hauschka, (1976) observed afferent connection to fetal CNS transplants in neonatal brain, while Bjorklund et al (1976) observed efferent connections from fetal CNS transplants in adult brain.
The transplantation strategy as it is applied to experimental and clinical Parkinsonism owes much to neuropharmacology. It remains a remarkable fact that destruction or pharmacological blockade of a single set of neurons in the brain, i.e. the dopamine system (which in rat comprises a total of only 40,000 cells) will render the animal unable to express normal motor behaviour. Perlow et al (1979) and Bjorklund and Stenevi (1979) using an experimental model of Parkinsonism (rats that had earlier received unilateral injections of 6-hydroxydopamine to destroy the dopaminergic neurons of the substantia nigra) were able to demonstrate the effectiveness of fetal dopaminergic neurons in ameliorating motor deficits. Olson et al (1979, 1980, 1985) investigated the long term behavioural pattern of neurons and reported the matura­tion of transplants of substantia nigra neurons and synthesis of dopamine. Analysis of these studies showed that the transplanted tissue grew, differentiated, formed fibre connections and was metabolically active. Later, animal models of various neurodegenerative disorders were used to properly demonstrate the clinical potential of fetal neural transplantation. Freed et al (1984) in a Parkinson-like model in rats showed that the asymmetry of movement was alleviated by striatal grafts proving that dopamine lost on destroying the pathway was restored. Fine et al (1985) demonstrated that hippocampal and ventrobasal forebrain grafts in Alzheimer-like rat model, not only improved signs but also showed cholinergic fibres growing into the respective regions depleted of these fibres. Bakay et al (1987) treated a primate Parkinson model induced by MPTP (1-methyl,4-phenyl, 1-2-5-6 tetrahydropyridine) with fetal substantia nigra and observed dopamine containing cells and fibres within the striatum. Banckiewicz et al (1988) argued that brain transplants do not need to contain dopamine to induce functional recovery in MPTP-induced Parkinsonian primate. Implant-induced and trophic factor mediated dopaminergic sprouting by the host brain plays a role in the behavioural recovery and may well be responsible for the clinical improvement seen in Parkinsonian patients after brain implants.

Over the past two decades, advances have been made in developing new techniques for replacing neuronal populations within damaged brain. Progress has
also been made in achieving better graft survival by the use of growth factors. Escobar et al (1993, 1994) reported accelerated behaviour recovery by nerve growth factor which was given simultaneously with cortical grafts in a rat model that showed inability to learn a conditioned taste aversion response due to insular cortex lesions. Russel et al (1994) observed that NGF stabilised acetylcholine turnover and therefore, helped to accelerate the behavioural recovery in insular cortical lesioned rats. Sprick and Sprick (1991), however, used, carbachol a cholinergic agonist to enhance graft function. This approval did not attract much attention since carbachol had transient effect due to its inability to cross the blood brain barrier. Based on the observation of Yoshida and Gage (1991) that basic fibroblast growth factor stimulates nerve growth factor synthesis and secretion, Mayer et al (1993a and 1993b) and Steinbusch et al (1990) used basic fibroblast growth factor to promote the survival of embryonic ventral mesencephalic dopaminergic neurons implanted in denervated rat caudate putamen. Although infusions of growth factors to implanted fetal neurons appeared to be more effective than supplementing cells with growth factors prior to grafting, the use of intracerebral cannulation evoked significant inflammatory response that reduced graft survival. This problem necessitated the use of modern molecular biological techniques to deliver growth factors in situ.

Recently, interest has been focussed on the application of gene therapy to the central nervous system, which involves the genetic modification of cells in vitro and subsequent implantation into the brain. The combination of these two techniques offers exciting avenues to replace chemical substances within the damaged brain. Groves et al (1993) reported repair of demyelinated lesions by transplantation of a purified O-2A progenitor cells that expressed a bacterial β-galactosidase gene which gives rise to β-galactosidase-positive oligodendrocytes which inturn help to remyelinate demyelinated axons within the lesion. Hoffman et al (1993) transplanted a polymer encapsulated cell line genetically engineered to release nerve growth factor and observed that NGF released as a result prevented lesion-induced loss of septal cholinergic neurons. Camarate et al (1992) used biodegradable polymer micro-
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spheres to obtain sustained release of NGF *in vivo*. Aebischar *et al* (1991) used a polymer encapsulated PC12 cell line genetically engineered to release dopamine to treat an experimental rat model of Parkinson’s disease. Takayama *et al* (1995) pursued a strategy of supplying basic fibroblast growth factor (bFGF) to grafted dopamine neurons by using cells genetically modified to produce bFGF as a localized source of the growth factor *in vivo*. Primary skin fibroblasts were genetically modified to release bFGF, mixed with fetal dopamine neurons, and implanted into rats with unilateral lesion of dopamine neurons within the substantia nigra. Results from this study indicated that such co-grafting improved the survival, growth and functional efficacy of transplanted dopaminergic neurons in rats with experimental Parkinson’s disease and offered a useful strategy for enhancing the clinical effectiveness of grafts in parkinsonian patients.

Silver *et al* (1996) isolated suprachiasmatic nucleus within a semipermeable polymeric capsule before transplantation, thereby preventing neural out growth but allowing diffusion of humoral signals. This approach helped to demonstrate that a diffusible signal is sufficient to restore rhythmicity in animals with altered circadian rhythms caused by ablation of the suprachiasmatic nucleus. Nikkah *et al* (1993) used a multiple microtransplantation technique to obtain enhanced functional efficacy of the transplanted neurons. Nikkah distributed the transplants over the entire damaged site in the brain to achieve a better repair of the damaged neural circuitry.

The use of fetal neural transplantation in animals with toxin induced neuronal degeneration has also received some attention although to a very limited extent. Arendt *et al* (1989) observed that cholinergic rich transplants were able to effectively ameliorate impaired memory function due to chronic ethanol treatment. Hodges *et al* (1991a) confirmed the findings of Arendt *et al* (1989) and reported lesions of the cholinergic basal forebrain projection system as a reason for the altered cognitive performance in rats chronically exposed to ethanol, and observed that only cholinergic rich transplants could reverse this altered behaviour and that other transmitter systems failed to do so.

The long term survival of neural grafts has received very little attention. Most of the investigation have not extended their studies beyond 3-4 months after implantation. Tandon (1992) and Gopinath *et al* (1996) conducted long term studies on the morphological behaviour of striatal transplants. A progressive loss of transplanted neurons by premature ageing was observed but survival of normal neurons within the transplant could be found at the end of two years. Previous studies of Gopinath *et al* (1991) showed cell death and rejection of transplants at 6-12 months post transplantation as was evident from vascular changes, presence of lipofusin in the neuronal cytoplasm and accumulation of lymphocytes around the transplants.

The immunological aspects related to neuronal transplantation have been thoroughly investigated (Sloan, *et al*, 1991). The concept that the CNS represents an immunologically privileged site has evolved since the early days of tissue transplantation to the brain (Barker and Billingham, 1977). The fact that this privilege is not absolute has been pointed out in several reports (Nicholas *et al* 1987; Gill and Lund, 1989; Geyer *et al*, 1985; Borlongan *et al*, 1996; Lopenlozano *et al*, 1997). Following a detailed review of the available information Rao *et al* (1988) concluded that "the grafts exist in a metastable immune balance, that can be disrupted by a variety of factors. Each one on its own may not be sufficient to provoke a rejection response, but once a critical threshold is reached a cascade of events may occur which precipitate
a classical immune response. Sloan et al (1991) reviewing the available information concluded that the neural tissue in itself is immunogenic and that failure of neural allo and xenografts to survive is due to immunological processes.

There seems little doubt that neural transplantation offers a real possibility for replacement or promoting regeneration of damaged or diseased adult central nervous system. Its utility in restoration or preservation of function has been amply demonstrated by a large number of studies, involving a variety of animal species and in several experimental models of human disease (Sladek, 1996). Clinical trials have provided enough evidence that at least for patients of Parkinson’s disease, there can be mild to moderate improvement in motor function (Olanow et al, 1996; Shults, 1992). Nevertheless, it is also obvious that translation of findings of animal experiments to human beings is not without its limitations and pit falls.

Lead and its associated toxicity

Lead (Pb) (atomic number 82; relative atomic mass, 207.19; specific gravity 11.34) is a bluish or silvery grey soft metal. It is relatively abundant in the earths crust and is found naturally throughout the world. The major natural sources of lead are volcanic emissions, geochemical weathering, and emissions from sea spray. It has been estimated that the world wide natural emission rates of lead are of the order of 19,000 tonnes/year (Nriagu and Pacyna, 1988). The average concentration of lead in the earths crust is between 10 and 20 mg/kg, in surface water it is about 0.012 µg/l and in air its concentrations are in the range of 0.01 to 0.1 µg/m³ (IPCS, 1995).

Occurrence

Lead occurs in a variety of minerals, the most important of which are galena (PbS), Cerrusite (PbCO₃) and anglesite (PbSO₄). Galena is by far the most abundant source of primary lead. Its physical and chemical properties have made it extremely useful industrially. Its principal use has been in the manufacturing of storage batteries, as a gasoline additive, in the preparation of paints, pigments and coloured inks. Other
diverse uses of lead include solders, ammunition, foil on wine bottles, cosmetics and folk medicines.

**Environmental contamination**

Environmental contamination by lead occurs chiefly as a result of anthropogenic activities. The biggest source of lead in the air is from automobile exhausts. Other sources of release into the air include emission from iron and steel production, smelting operations, mining, and lead-acid battery manufacturing factories. Municipal waste incinerators, sewage sludge, industrial waste water discharges all may contain lead at concentrations as high as 50 g/kg. Dusting and flaking of lead paint from surfaces can be a source of lead contamination in surface dust and soil near houses. Removal of lead-based paints from bridges and water towers using improper techniques can result in significant environmental contamination. Direct application of lead-contaminated sludge as fertilizers and residues of lead arsenate from use in agriculture can lead to the contamination of soil, surface water and ground water. In local aquatic environments, pollution can result from industrial discharges, atmospheric fall out, shot gun cartridges and sewage effluents.

**Human exposure**

Human beings can be exposed to lead and its compounds from breathing air, drinking water and eating food contaminated with Pb. Various sources of Pb exposure among human beings are shown in Fig. 1. In addition, exposure of certain groups within the general population may vary because of physiological, behavioural or other factors. For example, the fetus is exposed to lead via maternal transfer of internal or external doses (Barltrop, 1969), nursing infants may be exposed to lead in breast milk, the young child is exposed more intensively to dusts and lead through non food items. Alcohol consumption (Flora and Tandon, 1987) and cigarette smoking (Mussalo-Rauhamaa et al, 1986) increase lead exposure. Differences in diet (Sobel et al, 1940) may influence lead exposure markedly and some people may be exposed to lead
Fig. 1: Various pathways of human exposure to lead (IPCS, 1995).
through hobby or occupational activities in addition to their exposure as members of
the general population (IPCS, 1995).

Lead absorption

The absorption of lead from environmental sources is not dependent solely on
the amount of lead presented to the portals of entry. It is dependent on the physical
and chemical state in which the metal is presented and is influenced by host factors
such as age, physiological status, nutritional condition and possibly genetic factors.
Men engaged in heavy work breathe more air and eat more food than sedentary
individuals of the same weight.

Absorption after inhalation

Absorption of lead from air to blood involves two processes: the deposition of
air borne lead particles in the respiratory tract; and absorption and clearance from
the respiratory tract into the circulation. After deposition in the lungs, lead is almost
completely absorbed (Morrow et al, 1980). Larger particles settle in the nose,
pharynx, trachea or bronchii and are transported to the oesophagus from where they
enter the digestive tract. Young children inhale a proportionally higher daily air
volume per unit measure than do adults (Barltrop, 1972).

Absorption after ingestion

In case of older children and adults without occupational exposure, lead ab-
sorbed by the gastrointestinal tract comes from the intake of lead in foods, beverages
and soil/dust. Rate of absorption in adults is 10 to 15% of the ingested quantity
(Rabinowitz et al, 1980; Gross, 1981). In preschool children, there is concern over the
intake of both food and non food items (e.g. toys, soil, paint flakes). Young children
may take in lead from non food items, via normal mouthing activity, which in the
extreme is the behavioural trait pica, which refers to the ingestion of such materials
as soil, ash, paint chips and plaster. Lead absorption increases under fasting conditions
(Rabinowitz et al, 1980; Heard and Chamberlain, 1982) and can increase up to 60%
or more. Nutritional factors play an important role in the amount of lead absorbed (Sobel et al., 1940; Levander, 1979).

Dermal absorption

Inorganic lead compounds being lipophilic do not normally penetrate the skin but are able to pass more easily by way of hair follicles and sebaceous glands. Small quantities of inorganic lead in cosmetics can slowly be taken up by means of vesicles present in the skin. Moore et al., (1980a) in one study showed that the human skin absorbed an average of about 0.06% of the applied quantity.

Transplacental transfer of lead

Lead is readily transferred from the mother to the developing infant during pregnancy and accumulates in bone during gestation (Barltrop, 1969). Fetal Pb uptake is cumulative until birth (Rabinowitz and Needleman, 1982).

Distribution of lead

Rather than being distributed homogenously throughout the body, Pb is dispersed among several physiologically distinct compartments (Landrigan et al., 1985). Rabinowitz et al. (1976) proposed a three compartment model based on tracer and balance data from five healthy men, indicating relative proportioning of Pb between blood, soft tissues and bone (Fig. 2). The figure shows lead content and mean half life of Pb in each pool and the rate of Pb movement between pools. The blood compartment shows the shortest half life of 36 days, followed by soft tissue of 40 days and the bone compartment of 27 years. Bone contains most of the body burden of lead.

Once absorbed inorganic lead is distributed essentially in the same manner regardless of the route of exposure (Hammond, 1982). About 99% of blood lead is associated with erythrocytes (Everson and Patterson, 1980). In adults about 95% of the total body burden of lead is found in bone. In contrast in children bone lead accounts for about 73% of the body burden (Barry, 1975, 1981). This large pool in
Fig. 2: Relative proportioning of lead between blood, soft tissues and bone ($\lambda =$ half life, $\sim =$ approximately).
bone is possibly responsible for persistent blood Pb levels long after the exposure (O’Flaherty et al, 1982).

Metabolism of lead

Inorganic lead in the body is not known to be metabolised or biotransformed per se. It is absorbed, distributed, retained and slowly excreted (Hammond, 1982). Alkyl lead compounds in contrast are actively metabolised in the liver by oxidative dealkylation which is catalysed by cytochrome P450 (Jenson, 1984).

Retention of lead

The retention rate of lead is relatively high in comparison to other toxic metals since it accumulates in bone and teeth. Lead in bone accumulates with age until about 60 years of age, when changes in diet and/or mineral homeostasis lead to a net negative balance. However, after years of active accumulation, very little of the total body Pb is lost.

Excretion of lead

Lead is eliminated from the body in both urine and faeces. About 90% of the ingested lead is eliminated unabsorbed through faeces. The rate at which the body eliminates Pb depends on age, with young children excreting less than adults because of greater net movement of Pb to bone (Ziegler et al, 1978). Chamberlain (1983) analysed a number of clinical and epidemiological studies and produced an aggregate analysis showing that with the increase in Pb intake the excretion rate also increases.

Lead toxicity

Lead has long been recognized as a poison, from ancient times to the present (Oliver, 1914; Cantarrow and Trumper, 1944). In the second century BC, Dioscorides observed that "lead makes the mind give way" (Major, 1931). Benjamin Franklin documented colic and neurological signs (wrist drop) in typesetters and painters (Franklin, 1918). Hippocrates (Fifth to sixth century BC) described a case of severe
colic in a worker who extracted metals. His report is one of the first accounts of those symptoms that can definitely be attributed to lead poisoning (Castellino, 1995). The effect of Pb on the nervous system has been known since the days of the Roman empire. The Romans preserved their fruits and vegetables with Pb salts, cooked their food in leaded pots, added Pb to their wines. Their water was delivered in Pb pipes. Leaded cosmetics and medicaments were common and quite popular. Several literary references indicate that members of Roman aristocracy were over exposed to Pb and that caused reproductive failure and high incidence of sterility, still births and well known mental incompetence of the progeny of the aristocrates. As long ago as 1824, Henderson suggested that lead had played an important role in the decline of the Roman Empire. This question re-emerged with even greater force in 1960's. Numerous authors such as Gilfillan (1965), Nriagu (1983a,b) and Bisel (1983) were convinced that lead had serious adverse effects on the ruling class, resulting in "blindness, insanity and sterility".

Lead poisoning in children was recognised before the turn of this century by Australian physicians (Turner, 1897; Gibson, 1892), who recognised the source of this frank poisoning as lead based paints (Gibson, 1904). American physicians began reporting cases of lead poisoning in children early in the 20th century; by the mid 1920s lead based paint was recognised as a serious source of illness in children (Lin-Fu, 1985, 1972; Robin, 1989). Lead based paint was banned in Australia in 1920, remained in paint in the United States for another half century and continues to remain unabated in India.

Symptoms of lead exposure

Lead produces both acute and chronic toxicity. The hemopoietic, gastrointestinal, renal and nervous system are mainly involved in lead toxicity. Toxic manifestations of Pb intoxication are anemia, acute abdominal colic, acute and chronic encephalopathy and peripheral neuropathy (Gerber et al, 1980). Anemia associated with acute lead poisoning is so characteristic that hemoglobin levels have often been
used to monitor occupational Pb exposure (Kazantzis, 1989). Symptoms associated with exposure to Pb have been briefly summarized in Table 1.

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<th>Table 1</th>
<th>Symptoms associated with exposure to Lead</th>
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| 1. Acute lead poisoning (Rare) | a) Intestinal colic  
   b) vomiting  
   c) salivation  
   d) sweet metallic taste |
| 2. Chronic lead poisoning (Plumbism) | a) Hypochronic normocytic anemia  
   b) Basophilic stippling |
| 3. Neurological (lead encephalopathy) | a) Nausea,  
   b) Vomiting  
   c) Restlessness  
   d) Irritability  
   e) Convulsions |
| 4. Gastrointestinal (Lead colic) | a) Anorexia  
   b) Constipation  
   c) Metallic taste |
| 5. Neuromuscular (lead palsy) | a) Wrist drop  
   b) Foot drop  
   c) Fatigue  
   d) Muscular weakening |
| 6. Renal | a) Fanconi-like syndrome (reversible)  
   b) Chronic nephritis (irreversible) |

**Effects of lead on the haematopoietic system**

The principal clinical manifestation of the effect of lead on the haematopoietic system is anaemia but this occurs only with high levels of exposure that are rarely seen today. Lead interferes at several steps in the biosynthesis of haem. It inhibits enzymes such as δ-aminolevulinic acid dehydratase, ferrochelatase, coproporphyrinogen oxidase and increases the activity of aminolevulinic acid synthase as a consequence of feed-back regulation by haem (Moore et al., 1980b). Lead affects erythrocyte formation by impairment of globin and haem synthesis. Globin synthesis is inhibited by lead in rat bone marrow cells at concentrations as low as 1 μM/l (Dresner et al., 1982). Lead
also decreases erythrocyte survival through its inhibition of membrane bound \( \text{Na}^+ ,\text{K}^+ \)-ATPase (Ragavan \textit{et al}, 1981).

Inhibition of \( \delta \)-aminolevulinic acid dehydratase leads to accumulation of \( \delta \)-aminolevulinic acid in the blood and its excretion in the urine. Lead has also been reported to inhibit significantly the activity of the enzyme ferrochelatase, thus interfering with the incorporation of iron into protoporphyrin molecule. Consequently, iron is replaced by zinc and increased levels of zinc protoporphyrin occur in the blood and free erythrocyte protoporphyrin is excreted in the urine (Torra \textit{et al}, 1989).

Nephrotoxicity

Prolonged exposure to lead adversely affects the kidneys. Occupational exposure to Pb has been known to cause disturbed renal function (Lilis \textit{et al}, 1968). Chronic renal failure has also been reported in adults exposed to high doses of Pb in childhood (Henderson, 1958). A characteristic cellular reaction in the kidney after Pb exposure is the formation of intra nuclear inclusion bodies in the proximal tubule cells (Goyer and Rhyne, 1973). These were first described by Blackman (1936) in children dying from acute encephalopathy.

Renal effects of Pb have been divided into three stages in experimental animals (Goyer, 1989). Stage I nephrotoxicity is manifested clinically by a decrease in energy-dependent transport functions, including aminoaciduria and glucosuria (Chisholm, 1962; Goyer, 1971). Stage II or chronic irreversible nephropathy occurs slowly over months or years and usually with continuous Pb exposure. It is characterized by gradual tubular atrophy, interstitial fibrosis and impaired glomerular filtration (Cramer \textit{et al}., 1974; Wedeen \textit{et al}., 1975). These conditions can eventually lead to renal failure (Goyer, 1971). Stage III of renal toxicity by Pb is characterized by renal tubular neoplasia or adenocarcinoma (Van Esch, 1962; Dobryszynck \textit{et al}, 1984).

Immunological effects

The effects of lead on the immune system are diverse but not well documented. Lead reduces resistance and increase mortality of experimental animals when they are infected by a broad range of bacteria and viral agents (Koller, 1984). Lead impairs
antibody production in animals and generally decreases immunoglobulin plaque-forming cells (Koller and Roan, 1980).

Mutagenicity

Lead is thought to have genotoxic properties. However, lead-induced gene mutations in cultures of mammalian cells have only been observed at concentrations toxic to the cells. Studies on the production of chromosomal aberrations, sister chromatid exchanges and micronuclei by lead, whether in *in vitro* or *in vivo* conditions, have given mixed results.

Carcinogenicity

Several studies carried out in rats and mice have reported formation of tumors of the kidney during long term administration of lead (Van Esch and Kroes, 1969; Moore and Meredith, 1979). Cystic hyperplasia, late morphological manifestation of chronic lead nephropathy, is a risk factor for renal cancer (Bernstein *et al.*, 1987). Prior to adenoma formation in animals treated with renal carcinogens, cystic hyperplasia was reported (Dees *et al.*, 1980; Goyer *et al.*, 1981).

Neurological effects of lead

Gross toxic effects of lead on the nervous system were reported by ancient Greek physicians. Kazantzis (1989) gave a summary of such reports and those of Roman physicians. The syndrome known as "painters colic", which included abdominal pain, constipation and paralysis is now known as "lead encephalopathy".

Animal studies

Experimental studies using animal models have demonstrated that lead impairs learning and memory function at virtually all stages of the life cycle. Bushnell and Levin (1983) observed impaired learning ability, when rats exposed to lead were tested for their ability to choose between alternate arms of a radial arm maze. Cory-Slechta *et al.* (1985) demonstrated significant learning impairment in rats given
lead acetate in drinking water (25 mg/l) from weaning. More recently Cohn et al (1993) demonstrated selective effects of lead on learning processes, as distinct from non specific effects such as motivational levels, and sensory or motor impairment using a multiple schedule of repeated learning and performance.

Winneke et al (1977) observed that rats that had been exposed to lead while in utero, through mothers milk and directly in drinking water were slower to learn the difficult task of size discrimination and tended to repeat more errors than control subjects. Altmann et al (1993) demonstrated deficits of both active avoidance learning and in vitro hippocampal long term potentiation in adult rats that had received pre-weaning or pre- and post weaning dietary lead.

Similar effects have been noted in studies using non-human primates. Rice (1985) reported deficits of discrimination reversal performance in monkeys dosed orally with lead for the first 200 days of life. Lilienthal et al (1990) showed dose related increase in the percentage of errors in a discrimination learning task. Bushnell and Bowman (1979a) found that the learning impairment in lead exposed monkeys persisted upto the fifth year.

Several studies on experimental animals have shown perseverative effects (the tendency to respond repetitively and inappropriately even though environmental conditions have changed) which may underlie many of the lead induced changes in learning and other higher order behavioural processes noted in such studies (Cohn et al, 1993; Bushnell and Levin, 1983; Rice, 1984; Levin and Bowmann, 1986; Rice and Karpinski, 1988).

Persistence of lead induced changes in some higher order behavioural processes is suggested by a number of experimental animal studies. Bowman and his colleagues (Bushnell and Bowman, 1979a,b; Levin and Bowman, 1983, 1986, 1989) have demonstrated persistent neurobehavioural deficits extending upto 8 years after the termination of lead exposure and long after blood lead levels had declined to control levels. In rats, Cory-Slechta and Thomson (1979) showed that the levels of lead
exposure determine the persistence of lead effects, while Cory-Slechta (1990a,b) revealed that apparently transient effects of lead on behaviour could re-emerge with changes in the reward contingencies of the environment. Munoz et al (1986) found deficits in both spatial and visual discrimination performance of rats at several months of age resulting from pre-weaning exposure via the dams. Altmann et al (1993) noted same out-come in a study where deficits in adult rats were observed following pre-weaning exposure.

**Human studies**

Despite the long recognition of lead poisoning, new clinical cases continue to be the subject of published reports (Cueto et al, 1989; Zukerman et al, 1989; Friedman and Weinberger, 1990; Gupta et al, 1990; Sharma et al, 1990; Verrula and Noah, 1990). Although important, the numbers of these cases is small compared with the population at risk to the potential effects of lead from environmental and dietary sources.

Lilis et al (1977) in a study of 158 secondary lead smelter workers found that 64% reported CNS symptoms with elevated blood lead levels. Fischbein et al (1979) noted CNS symptoms in 21 out of 81 employees working in firing ranges. Karim et al (1986) studied 92 lead acid battery workers and compared them with 40 oil mill worker controls. They found CNS symptoms in 50% of lead exposed workers which correlated with their blood lead levels. Impairment of psychological and neuropsychological test performance has been reported for lead exposed workers (Haenninen et al 1979; Parkinson et al, 1986; Araki et al, 1986a,b; Stollery et al, 1989, 1991).

The critical flicker fusion test has been reported to be a sensitive indicator of CNS changes associated with exposure to lead. Wooler and Melamed (1978) and Williamson and Teo (1986) found significantly lower critical fusion in subjects with blood lead levels above 60 μg/dl.

Stollery et al (1989, 1991) and Stollery, (1996) have reported that occupational lead exposure impaired performance in a range of test that included sensory motor reaction time, memory, attention, verbal reasoning, and spatial processings. Lead
exposure impaired both the speed of making simple movements and decisions. The results of Araki et al (1986b, 1987, 1992) using short latency somatosensory and visual evoked potentials and auditory event related potential (P300) indicate that subclinical electrophysiological effects of lead occur not only in peripheral nerves but also in the CNS.

Peripheral neuropathy is a common sign of chronic, high level lead exposure manifesting as weakness in the upper or lower limbs. At lower levels of exposure, nerve conduction velocity (NCV) has provided a more sensitive indicator of peripheral nerve dysfunction. However, studies on NCV have yielded mixed results due to differences in methodological features (such as in the handling of confounding variables such as nerve temperatures, age of subject, type of nerve (Seppalainen and Hernberg, 1980; Davis and Svensgaard, 1990).

The majority of the epidemiological research on the health effects of lead has been focussed on children because, in comparison with adults, they are more vulnerable to lead in several respects (Davis and Grant, 1992). Children typically engage in hand to mouth activities that result in greater ingestion of lead than adults normally experience. Also, because of their greater absorption and retention of lead, body burdens in children resulting from a given external exposure level tend to be higher than adults. Furthermore, the relatively greater exposures and body burdens of children occur during sensitive periods of development. Finally children are generally more sensitive to the toxicological effects of lead at a given exposure level. The lowest observed effect level (LOEL) for various end-points (e.g. encephalopathy, anaemia, reduced haemoglobin, elevated erythrocyte porphyrin, slower nerve conduction velocity, impaired neurobehavioural function) are lower in children than in adults (US EPA, 1986, 1990).

Various population based cross sectional studies and epidemiological studies on children have in addition to intelligence tests, included extensive batteries of tests covering several other areas of psychometric functioning such as academic attainment,
behaviour, gross motor abilities and fine motor coordination, reaction time, visuo-spatial skills and learning and memory. Although findings from these studies have been less consistent and difficult to interpret, nevertheless these studies highlight the toxicological manifestations of lead exposure and do provide support for the reversibility of behavioural deficits (Needleman et al, 1979; Winneke et al, 1983; Fergusson et al, 1989; Rabinowitz et al, 1991; Silva et al, 1988; Bellinger et al, 1984, 1992; Needleman et al, 1990; Dietrich et al, 1987, 1991, 1992, 1993; Ernhart et al, 1989a,b; Cooney et al, 1989a,b, 1991; McMichael et al, 1988; Baghurst et al, 1992).

Mechanisms of lead induced behavioural toxicity

While the mechanisms underlying lead induced behavioural toxicity have yet to be adequately determined, experimental animal literature provides suggestive leads. The early experimental studies of Pentschew (1965) and Pentschew and Garro (1966) demonstrated in rodents that the pathogenesis of acute high dose encephalopathy was secondary to increased permeability of capillaries in the brain, leading to leakage of fluid and red blood cells. These changes are similar to those occurring in children with acute lead encephalopathy characterized clinically by coma, convulsions and death, and identify the brain microvasculature as the primary target for lead in the central nervous system following high level exposure to lead.

Changes in microvasculature morphology are not evident with low level lead exposure, but it is the vulnerability of the blood-brain-barrier that permits exposure of the brain to lead. Exposure of the developing fetus to lead results in higher uptake of lead in the brain than from later exposures (Rossouw et al, 1987). The development of resistance to acute encephalopathy in rat pups during the first few days of life is thought by Holtzman et al (1984) to be related to maturation of the blood brain barrier and possibly to the ability of the older animals to sequester lead in protein complexes. Thus, neuropathological changes may relate more to the issue of exposure than to mechanism of effect.
Recently, the focus of mechanistic studies has involved biochemical and neurochemical changes. Biochemical changes in synaptic transmission are likely to be related to problems in dendrite-nerve organisation and function (Goldstein, 1990). There is continuing reorganisation of dendrite and nerve terminal connections throughout early months of neural development. A number of biochemical and functional studies concerning possible mechanisms of lead effect suggest lead may disrupt this process and its function through early childhood (Cookman et al, 1987; 1988; Bielarczyk et al, 1994, 1996; Tian et al, 1995).

Other biochemical effects from lead exposure which may be the basis of important neurobiological mechanisms of lead-induced behavioural toxicity include changes in protein-kinases, with implications for neurotransmitter system disturbances. At least three protein kinases (protein kinase C, calmodulin kinase and cyclic AMP-protein kinase) present in nerve terminals involved in modulating the release of neurotransmitters have been shown to be affected by lead. However, the relevance of these findings to cognitive function has not been established (Goldstein, 1990).

From both *in vivo* and *in vitro* studies, lead exposure has been reported to have effects on virtually all neurotransmitter systems. Depending on the stage of development these include dopaminergic, cholinergic, serotonergic, GABAergic, glutamnergic and opiate systems. Probably the most extensively investigated systems have been the dopaminergic and the cholinergic system where a number of changes at the biochemical and receptor level have been described. One of the difficulties, however, is determining the relationship of the reported changes at the biochemical receptor level to changes in behavioural function (Cory-Slechta, 1995).

The subtle changes in the cytoarchitecture of the brain as a result of lead exposure have recently been attributed to defects in the polyamine pathway. The polyamine pathway (Fig. 3) is an essential component of all biological systems, and polyamines are considered as essential for cell growth and proliferation (Auvinen et al, 1992; Marton & Pegg 1995). The rate limiting enzyme of this pathway, Ornithine decarboxylase (ODC) has been found to be particularly susceptible to the toxic effect of lead.

Studies of Zawia et al (1994a,b) indicate that exogenous Pb produces distinct ontogenic effects on ODC activity in the hippocampus and the cerebellar region of the brain. These alterations in ODC activity can result in biochemical changes which may significantly interfere with normal brain development. Adhami et al (1996) reported that the levels of polyamine (putrescine, spermidine and spermine) were significantly decreased following lead exposure. Further the decrease in polyamine level was observed both in the neuronal as well as glial cells of cerebellum and hippocampus as a consequence of which migration and maturation of differentiating neuronal and glial cells during the critical stages of brain development is affected resulting in perturbed neurobehavioural sequelae. Recently polyamines have been implicated as important therapeutic agents (Janne et al, 1991; Gilad et al, 1996).

Iron-deficiency

Iron has been used since early times as a metal capable of restoring strength to people suffering from weakness. Physicians in Egypt, Greece and India used iron in their 'therapeutics'. In the 17th century, Syndenham identified the value of iron in chlorosis, the 'green sickness' of iron-deficient anaemic girls (Yeduda and Youdim, 1989a).

Iron is the most abundant trace metal in the body and brain. In human and rat brain it increases with age and reaches its maximum values at the age of 30-40 years and 4-5 weeks postnatal respectively (Yehuda and Youdim, 1989b). Within the brain iron shows an uneven distribution being highest in the basal ganglia (Spatz, 1922a,b;
Fig. 3: The polyamine biosynthetic pathway.
Riederer et al, 1992). Although the function of regionally high brain iron content is unknown, the homeostasis of brain iron is thought to be necessary for normal brain function, especially in learning and memory (Youdim et al, 1989; Yehuda and Youdim, 1989a; Pollit and Metallinos, 1990; Youdim, 1990, Pollit, 1993).

Requirement and sources of iron

The need for iron in the human diet varies greatly at different ages and under different circumstances and is determined by the requirement for tissue growth and hemoglobin synthesis. Losses of iron occurring in urine, faeces and sweat and in the females additional losses during menstruation, gestation and lactation need to be supplemented. Requirement for iron is significantly high during the first two years of life, during the periods of rapid growth, haemoglobin increases in the adolescence and throughout pregnancy in women. Clinical studies have indicated that an additional 0.8-1.0 mg of iron per day is needed to maintain normal haemoglobin concentration in children and adolescents. The average adult human male of 75 kg absorbs approximately 1 mg iron per day from dietary sources and loses about the same amount ranging from 0.4-0.2 mg depending upon the iron content of exfoliated cells. The recommended daily amount of iron suggested are-infants: 10-15 mg, children (1-3 years of age): 15 mg, males (11-18 years): 18 mg and after 19 years: 10 mg and females (11-51 years): 18 mg, and after 51 years: 10 mg.

Iron-deficiency and brain function

Iron-deficiency is one of the most widely recognised causes of anaemia the world over (Baker, 1978; Oski and Honig, 1978; Oski et al, 1978, 1983; Lozoff et al, 1985; Walter et al, 1983, Yehuda and Youdim, 1989a, Pollit, 1993). Octave et al (1983) reported that more than 500 million people throughout the world are iron-deficient, but a clear cut relationship between iron-deficiency and biochemical, physiological and behavioural parameters is yet to be established (Youdim, 1989).

Published literature dealing with the effect of nutritional iron-deficiency, suggests that iron-deficiency in children induces behavioural abnormalities that in-
eludes reduction of learning and cognitive process (Lozoff, 1985; Pollitt, 1993, Weinberg et al, 1979, 1981). Yehuda, Youdim et al (1986) examined the ability of iron-deficient rats to learn a water maze and found that iron-deficient rats learning capacity was significantly inferior in comparison with control rats. Further, the longer the duration of iron-deficiency the greater was the deficit, and the striking finding was that the deficit in learning was evident even when the level of haemoglobin did not reach a significantly decreased level. A similar deficit in learning and memory was found in iron-deficient rats when they were trained in a Morris water maze (Yehuda and Youdim, 1989a). No restoration of learning and/or memory was found when the rats were fed on an iron supplemented diet, suggesting irreversible changes as a consequence of iron-deficiency. In contrast, other effects of iron-deficiency such as motor activity, stereotypic behaviour, thermoregulation show reversal on iron supplementation. While the level of haemoglobin in the blood is restored during iron supplementation, the level of brain iron remains low for a very long time after iron-deficiency treatment (Ben-Shachar et al, 1985). This might explain some of the long term effects of iron-deficiency treatment on cognitive function.

Youdim et al (1989) showed that the effects of iron-deficiency are mediated by a decrease in functional activity level of dopamine receptor leading to a modification in dopamine mediated behaviour. Decrement in iron dependent dopamine D2 receptors in the cortex alters dopamine neurotransmission, which in turn, impairs cognitive function (Liebel et al, 1979; Youdim, 1988, 1990; Youdim et al, 1989).

Glover and Jacobs (1972) showed that iron-deficient rats exhibited lower motor activity level compared to control rats. Youdim and Green (1977) confirmed these results. Youdim et al (1981) using an LKB Animex(TM) activity meter found that iron-deficiency state induced a complete reversal of the circadian cycle of motor activity and also found that the general level of motor activity during a 24 hour period was lower in iron-deficient rats. The motor activity cycle, however, returned to normal after treatment with ferrous sulphate.
Although, iron-deficiency in rats reduces brain non-heme iron by 30-40% (Dallman, 1974, Ben-Shachar et al, 1988) there is resistance to change in the brain enzymes activity (Youdim and Green, 1977) and certain receptors (Ashkenazi et al, 1982; Youdim et al, 1983) and neurotransmitter metabolism. The brain has a greater tendency to retain iron and resist depletion than does the liver. During iron-deficiency, liver stores of iron are exhausted considerably faster (90%) and prominently than brain stores (Dallman and Spirito 1977; Ben-Shachar et al 1986). This slow loss of brain iron is matched by the reduced rate of recovery of the metal after iron sulphate therapy. Thus, it is apparent that the turnover of iron in the brain is extremely slow and its storage capacity is significantly limited compared with liver.

Iron-deficiency in children, assumes significance since from the age of five months to the end of the second year, they live in a physiological state called latent iron lack. The plasma iron level is low and the iron binding capacity raised. Such children readily slip into overt iron-deficiency. It has been reported by Walter et al (1983) that from birth to age 15 months as iron-deficiency progresses to anaemia, adverse effects on both mental and motor development are produced. Oral iron therapy for 3 months failed to reverse the change despite the fact that anaemia had been corrected.

Iron-deficiency and lead exposure

The principal causes of iron depletion are related to chronic blood loss, increased physiological demands for iron or intestinal malabsorption syndromes, and most of these cases are ultimately the result of or complicated by diets containing inadequate iron. When body iron stores are depleted, anaemia develops. Since anaemia occurs as a late manifestation of iron deficit, the incidence of latent iron-deficiency is undoubtedly quite high throughout the world. These facts are of special interest in metal toxicity studies. The toxicity of many metals has been well established in both men and animals. However, most animal studies are performed with the use of "complete" diets, and retrospective studies in humans were done without close
scrutiny of the body iron status (Ragan, 1977). Therefore, there is a paucity of information regarding the absorption and/or toxicity of pollutant metals in relation to iron-deficiency. Experimentally, iron-deficient rats have been reported to be more susceptible to lead toxicity than iron replete rats, and that lead absorption is increased by the iron deficit (Ragan, 1977; Cerklewski, 1980).

In developing countries such as India, an important consequence of iron-deficiency is an increased risk of childhood lead poisoning. Surveys show that young children with iron-deficiency have a prevalence of lead poisoning 3-4 times higher than children without iron-deficiency (Yip, 1990). This association is partly socioeconomic: poor children are more likely to have nutritional disorders and are more likely to live in inadequate housing where risk to lead exposure is greater (Yip, 1994). However, there is a strong evidence of a direct association between iron-deficiency and lead toxicity, related to the fact that iron-deficient individuals have increased efficiency for iron absorption (Dallman, et al, 1980). This increased absorptive capacity is not specific for iron, and the absorption of other divalent metals, including toxic heavy metals such as lead and cadmium, is also increased (Watson et al, 1980; Ragan, 1977). The microcytic anaemia thought in past to be due to lead poisoning is in fact iron-deficiency anaemia which is frequently observed among lead poisoned children (Clark et al, 1988).

Prevention of iron-deficiency would reduce the number of children who are susceptible to lead poisoning through greater lead absorption, and studies suggest that iron treatment of children with lead poisoning may also reduce their lead burden (Ruff et al, 1993).

Symptoms of fatigue, irritability, apathy, listlessness, lack of ability to concentrate, inattention, hypoactivity and/or hyperactivity typical of iron-deficiency have been found to be similar to those observed during prolonged exposure to low levels of lead (Miller et al, 1990). Interference with iron absorption and/or metabolism by
lead exposure could result in a synergism of deleterious effects (Yip et al., 1984; Szold, 1974). Although behavioural perturbations described for iron-deficiency are similar to those that accompany lead exposure, there is little information on the biochemical/metabolic systems specifically responsible for the behavioural effects.