REVIEW OF LITERATURE
Review of Literature

2.1 Poison and Poisoning: Historical Back Ground:

Poisons and poisonous substances that played a dominant role in the affairs of mankind, producing serious injury or death, have been documented from the times of ancient Egyptian magicians, physicians and priests. These are first documented recognition of the existence of these poisons. The medical records of ancient Egypt include a practicing physician's formulary containing about 260 prescriptions, some of which are for various kinds of animal stings, including the poisonous puffer fish. The Bible and Talmudic records document the toxic effects of dinoflagelates (a group of algae). Other records list some 760 medicinal plants and mention venomous animals and antidotes for bites and stings, and charms against snake poison (Halstead B.W., 1978).

In ancient China, toxicology was a comparatively well developed science. The ancient Greeks were probably the first to dissociate medicine from magic. Hippocrates, while introducing rational medicine
described a number of poisons. Mithradates VI, king of Pontus in the 2nd century B.C., is said to have been one of the first to study intensively the art of poisoning and the preparation of antidotes to poisons.

During the middle ages, poisoning became a very popular way to dealing with personal and political problems. Individuals skilled in the art of poisoning gained notoriety and in some cases, great financial reward. The French physician, Jacques Grevin published his book DEUX LIVERS DES VENINS in 1568. In 1702, the British physician Robert Mead published his "Mechanical taconite of poisons". Important toxicological contribution of the French physician M.I.B. Orifila (1787-1853), who has accrued the name of father of modern experimental toxicology. His Traite Detoxicologie first published in 1814, went through many editions and translations.

Experimental research based on sophisticated analytical procedures accelerated in the early 20th century. During World War II, environmental and biological poisons become medically important in many military operations. A rapidly expanding world
population in the Post War Years has increased the use of various pesticides in a continuing effort to feed the millions. This has increased the risks of toxicity, both intentional and accidental, especially in the underdeveloped countries like ours, where illiteracy, poverty and lack of infra structural facilities combine to take a heavy toll.

2.2 Aluminium phosphide: Nature of poison

Aluminum phosphide (AIP₃ Celphos, Quickphos, Alphos, etc.), is a solid fumigant. AIP is commercially available as solid pellets, each of 3.0g, in air sealed metal tubes.

Ingredient of pellets:

i. 56% \( \text{PH}_3 \) (Active ingredient)

ii. 44% Ammonium carbonate

On contact with moisture or hydrochloric acid, the pellets rapidly release phosphine (\( \text{PH}_3 \)) gas which is the active agent, as well as \( \text{CO}_2 \) and \( \text{NH}_3 \). Each 30g pellet of AIP can liberate 1.0 g of \( \text{PH}_3 \). The human toxicity is due to inhalation of phosphine from the fumigated
grain or after ingestion of AIP.

\[
\text{AIP} + 3\text{H}_2\text{O} \rightarrow \text{Al} (\text{OH})_3 + \text{PH}_3
\]

\[
\text{AIP} + 3\text{HCl} \rightarrow \text{AlCl}_3 + \text{PH}_3
\]

### 2.3 Physico-Chemical Properties of AIP:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Name</td>
<td>Quickphos (Celphos)</td>
</tr>
<tr>
<td>Chemical name</td>
<td>Aluminum phosphide</td>
</tr>
<tr>
<td>Active Ingredient</td>
<td>Phosphine</td>
</tr>
<tr>
<td>Structural formula</td>
<td>AIP (Al≡P)</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>57.00</td>
</tr>
</tbody>
</table>

### 2.4 Properties of Phosphine (PH₃)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
</table>
| Structure Formula               | \[
\begin{array}{c}
\text{P} \\
\text{H} \\
\text{H} \\
\text{H}
\end{array}
\]
| Molecular Weight                | 34.00          |
| Solubility                      | Soluble in water and inorganic solvents |
2.5 Lethal Dose of PH$_3$

The lethal dose for 70 kg human being is 150-500 mg determined recently in most cases of acute intoxication reported. Phosphine is dangerous to human life at a concentration between 400-600 ppm. At 1000 ppm, it is rapidly lethal (WHO, 1988).

A dose of less than 0.5 g of AlP has been reported to be lethal for an adult individual (Gosseline et al. 1984). However, other authors have quoted doses as low as 0.01 g (Sidney, 1980).

2.6. Aluminium Phosphide Poisoning in Humans:

The 1$^{st}$ reported case of toxicity due to AlP ingestion was by Zipf et al., (1967). Prior to this, toxicity due to inhalation of PH$_3$ has been reported. Gessner (1937) reported 12 cases of illness beginning with nausea and including one death in a house adjacent to a warehouse in which bags of AlP were stored. As recently as 1980, 31 crew members aboard a freighter carrying fumigated grain, after inhaling phosphine, one child was died (Wilson et al. 1980).
In India, the 1st case of AIP toxicity was reported in 1981, and since then it has assumed epidemic proportions in the entire wheat belt of North India (Bajaj and Wasir, 1988). This is clearly the case in Haryana (Siwach et al., 1988), Chandigarh (Singh et al., 1985), Western U.P. (Ram et al., 1988), M.P. (Caha et al., 1988) and Rajasthan (Saraswat et al., 1985).

The true magnitude of the problem is not completely known. Many deaths from AIP poisoning occur before the victim can be hospitalized, and practically no reports are available from non-teaching hospitals, peripheral health centres and private practitioners. Even teaching hospitals provide mope reports. It is estimated that less than 10-15% of patients reaching a medical college hospital have actually been reported, and these in turn form only about 5% of the total victims (Bajaj & Wasir 1990). Even in 1988, it had been suggested that the number of deaths, exceeded the number of reported casualties in the Bhopal gas tragedy (Kobra and Narayanan, 1988). During Iran and Iraq War, in 1985, the dangerous biological bombs used by Iraq released PH₃ gas. It is clear that AIP
ingestion is now the single most frequent mode of suicide.

The poison is usually taken by young adults, mostly teenagers by suicidal intent or occasionally by accident.

2.7 Clinical Manifestations of AIP Toxicity: Signs and Symptoms

The signs and symptoms of AIP toxicity depend on the dose and severity of poisoning. Mild poisoning manifests as epigastric burning and nausea, with tachycardia and atrial ectopy. Patients with moderate to severe toxicity were found with vomiting and epigastric burning and are usually in cardiovascular shock with thready pulse, cold extremities and restlessness (Bajaj et al., 1988).

Phosphine is a systemic poison and it affects the cardiovascular, gastrointestinal, renal, respiratory, central nervous system and hepatobiliary system.

Feature of above systems

Cardiovascular: The heart is involved in nearly 50% of cases.
(i) Elevation of serum levels of cardiac enzyme CPK-MB and LHD (Bajaj et al., 1988).

(ii) Hypokinesia of the left ventricle, with decreased ejection fraction in the first 4 days, hypomagnesemia (Mg depletion). Bajaj et al., 1988).

(iii) Histopathological studies of myocardial tissues show myocarditis and inflammatory cells (Siwach et al., 1988).

(iv) ECG changes comprise ST-T changes (elevation/depression, caving). Circulation disturbances Sino-Atrial, atrio-ventricular, bundle branch blocks and rhythm disturbances (ventricular and superventricular) [Katira et al. 1990] as well as atrial infarction (Jain et al., 1985).

**Respiratory:** Adult respiratory distress syndrome has been reported in severe cases cough, dyspnoea and cyanosis (Chough et al., 1988).

**Renal:** Renal failure alongwith hepatitis and bleeding diathesis has been reported (Gupta et al., 1990).
**Hepatobiliary:** Jaundice and hepatitis.

**CNS:** Inhaled phosphine is absorbed through the lungs and reaches the nervous system, and liver thereby producing neurological and hepatic symptoms (Childs and Choates, 1971). Symptoms such as headache, dizziness, altered mental state, restlessness without alteration in consciousness, convulsions, acute hypoxicencephalopathy and coma, follow soon.

**Gastrointestinal:** Common findings are nausea, vomiting, diarrhea etc.

### 2.8 Detection of the Poison

Phosphine poisoning may be detected by means of a simple test using filter paper impregnated with fresh silver nitrate (0.1 M) solution (Mittal et al., 1992). The test is performed with the help of gastric aspirate. The AgNO$_3$ paper test is based on the capacity of PH$_3$ to reduce AgNO$_3$ which gives a black precipitate.

\[
\text{PH}_3 + 8 \text{AgNO}_3 + 4 \text{H}_2\text{O} \rightarrow 8 \text{Ag} + \text{H}_3\text{PO}_4 + 8 \text{HNO}_3
\]

Black

The sensitivity of this test is high and it affords a ready means of confirming poisoning by AlP, especially
when the history is not forthcoming.

2.9 Mechanism of Action of Phosphine:

The mechanism of action of PH₃ is not well understood. The lethal properties of AlP are due entirely to the liberation of PH₃. The mode of action of PH₃ has been studied in numerous animal models. It has been shown by various investigators to act on the respiratory chain. PH₃ is liberated during the oxidation of fatty acids and amino acids and nearly all of that released from the oxidation of carbohydrates is made available within the mitochondria as reducing equivalents (-H or electron). Mitochondria contain the series of catalysts known as the respiratory chain that collect and transport reducing equivalents and direct them to their final reaction with oxygen to form water.

Lipoate $\rightarrow$ NADH $\rightarrow$ NADH-Q-reductase $\rightarrow$ Q $\rightarrow$ Cyt reductase $\rightarrow$ Cyt C $\rightarrow$ Cyt Oxidase $\rightarrow$ O₂

(Components of the respiratory chain in mitochondria)
Cyt : Cytochrome ; Q : Ubiquinone

The terminal cyt. is responsible for the final combination of reducing equivalents with molecular oxygen. This is the only irreversible reaction in the respiratory chain, and gives direction to the movement of reducing equivalents in the respiratory chain, and to the production of ATP.

Oxidation and phosphorylation are tightly coupled. Nakakita et al. (1971) carried out in vitro studies in rat liver mitochondria showed phosphine to be potent inhibitor of ADP and ion-stimulated respiration. However, no attempt was made to identify the target site (Nakakita et al., 1971). Chefurka et al. (1976) investigated the basis of the respiratory inhibition of phosphine in mitochondria from mouse liver and housefly flight muscles. Their conclusion was that phosphine is an inhibitor of state 3 and 4 respiratory activity by virtue of its direct inhibition of electron transport due to interaction with cytochrome oxidase.

The inhibition kinetics suggested non competitive inhibition of this site; oxidative phosphorylation was not
affected. Chefurka et al (1976). Studied the nature of the interaction between phosphine and purified cytochrome oxidase by absorption spectrometry and circular dichroism and noted a conformational change in the haem moiety of cytochrome oxidase. They concluded that cyt. was the major site of action of phosphine (Khashi and Chefurka, 1976). In the event of inhibition of cytochrome oxidase, oxygen undergoes an alternative pathway of reduction resulting in formation of free radicals. (Fridovich, 1978b).

Price (1980) studied the inhibition of cytochrome C-oxidase by phosphine in vitro in both susceptible and resistant strains of R. dominica but found little inhibition of cytochrome oxidase. The toxicity of phosphine to insects is oxygen dependent (Bond et al., 1967). In the absence of oxygen, phosphine is virtually nontoxic and is not absorbed to any appreciable extent. Price et al. (1982) and Price and Dance (1983) suggested that this may be due to the effect of phosphine on the haem containing enzyme catalase. Catalase is an enzyme which reduces hydrogen peroxide to water in the body; it is suggested that it also plays a vital role in the
detoxification of free oxygen radicals (Master and Holmes, 1976). Bolter and Chefurka (1989) observed that phosphine reduces cytochrome C-oxidase but none of the other cytochromes in the electron transport chain.

Thus whether by inhibition of cytochrome oxidase or of catalase, phosphine toxicity would be expected to lead to over production of free oxygen radicals.

2.10 Free Radicals:

Electrons in the atoms are present in orbitals, each of which can hold a maximum of two electrons, spinning in opposite direction. A free radical is defined as any atom or group of atoms, or molecule capable of independent existence in a particular state with one or more unpaired electrons in the outer orbital. These are a highly unstable and reactive species (Del Maestro, 1980). Free radical reactivity is accounted for by the strong tendency of the unpaired electron to interact with other electrons to form an electron pair and thus a chemical bond. Free radicals tend to give rise to chain reactions, which may be conveniently divided into three stages (1) Initiation (ii) propagation (iii) termination.
i. **Initiation** reaction by which the free radicals are formed (Pryor, 1966).

ii. **Propagation** reaction in which the free radical chain transfer reaction.

iii. **Termination** reactions resulting in removal of free radicals from the propagation pool.

The presence of an unpaired single electron in its outer orbital is conventionally represented by a superscript dot, \( \cdot \).

### 2.11 Oxygen Free Radicals:

Molecular oxygen in its ground state is a bi radical possessing two unpaired electrons in its outer orbital with parallel electron spin (Tabue, 1965; Ogryzlo, 1978). This arrangement prevents the direct addition of a pair of electrons (which would have one parallel and one antiparallel spin) to the molecule necessitating an electron spin inversion before an oxidation reaction is possible. The spin inversion is a slow process compared to the life-time of collisional complexes \( \alpha \) relatively weak oxidant (Toube, 1965), the restriction in the oxidising
when it undergoes univalent electron reduction along with electron spin inversion, results in the formation of toxic intermediates, the free oxygen radicals.

During oxidative phosphorylation, the mitochondrial cytochrome oxidase enzyme system links production of adenosine triphosphate to controlled tetravalent reduction of molecular oxygen ($O_2$) to water (Antonim. et al. 1979). In this process, the partially reduced oxygen free radical intermediates remain lightly bound to the active sites of the enzyme and pose no threat to the cells.

However, in the presence of intercellular oxygen, this reduction of oxygen is frequently incomplete and the univalent reduction pathway predominates over the tetravalent pathway leading to the inadvertent production of toxic intermediates oxygen species, superoxide anion radical ($O_2^-$), hydroxyl radical ($OH^-$) and hydrogen peroxide ($H_2O_2$).

\[
\begin{align*}
O_2 & \rightarrow O_2^- \\
& \rightarrow H_2O_2 \\
& \rightarrow H_2O \\
& \rightarrow H_2O \\
OH^- & \rightarrow H_2O
\end{align*}
\]
### 2.12 Biological Sources of Free Radicals:

Many enzymes in the body produce univalent production of molecular oxygen leading to the formation of toxic free radicals. In addition, these reactive metabolites may arise from the action of various environmental agents. Possible sources of free radicals are indicated in the following table (Sinclair et al. 1991).

<table>
<thead>
<tr>
<th>Source</th>
<th>Mechanism</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular metabolism</td>
<td>Oxyhaemoglobin metabolism</td>
<td>NADPH oxide</td>
</tr>
<tr>
<td></td>
<td>Enzymic activity</td>
<td>Xanthine oxidase</td>
</tr>
<tr>
<td></td>
<td>Arachidonic Acid metabolism and PG synthesis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitochondrial electron transport Auto-oxidation</td>
<td>Endoplasmic reticular oxidation</td>
</tr>
<tr>
<td>Environmental</td>
<td>Catecholamines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Riboflavin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meluminthiols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flavin derivatives</td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td>Paracetamol</td>
<td></td>
</tr>
<tr>
<td>Pesticides</td>
<td>Halothane</td>
<td></td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td>Paraquat etc.</td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 2.13 Biological Consequences of Free Radical Damage:

Free radicals mediate injury at several sites including cell membrane lipids, the sulfhydryl group of protein and nucleotides of DNA (Llebanoff, 1988; Slater, 1984; Inlay and Linn, 1988; Dormandy, 1988). This injury occurs through several mechanisms. (Del Maestro, 1980).

**i.** Peroxidation of polyunsaturated fatty acids and the subsequent disruption of cell and organelle membranes.

**ii.** Lysosomal membrane disruption resulting in the autophagocytic vacuole formation.

**iii.** Protein damage leading to fragmentation, cross linking and aggregations of proteins.

**iv.** DNA degradation resulting in mutations and potential neoplastic transformation.

**v.** Membrane phospholipase activation leading to release of prostaglandins and various endoperoxides.
vi. Noxious products of lipid peroxidation may be transferred by the circulation to distant sites where they may provoke further damage.

2.14 Protective Mechanism Against Free Radical Damage:

Normally, a small amount of free radicals is produced in the body with its attendant risks of cell injury. The free radicals may decay spontaneously, but several cellular mechanisms exist for both enzymatic protection and non enzymatic antioxidant mediated protection.

2.14.1 Enzymatic Protection:

i. Superoxide Dismutase (SOD)

This enzyme converts superoxide : anion \( (O_2^-) \) to \( H_2O_2 \)

\[
\text{SOD} \quad O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

SOD increase the rate of intracellular dismutation of superoxide by a factor of \( 10^9 \) (Fridorich, 1978a). The acceleration of this reaction ensures that no superoxide anion is available to react with \( H_2O \) to form hydroxyl.
radical through the metal catalysed Fenton reaction. The cells are capable of increasing the synthesis of SOD in response to hyperoxident stress (Firdorich, 1983).

Two enzyme systems exist to catalyse the breakdown of $\text{H}_2\text{O}_2$ produced by the univalent reduction of superoxide anion (Roose et al., 1980; Chance et al., 1979).

ii. Glutathione Peroxidase (GSHPx):

At low concentrations, most of the $\text{H}_2\text{O}_2$ is removed by reaction with reduced glutathione (GSH). GSHPX is a selenium dependent enzyme present mainly in the cytoplasm. It catalyzes the transfer of hydrogen from the sulfhydryl (-SH) to a hydroxyl radical or to $\text{H}_2\text{O}_2$.

$$\text{2OH}^- + \text{GSH} \rightarrow \text{2H}_2\text{O} + \text{GSSG}$$

(Oxidized glutathione)

$$\text{H}_2\text{O}_2 + 2 \text{GSH} \rightarrow \text{2H}_2\text{O} + \text{GSSG}$$

The enzyme glutathion reductase (GR) catalyzes the regeneration of reduced glutathione (GSH) from GSSG by using NADPH formed by the hexose monophosphate shunt pathway. Glutathione is a key factor in the
detoxification of reactive oxygen intermediates and electrophilic metabolites (Curello et al., 1987).

iii. Catalase: At high concentration of $\text{H}_2\text{O}_2$, which is mainly present in the peroxisomes, catalase becomes important.

$$\text{catalase}$$

$$2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$$

2.14.2 Nonezymatic Protection: Apart from enzymes, endogenous as well as exogeneous antioxidants may either block the initiation of free radicals or inactive them.

2.15 $\alpha$-Tocopherol (Vitamin E):

About thirty years have passed since the existence of vitamin E was recognized. Alpha tocopherol the substance with highest known vitamin E activity was synthesised about twenty years back. The principal group of compounds having vitamin E activity are the tocopherols. The seven tocopherols have been found to occur naturally.
The biopotencies of various tocopherols differ somewhat, depending on the assay criteria and animals used. α-Tocopherol has the greatest biological activity, however. This could be a reflection of its greater susceptibility for methylation. Such methylation will prevent side reactions and lower the oxidation-reduction potential of hydroquinone-quinone system. The features of tocopherol chemistry that appear most salient to possible biological function are its lipid solubility and oxidation properties. They play a physiological antioxidant role (potentiated by ubiquinone Q10) at the cellular level by counteracting lipid peroxidation increasing membrane phospholipids. Vitamin E acts as a \( \text{H}^+ \) donor in Krebs Cycle, between the steps of flavin coenzymes (FMH and FAD) and the cytochrome system (Butturini, et al. 1955).

The identification of vitamin E as a fat-soluble vitamin, its occurrence in vegetable oils, its storage in association with body lipids and its possible function as a biological antioxidant suggest that close relationship exists between vitamin E and various phases of lipid metabolism. When vitamin E is deficient or completely
lacking, there is uncoupling of phospholipids and proteins, necessary for formation of cell membrane. In studies in humans, the administration of tocopherol to healthy male subjects resulted in increase in plasma cholesterol values (Gray and Lon. 1958.) Grey (1959) found levels of phospholipids and cholesterol esters in livers of rats fed 100 mg α-tocopherol acetate daily when compared from unsupplemented, rats presumably due to increased hepatic lipid synthesis in the supplemented animals.

After the pioneering works of Kudrjashow (1937) and Davis and Moore (1941) an appreciable number of scientists have been dealing with the relationship between vitamin E and lipid peroxidation. The contribution of Tappel's laboratory has generally been recognized (Tappel, 1962, 1972, 1973). The increase in lipid peroxidation resulting from E-avitaminosis was proved beyond question. The activation of lipid peroxidation increases the requirement of vitamin E (Willing, 1970, 1972). Synthetic antioxidants prevent symptoms of E-avitaminosis, though not completely (Rods, 1967; Tappel, 1972). The toxic action of lipid
peroxidation products (Holman and Greenberg, 1958) is usually inhibited by α-Tocopherol (Kokathur et al., 1966; Privett and Cortesi, 1972).

α-Tocopherol is a powerful chain breaking inhibitor of lipid peroxidation. Vitamin E acts in the lipid phase to refuse the lipid peroxy radicals (Clavce, et al., 1979; Halliwell and Gutteridge, 1985).

2.16 Free Radicals in Poisoning:

The central role of free radicals in toxicity due to many chemicals is established. For example, the toxicity of the herbicide paraquat is due to enzymatic reduction to paraquat-pyridinyl cation radical and its reoxidation by hydroxyl radicals and by the Fenton and Heber-Weiss reactions (Osheroff, et al., 1985)

In AlP poisoning, the inhibition of either cytochrome oxidase or catalase would be expected to give rise to an abundance of $O_2^-$ free radicals, (Clough, et al., 1992).

Although abundant literature is available on the effects of AlP on different organ systems, the neurotoxic
effect of AlP on different regions of brain has not been reported so far.

2.17 Treatment of AlP Poisoning:

To date, there is no known specific antidote for AlP poisoning, therefore, the therapeutic part is mostly restricted towards symptomatic treatment and maintenance of vitals.

The manual of Indian standard code for safety for AlP poisoning suggests:

i. Induction of vomiting by administration of 0.2% CuSO$_4$ solution which acts as an emetic and also forms insoluble cupric phosphide which can be taken out by gastric lavage with 1:5000 KMNO$_4$ solution.

ii. 25.0ml of milk of magnesia or white beaten of 2 or 3 eggs should be given.

iii. Vasopressors (Dopamine, noradrenaline) for maintenance of blood pressure.
iv. Urinary pH is made alkaline by giving sodium carbonate (Na$_2$CO$_3$)

v. Pulmonary oedema is treated on standard lines. Intranasal oxygen, steroids and hypertonic solution of glucose may be given.

vi. Haemodialysis may be required for renal shutdown which may be an outcome of shock syndrome due to direct tissue toxicity.

In the present study, effect of $\alpha$-Tocophrol (vitamin E) was studied in different regions of rat brain. To date no report is available on the antioxidant effect of vitamin E on the treatment of CNS toxicity due to AlP inhalation.

2.18 The Lipids:

Lipids are essential components of all cell membranes and are involved in numerous biological process they are also a major components of several critical enzyme systems, include a potent class of hormones, and are also implicated in the cellular transport of some components (Spectator and
Brenneman, 1973). Tumors of cancer require lipids so that membrane synthesis can proceed at a significant rate to permit rapid growth. If the availability of lipids is sufficiently reduced then the growth of tumor is also slowed down (Spectator and Brenneman, 1973). Lipids are the most concentrated source of energy to the organism, they are stored in a relatively water free state in the tissues, in contrast to carbohydrates which were heavily hydrated. The lipid depots serve as a reservoir of energy, available in times of restricted nutrition for the operation of the numerous endogenic processes essential for maintenance of life. Lipids work as insulators of heat. The subcutaneous lipid depots also insulate against mechanical trauma (White et al., 1978). Lipids are chemically triacylglycerol in human adipose tissue (99%).

The quantity of body lipids is increased by excessive nutrition, and conversely, during periods of prolonged fasting the amount of body lipid decreases. Fatty acids are used by the body tissues as their prime energy source (Fritz, 1961) and direct evidence for the uptake and utilization of fatty acids from the circulatory
blood by individual organs and tissues in the fasting state has been obtained (Shipp et al., 1961; and Gousios et al., 1963). Depot lipid is continuously being mobilized, new lipid is continuously being deposited, and the constancy of the quantity of depot lipid is the result of a relatively precise adjustment of the rates of these two processes. Almost 10% of the fatty acids in the depot lipid is replaced daily by new fatty acids in the body.

2.18.1 Brain Lipids:

"There is no other organ in the body that contains such a high proportion of lipids as the brain".

Studies of lipids in the nervous system form an important part of neurochemical investigations. Among various body organs, the brain is one of the richest in lipids, comprising over half of the total dry weight (Brante, 1949; Balakrishnan et al., 1961; and Suzuiki, 1981). It contains a unique structure, the myelin sheath, which have the highest lipid concentration of any normal tissue or subcellular components, except for adipose tissue, and which has been the subject of intensive and extensive studies in recent years. Myelin is present in all parts of the nervous system but is more concentrated
in areas composed mainly of fiber tracts, such as the white matter of brain and spinal cord and in peripheral nerve trunks, such as sciatic nerve. The abundant lipids of CNS are located in both cellular and subcellular membranes and in the myelin sheath. Different types of membranes accumulate different types of lipids (Horrocks et al., 1975). Mammalian brain white matter contains about 50% myelin on a dry weight basis. Even in the whole brain of an adult rat, myelin is about 25% of dry weight and accounts for more than 40% of the brain lipid (Northon and Poduslo, 1973). Early analysis of white matter revealed that cholesterol, sphingomyelin, and cerebroside were present in larger amounts than in the grey matter (Johnson et al., 1948). Cumings (1953 and 1955) compared the lipids of demyelinated lesions in multiple sclerosis with those of normal areas of the brain and found a decrease in sphingomyelin, cerebroside, and free cholesterol. Similar findings were made in demyelination resulting from Walarian degeneration of the peripheral nerve (Rossiter, 1961 and Berry et al., 1965). The rapidly increased myelin content is closely related with increase in brain weight (Smith et al., 1983).
Waelsch, Sperry and Stayanoff (1941) have studied that after birth lipid is deposited in the brain as a result of two processes, (a) growth and (b) myelination. Immediately after birth and before myelination is complete there is active deposition of brain lipids (Fries, Changus and Chaikoff, 1940). The important lipids of the CNS are cholesterol, cerebrosides, phospholipids, lecithin, sphingomyelin & cephalin (Johnson, et al. 1948). Galli et al. (1970) indicated that rat brain may have sphingomyelin levels as high as in human brain. About 50% of all lipid in white matter or 30% of total brain lipid, has been estimated to belong to the myelin sheaths in rat brain. The complete lipid analysis of myelin from different species has been published by various workers (Autilio et al., 1964; Eichberg et al., 1964; and O'Brein, 1965). The most rapid increase in lipid content of brain begins after the periods of greatest increase of DNA and protein. Changes in specific lipids have been well documented (Rouser and Yamamoto, 1969 and Wells and Dittmer, 1967). Changes in fatty acid concentration and degree of saturation have been determined for several groups of lipids (Rouser and Yamamoto, 1969 and O'Brien, 1979).
Studies from this laboratory have shown that pesticides perturb the levels of total lipids in the rat CNS (Tayyaba and Hasan, 1980; Islam et al., 1983; Tayyaba and Hasan, 1985; Hasan and Khan, 1985; and Naqvi et al., 1988; Gupta and Hasan, 1988).

The available literature on different lipids indicate that the knowledge of pesticide toxicity on brain lipids is inadequate. The different parts of the brain show regional variations in the lipid contents. Since the brain is a heterogeneous organ composed of many structural and functional components with markedly different levels of functional and metabolic activity, it is reasonable to investigate the neurotoxicants' influence on discrete brain areas. The present study deals with the effect of phosphine inhalation on different lipid levels in various regions of CNS of the rat brain.

2.19 Cholesterol:

The myelin of mammalian CNS is composed of 25 to 28% cholesterol, 40 to 45% phospholipid and 27 to 30% galactolipids. Brante (1949) estimated that myelin sheath lipids were 25% cholesterol, 46%...
phospholipides and 29% galactolipids. The data for lipid composition are expressed in mole percent. Most of the preparations analysed so far contain cholesterol, phospholipid and galactolipid in molar ratios ranging from 2: 2: 1 to 4: 3: 2. Thus, cholesterol constitutes the largest proportion of lipid molecules in myelin. It contains approximately 25% of myelin lipid by weight (Soto et al., 1966 and Cuzner, 1965). The structural matrix of cell membranes is a lipid bilayer with variable amounts of cholesterol and glycolipids (Tanford, 1978).

Cholesterol is the only sterol present in normal adult brain in significant amounts. The alcohol group (-OH) at position 3 may be esterified with a long-chain fatty acid. Esterified cholesterol is present in normal brain only in very low concentrations. In adult brain it is unesterified form (Davison, 1965). Unesterified cholesterol has been suggested as a lipid that is characteristic of myelin sheaths. It occurs in white matter in amounts greatly exceeding those in grey matter (Johnson, 1949 and Brante, 1949). Along with cholesterol in CNS, there occur very small amounts of other sterol (upto 1%) (Cook, 1958). In rat brain, total
levels of sterol esters increase from birth to 40 days (Eto and Suzuki, 1972). Kritchevsky and Holmes (1962) found varying amounts of the sterol in the newborn rat brain. Recently, Fumagalli and Paoletti (1963) and Fumagalli et al. (1964) reported that desmosterol accounted for up to 7% of the human and rat fetal and neonatal brain sterol content. Desmosterol which is the immediate metabolic precursor of cholesterol (Fish et al., 1962) and has an additional double bond at C\textsubscript{24}, is present in normal developing brain in measurable amounts just prior to myelination (Kritchevsky et al., 1965 and Paoletti et al., 1965) and also in the myelin sheath itself in the early stage of myelination (Smith, et al., 1967). Laatsch, et al. (1962) demonstrated that cholesterol accounts for 18-20% of the dry weight of the myelin fraction and that about 70% of the total brain cholesterol is present in the myelin. Cholesterol accounts for about 10% of dry weight of the brain in contrast to less than 11% found in most other organs.

Biosynthesis of cholesterol in brain is most rapid during the period of active myelination. But adult brain retains the capacity to synthesize cholesterol when
precursors such as acetate or mevalonate are available. Acetate and its precursors are transformed through mevalonic acid to cholesterol. The adult human brain contains 25g of cholesterol, but this amount at birth is only 2g (Waelsch et al., 1940 and 1941). On pH 7.2 cholesterol ester hydrolase is one of three such hydrolases in brain that can be distinguished by their pH optima and response to detergents (Eto and Suzuki, 1973 and Igarashi and Suzuki (1977).

Although most of the cholesterol in brain appears to be synthesized from endogenous precursors, experimental evidence indicates that a small amount of systematically injected cholesterol can be taken up intact and that the rate of uptake is greatest when the rate of cholesterol deposited in brain is most rapid, i.e., during active myelination (Dobbing, 1963). Once deposited in brain, cholesterol, particularly that incorporated into myelin, is relatively inert metabolically (Davison et al., 1958 and Khan and Folch, 1967). Further studies of incorporation of labelled cholesterol or labelled acetate into brain tissue indicate that the cholesterol of adult brain is relatively inert (Waelsch et al., 1940; Bloch et
al., 1943; Syere et al., 1950; and Van Bruggen et al., 1953). After intracerebral injection of labelled cholesterol acetate in rats, however, some label is incorporated into cholesterol and appears to remain there indefinitely (Nicholas and Thomas, 1959). Paoletti (1971) has substantiated the observation that microsomes are the subcellular sites of brain cholesterol biosynthesis.

Earlier findings from this laboratory have shown alterations in the levels of cholesterol in discrete brain areas following chemical stress (Tayyaba and Hasan, 1980; Islam et al., 1983; Hasan and Khan, 1985; Tayyaba and Hasan, 1985; Vadhva and Hasan, 1985; Naqvi et al., 1988 and Gupta and Hasan, 1988; and 1992).

Till date, no attempt has been made to evaluate the effect of phosphine in the various regions of the rat brain and spinal cord cholesterol. Therefore, it would be of interest to estimate the levels of cholesterol quantitatively after the administration of A1P solution by gavage.
2.20 Lipid Peroxidation:

The lipids in membranes of cells from higher organisms contain large numbers of polyunsaturated fatty acid side-chains. Such fatty acids are prone to undergo a process known as "lipid peroxidation", which involves the generation of carbon radicals, followed by production of peroxide radicals (Sohail, 1981). Lipid peroxidation has been broadly defined as the oxidative deterioration of polyunsaturated lipids and involved in many disease processes and chemical toxicities (Tapple, 1973 and Tappel and Dillard, 1981). Lipid peroxidation is found to be affected in certain clinical disorders such as under nutrition, cancer, aging, hypoxia etc. It has been claimed that lipid peroxidation in vivo has been of basic importance in aging, damage to cells by air pollution, toxic chemicals and in oxygen toxicity (Tapple, 1970). It is believed that lipid peroxidation of biomembranes is one mechanism whereby a foreign chemical may be toxic to cells, and this has evoked considerable interest in understanding the mechanism of this phenomenon in various biological systems (Bus and Gibson, 1979 Msayuki et al., 1996). According to Demopoulos et al.,
(1979) the loss of membrane integrity during pathological free radical mechanism leading to lipid peroxidation and degeneration of phospholipids are important factors which irreversibly damage brain cells in ischaemic and other adverse conditions. The rate of lipid peroxidation is also highly catastrophic to the integrity of cellular membranes and to membrane bound enzymes (Recknagel and Glence, 1977 and Zebra, et al., 1990). It is appropriate to recall here that, as well as being destructive at higher concentrations, lipid peroxides also appear to have an essential enzyme activating role in the arachidonate metabolizing pathways (Helmer and Lands, 1980; reviewed by Cleveland, 1984). Free radicals have long been suspected as intermediates of biological oxidative processes. They are highly reactive transient chemical intermediates, the concentration of which is increased by high energy irradiation to the oxygen radicals themselves, certain products of radical induced lipid peroxidation, including a series of aldehyde, may be toxic to invading organisms and host cells (Morel et al., 1983). Being much more stable than free radical, these toxic compounds can
cause injury some distance from the site of radical
generation. Kartha and Krishnamurthy (1978) reported
that among the different tissues from normal rats, the
brain showed a considerably high degree of peroxidation,
while the homogenate of other body parts showed
comparatively low lipid peroxidation. When lipids react
with oxygen radicals they undergo a series of molecular
rearrangements termed peroxidation and form a series of
oxidation derivatives, including lipid hydroperoxides
and aldehydes (Esterbauer, 1982 and Ji, L.L. and Fu, R.
1992). Some of these products of lipid peroxidation are
toxic to various cells, including endothelial cells
(Sasaguri et al., 1984). Lipid hydroperoxides decompose
to produce aldehydes (e.g. malondialdehyde) and other
products, including gaseous hydrocarbons such as ethane
and pentane (Pryor, 1978 and Cohen, 1979). Their
decomposition is catalyzed by transition metal ions and
by haem compounds (Svingen et al., 1979; and Kohn and
Kessel, 1979). Lipid hydroperoxides and some of their
degradation products are highly cytotoxic: they cause
extensive damage to enzymes and to membranes,
producing a decrease in electrical resistance and
membrane fluidity and eventual loss of membrane integrity (Gutteridge et al., 1979; Gardner, 1979; and Pauls and Thomas, 1980). Further, there is some evidence that malondialdehyde is a mutagen. Disruption of lysosomal membranes by lipid peroxidation can spill hydrolytic enzymes.

The peroxidation observed was probably initiated by traces of metal ions, especially iron, contaminating the reducing agents. Since \( \text{Fe}^{2+} \) is a good initiator of peroxidation, these compounds probably serve to keep the iron in the reduced form so as to allow continuation of peroxidation (Wills, 1960 and Gutteridge et al., 1979). As a second protective mechanism, the chain reaction of lipid peroxidation can be effectively inhibited by abstraction of H from another donor (often called a "scavenger") to yield a donor radical that is relatively unreactive.

\[
\text{Lipid-O}_2+\text{donor-H} \rightarrow \text{Lipid-}00\text{H} + \text{donor}
\]

It is well known that unsaturated fatty acids or lipids will undergo oxidation in the presence of oxygen. The progress of this reaction can be monitored in several
ways. As oxidation proceeds, it is possible to observe: (1) an increase in absorbance at 233 nm; (2) $O_2$ uptake; (3) an increase in the rate of lipid peroxidation present; and in some cases, (4) an increase in the amount of malondialdehyde formed which is usually quantified by its highly coloured reaction product with thiobarbituric acid (TBA) test (Barber and Bernheim, 1969).

The increase in absorbance at 233 nm is attributed to the formation of conjugated diene systems in unsaturated lipids. Oxygen uptake results in the formation of peroxides whose concentration can be determined using an iodometric procedure. The TBA test for lipid peroxidation is a sensitive and widely used assay for malondialdehyde formed during lipid peroxidation.

Lipid peroxidation is an autocatalytic free radical process (Pryor, 1978). Free radicals are short lived, highly reactive chemical species involved in a variety of functions, namely, oxidation of unsaturated fatty acids in cell membranes (lipid peroxidation), damage of DNA.
modulation of nucleotide cyclase activities and synthesis of prostaglandins and lipo-peroxides. These free radicals are usually produced in biological system during antimicrobial defense, through the action of mixed function monoxygenases, by various oxidative enzymes, such as xanthine oxidase and by auto-oxidation mediated by heavy metals and quinones (Proctor and Reynolds, 1984; Richter, 1988; Simic, et al., 1989). The $H_2O_2$ and other reactive $O_2$ species, if not scavenged efficiently, are known to give rise to potentially toxic intermediates, namely, hydroxy radical ($OH^\cdot$) and singlet ($O_2^\cdot$). These oxidants, in the presence of metal ions, result in the formation of lipid peroxides (Lai and Piette, 1978; Lai et al., 1979a).

Initiation of lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom. Since a hydrogen atom has only one electron, this leaves behind an unpaired electron on the carbon atom. The carbon radical in a polyunsaturated fatty acid tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which rapidly reacts with $O_2^\cdot$ to give...
hydroperoxy radical. Hydroperoxy radicals abstract hydrogen atoms from other lipid molecules and so continue the chain reaction of lipid peroxidation. The hydroperoxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxide (Barber and Bernheim, 1967). The classically accepted mechanism of free radical lipid peroxidation is outlined below.

\[ \text{LH = fatty acid; LOOH = lipid hydroperoxides; L}^\cdot = \text{lipid alkyl radical; LOO = lipid peroxy radicals} \]

**Initiation**

\[ \text{LH} + \text{O}_2 \rightarrow \text{Free radicals, L}^\cdot \quad (i) \]

\[ \text{LOOH} \rightarrow \text{Free radicals, L}^\cdot \quad (ii) \]

**Propagation**

\[ \text{L}^\cdot + \text{O}_2 \rightarrow \text{LOO}^\cdot \]

\[ \text{LOO}^\cdot + \text{LH} \rightarrow \text{LOOH} + \text{L}^\cdot \]

**Termination**

\[ \text{LOO}^\cdot + \text{LOO}^\cdot \rightarrow \text{LOOL} + \text{O} \]

\[ \text{LOO}^\cdot + \text{L}^\cdot \rightarrow \text{LOOL} \]

\[ \text{L}^\cdot + \text{L}^\cdot \rightarrow \text{LL} \]
Hydrogen abstraction - by a
(A) Previously formed peroxide radical or by a species such as 
OH* generated from O2*

(B) Rearrangement

(C) Conjugated diene (can be detected by absorbance at 233 nm)

(D) Peroxide radical: abstracts H* from another chain so causing a chain reaction

(E) Hydroperoxide

Fragmentation products including (especially malondialdehyde)

Mechanism of peroxidation of polyunsaturated fatty acids.
Mechanism For Lipid Peroxidation: Dahle et al., (1962)

Lipid peroxidation is aimed to explain the following observations:

The increase in absorbance at 233 nm in the early stage of the oxidation, the appearance of lipid peroxides as intermediate in the reaction, the inhibiting effect of chain breaking antioxidants on the reaction, and the more facile production of malondialdehyde as quantified by the TBA test from linolenic or arachidonic acids when compared to linoleic acid. The steps in the oxidation of a diene and a triene fatty acid system are illustrated in Fig. A. This free radical chain reaction is initiated when some unidentified free radical abstracts a methylene H atom from the unsaturated fatty acids. The resulting free radical is stabilized by resonance, with several of the resonance forms adopting a conjugated diene system. Oxygen adds to the lipid free radical producing a hydroperoxy radical which may abstract a hydrogen atom from another unsaturated fatty acid (thereby propagating the chain reaction) to form a
lipidperoxide. In this mechanism, malondialdehyde is produced by the triene but not the diene system because only with the former is it possible to obtain a hydroperoxy radical with a double bonds to the peroxy radical. It is possible to obtain the cyclic peroxide II in triene systems which is the non-volatile precursor of malondialdehyde. Pryor, et al., (1976) have criticized this mechanism because of the assumption that only methylene hydrogen atoms can be abstracted in the step. If hydrogen abstraction can also occur at alkylic positions at the ends of the alkene systems then linoleic acid can also produce the cyclic peroxide II. Since both linoleic and linolenic acids can form II, then Dahle’s mechanism does not explain the difference in the state of malondialdehyde formation from linoleic and linolenic acids. Pryor, et al., (1976) also suggest that the fatty acid free radical I abstracts a hydrogen atom internally. Triene systems produce a more suitable bicyclic free radical III than diene systems since they are stabilized by the third double bond.

The endoperoxide is the non-volatile malondialdehyde precursor in Pryor’s mechanism. The
endoperoxide has a structure related to those of the endoperoxides produced in bio synthetic sequence leading to prostaglandins and have also shown that many cyclic peroxides produced during the oxidation of unsaturated fatty acids give a positive TBA test (Pryor et al., 1976). In fact, of the 5 cyclic peroxides only the one with dioxygen functionally on a tertiary carbon failed to give a positive TBA test. Hence, there is probably more than one malondialdehyde precursor. However, the activation of O$_2$ is not the only mechanism of activation of lipid peroxidation. But it is also the process of a chain reaction by its nature, and the influence on the proceeding of the reaction may be the key mechanism of lipid peroxidation regulating in the cell. Studies from our laboratory (ZBRK) have shown that the lipid peroxidation 'in vivo' is of basic importance in aging, in damage to cells by toxic gases, heavy metals and organophosphate pesticide neurotoxicity (Gupta and Hasan, 1988; Haider and Hasan, 1984; Haider et al., 1981; Hasan and Ali, 1981; Bano and Hasan, 1989; Vadhva and Hasan, 1986; and Naqi et al., 1988; Myshkin et al., 1992; Milan and Zuzana, 1996 and Masayuki, et al. 1996).

Fig. A.

Oxidation of a diene (A) and a triene (B) fatty acid system adapted from the mechanism proposed by Dalgleish et al. (1962).
The problem of controlling mechanisms of lipid peroxidation in the cell has become more and more complex.

To date, the effect of AIP pesticides on brain and the rate of lipid peroxidation are limited particularly the effect of AIP on the lipid peroxidation is not known. Therefore, it would be of particular interest to investigate the rate of lipid peroxidation in discrete brain areas after the administration of AIP solution.

2.21 Sulfhydryl Groups (Thiol Groups; SH):

They play a pivotal role in many important enzymes by acting as active enzymatic sites (Hoch and Vallee, 1959). In principle, any enzyme bearing an accessible thiol essential for activity is capable of forming protein mixed disulfides or intramolecular disulfides can increase or decrease catalytic activity and examples of both are known. Furthermore, the extent of enzyme-s-thiolation would depend on the thioldisulfide redox potential as well as the nature of the small
disulfide and the microenvironment around the accessible protein thiol. These parameters are at least potentially capable of conforming to the specificity required for a biological control mechanism through signals transmitted by changes in the thiol - disulfide redox potential as a function of different metabolic states.

Sulfhydryl groups derived from the side chain of cysteine residues, occur in a number of enzymes. Sulfhydryl (-SH) groups and disulfide (-SS groups) bond of cysteine are highly reactive and apparently involved in the maintenance of the conformation and biological activity of certain proteins. As the receptors are protein in nature, the reagents which modify -SH groups may influence the interaction of neurotransmitters with their recognition sites (Sobrino and Del Castillo, 1972).

Sulfhydryl groups play an important role in GST induced detoxification against electrophilic xenobiotics and toxicants by conjugating with such compounds and thus neutralizing their electrophilic sites (Habig et al., 1974).
Glutathione has been considered to function as biological antioxidant. It plays a pivotal role in the destruction of free radicals as well as inorganic and organic peroxides (Sohal et al. 1984; Ji, L.L. and Fu. R. 1992). GSH is a naturally occurring and widely distributed tripeptide. It consists of glycine, cysteine and glutamic acid moieties (Allen and Balin, 1989). It is the major non protein thiol compound present in cells in concentrations which range between 0.1 and 10 mM (Kosower, 1976a). It is synthesized intracellularly by the consecutive actions of glutamyl cysteine synthase and GSH synthase. Its concentration is dependent on metabolic rate and the level of oxidative stress (Allen et al, 1985a). It has been implicated in a wide variety of biological functions, such as the maintenance of cell membranes, destruction of metabolic peroxides and free radicals, detoxification of foreign compounds, removal of $H_2O_2$, maintenance of thiol group of enzymes and proteins, control of redox status, disulfide exchange reactions and transport of amino acids and peptides across membranes (Hazeltona and Lang 1980 and; Ziegler, 1985).
Katoh et al. (1989) observed an enhanced level of lipid peroxides associated with the GSH depletion. The role of GSH in peroxidation is evidenced by the inhibition of oxidative stress induced by different compounds such as ascorbate, NADPH-BrCl, and NADPH-Fe²⁺ (Tampo and Yanaha, 1990). The GSH was observed to protect rats from toxic species engendered by hyperoxia (Van et al., 1985). White et al. (1988) observed that GSH redox cycle increases survival and detoxification of H₂O₂ in hypoxia pre-exposed rats and contributes to tolerance to hyperoxia. Gupta et al. (1986) observed a significant increase in GSH level with antioxidant in mice. Rotruck et al. (1972) reported that Se-glucose - GSH system plays a dual role in the preservation of the integrity of the cell membrane and of haemoglobin against haemolysis and oxidative damage.

In 1970, Tappel had suggested that deficiency of total and free sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxy acids, causing accumulation of peroxides in various regions of the brain.
The concentration of oxidized glutathione or glutathione disulfide (GSSG) reported for various tissues range between 4 and 50 mM (Tietze, 1969). A slight increase in the concentration of GSSG even in the presence of a large excess of GSH, has effects of potential physiological importance (Kosower and Kosower 1974 a). One potent physiological function of the activity of GSSG in inhibiting protein synthesis might be as a control mechanism. If the concentration of GSSG within the cell rises above a certain level, initiation factors are converted into an inactive form and the total rate of protein synthesis decreases. According to Zehavi-Willner et al. (1971) alterations in the GSH/GSSG ratio may also be related to the enhanced rate of protein synthesis. GSH/GSSG ratio is not affected by exercise or by melatonin treatment (Masayuki, et al. 1996).

2.22 Superoxide Dismutase (SOD):

Oxygen is utilized by all aerobic organisms that must have some mechanism by which they can minimize toxicity. One mechanism is the production of superoxide
radical and its dismutation reaction, catalyzed by the enzyme superoxide dismutase (Harman, 1956; 1971). The superoxide anion is a free radical formed by one electron transfer to oxygen by so many spontaneous and enzymatic oxidations (Mishra & Fridovich 1972; Marklund & Marklund, 1974).

\[
\text{O}_2 + e^- \rightarrow \text{O}_2^-
\]

Superoxide dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (Hydrogen peroxide) (Klug, et al. 1972).

\[
\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

This is analogous to the dismutation of hydrogen peroxide to oxygen and water catalyzed by catalase. Ordinarily, electrostatic repulsion between two molecules of superoxide anion. Their approach to one another: SOD overcomes the barrier greatly increases the dismutation rate (Fridovich, 1976; 1978).

SOD appears to protect against the toxic effects of the \( \text{O}_2^- \) free radical and thus provides a mechanism
whereby an organism can avoid possible deleterious effects of this radical or other free radicals which might be produced by its further reaction with cellular components (Fridovich, 1975; McCord et al., 1971). Superoxide arises naturally in some enzymatic reactions (Fridovich, 1978) such as those catalyzed by xanthine oxidase, dihydro-oratic acid oxidase, aldehyde oxidase, tryptophan dioxygenase, or during autoxidation of tissue constituents such as reduced flavins of ascorbate or more dramatically during the rapid spontaneous auto-oxidation of certain neural toxins such as 6-hydroxydopamine or 6-aminodopamine (Cohen and Heikilla, 1974). Superoxide radical at neutral pH can act either as a weak oxidizing agent, e.g. with catecholamines, or as a strong reducing agent, e.g. with cytochrome C.

The SOD enzyme was first discovered in 1969 by McCord and Fridovich and several forms have been identified. They identified the enzymatic activity associated with erythrocuprein, a copper-zinc protein of erythrocytes. The copper is associated with enzymatic activity, whereas the zinc is structural. Similarly, SOD activity is associated with a family of copper-zinc
proteins, cerebrocuprein in brain (Fried, 1979) and hepatocuprein of liver. In mammalian tissues, a second form exists in which manage the prosthetic group (Fridovich, 1976). SOD is localized to mitochondria, whereas the Cu-Zn SOD is cytoplasmic. However, this distribution does not hold in other species.

Regional distribution in the rat showed a relatively homogenous distribution in brain about a twofold range from the highest area (medulla oblongata) to the lowest area (cortex), also subcellular distribution studies in the rat showed the highest level in the cytoplasm while myelin has very low levels. Thomas et al., (1976) indicated that very high levels of activity are present in liver, while the adrenals, kidney and red blood cells have intermediate activity, and lower activities were found in most other tissues including brain (Fried and Mandel (1975).

The principal causes of peroxidative damage has been implicated (Barber and Berheim, 1967; Hougarrd, 1968). Such damage is at least partially associated with the free radicals. The reduction in SOD activity as a
function of age could result in an impaired protection against the toxic effects of O₂ and thus might lead to serve cellular damage (Kellogg and Fridovich, 1976; Vanella et al. 1982, Tayarami et al. 1989). No report, however, is available to date on the effect of AIP toxicity on the SOD activity.

2.23 Glutathione Reductase (GSSG-R or GR):

Glutathione reductase, a heat labile enzyme catalyzes irreversible conversion of GSSG to GSH and accounts for very high GSH:GSSG ratio in the cells. The reaction takes place according to the following equation.

\[
\text{GSSG-R} \\
\text{GSSG + NADPH} \xrightarrow{\text{H}^+} 2\text{GSH} + \text{NADP}^+
\]

GR is reckoned to be as ubiquitous as glutathione and has been studied in various tissues (Ray and Prescott, 1975; Ormstad et al., 1979). This enzyme is isolated from human platelets (Moroff and Kosow, 1978), leucocytes (Ogus and Tezcan, 1981) and erythrocytes (Chang et al., 1978). The primary and unambiguous role of glutathione reductase is of course, to regenerate reduced GSH that has been oxidized (i) non-specifically
by oxygen radicals or peroxides, (ii) enzymatically through the GSH peroxidase reaction, (iii) spontaneously or enzymatically by means of thiol-disulfide exchange reactions or (iv) possible by other redox reactions.

Glutathione reductase has been illustrated to be an inducible enzyme when rat liver cells were treated with various compounds which suggests that GR is of great importance for the protection of cells against toxic agents (Carlberg et al., 1981). The destruction of GSHPx, GSSG-R and SOD activities was found to be the underlying cause of free radical damage caused by reperfusion injury of rat kidney (Okajima, 1990). Benzi et al. (1989) measured the activities of enzymes related to the anti-oxidant system in different regions of brain of rats. In general, both SOD and GR tended to decline during the last half of life. The GSSG-R activity was maximum at 25 days after birth in rats, afterwards the activity decreased continuously in adults but again increased during the developing period, especially in female rats (Santa and Machado, 1986). Stohs et al. (1984) reported that GR activity and GSH content were higher in erythrocytes from mature and middle aged
humans followed by a considerable decline contributing to senescence and increased susceptibility to carcinogenesis and drugs.

2.24 Glutathione Peroxidase (GSHPx; Glutathione: \( H_2O_2 \) Oxireductase):

GSHPx to be a peroxidase in red blood cells and in a variety of tissues (Mills, 1957). The enzyme would be detoxify lipid peroxides by converting the peroxides to their corresponding monohydroxy unsaturated fatty acids (Little and O'Brien, 1968). The reduction takes place at the expense of donor substrate, GSH, which is hydrogen donor to reduce hydroperoxides to the corresponding alcohols.

\[
2\text{GSH} + \text{ROOH} \rightarrow \text{ROH} + H_2O + \text{GSSG}
\]

Where \( R = CH_3 \) or any alkyl group.

These acceptor substrates comprise a variety of biochemically important compounds, such as unsaturated lipids, steroids, nucleic acids (Flone and Gunzler, 1974) and prostaglandins (Nugteren and Nazelhof, 1973).

The enzyme occurs in two forms (i) selenium dependent GSHPx (it catalyzes the reduction of all
hydroperoxides including \( \text{H}_2\text{O}_2 \) and (ii) selenium independent GSHPx (it catalyzes the break-down of only organic hydroperoxides).

The presence of peroxidase in various tissues and the ability of the enzyme to metabolize peroxides of any structure at similar rates has led to the suggestion that GSHPx is the main product within the mammalian cell from peroxidative damage (Chow and Tappel, 1972; Chow et al., 1973). Multiple cellular functions [regulated by GSHPx such as cell division (Kosower and Kosower, 1974a) pentose phosphate shunt (Eggleston and Krebs, 1974) and mitochondrial oxidation of 2-oxoacids (Sies and Moss, 1978). The role of the GSHPx in maintaining the integrity of the erythrocytes membrane has been extensively studied (Beutler, 1972).

Owing to the high concentration of polyunsaturated fatty acids to peroxidative damage GSHPx could provide a mechanism to protect brain tissue against this type of damage. A limited number of studies have been performed with rat brain. A species comparison of levels of GSHPx in the cytosolic fraction was carried out (Demrchine et al.).
1974). The study was performed with unperfused brains may be object to contamination by erythrocytes which contain much higher levels of enzyme.

The increased concentration of GSHPx in growing mouse kidney was reported (Su et al., 1979 and Barlow-Walden, 1995), while blood GSHPx showed an increase only in vitamin E supplemented animals (Pieri, et al., 1994). Other tissues, like lung, liver, uterus and spleen, do not show any increase in the enzyme activity. The GSHPx and catalase increased with age and decreased their highest values by adulthood or senescence respectively in the subendocardial region of heart (Simonetti et al., 1990). Hazelton and Lang (1985) observed GSHPx and SOD showed a lower constant specific activity during the development with a post-natal increase up to adult age of isolated hepatocytes. GSHPx activity inhibited by H$_2$O$_2$ (Ochi, 1990b) but the H$_2$O$_2$ cumene hydroperoxide and t-butyl hydroperoxide as substrates increased GSHPx activity more than 1.5 fold over the period of 1-12 months and remained high in old rats (Lemeshko et al., 1985). Following vigorous exercise, plasma lipid peroxide concentration was
increased and GHSPx activity significantly reduced (Masayuki, et al. 1996).

2.25 Glutathione-S-Transferase (GST):

It is a non selenium dependent glutathione peroxidase (Sies et al., 1979). GST was first identified in rat liver cytosol (Both et al., 1961; Coombs and Stakelum, 1961). The enzyme was subsequently named glutathione-s-aryl transferase. Later on, several other GSTs were demonstrated depending upon the substrate specificity. GSTs are of three types-

i) Glutathione-s-alkyl transferase as a catalyst in numerous reactions in which glutathione participates as a nucleophile (Johbson, 1966);

ii) Glutathione-s-epoxide transferase, active towards the conjugation of active epoxides with glutathione (Boyland and Williams, 1965).

iii) Glutathione-s-alkene transferase, catalyzing the conjugation of unsaturated compounds with glutathione (Covalent linkage).
The concentration of GST is, in general, high in mammals (upto 10% of cytosolic proteins in some organs). In other species (Shark) the level of activity is quite low (Sugiyama et al., 1981). The GSTs are a family of multifunctional proteins that function both as important enzymes of detoxification and intracellular binding proteins (Boyer, 1989). As enzymes, they catalyze the reaction between nucleophil reduced GSH and large number of electrophilic compounds such as polycyclic aromatic hydrocarons, aromatic amines, azodyes, alkylating agents, carcinogens and neurotoxins (Habing et al., 1974; Smith et al., 1977 & Chasseaud, 1979). Additionally, a number of endogenous compounds, including prostaglandins, leucotrienes, organic hydroperoxides (including lipid hydroperoxides and products of lipid peroxidation) and steroids act as substrate for GST (Jakboy, 1978; kaplowitz, 1980). GST catalyzed reactions produce two types of products (Douglas, 1987).

i) a stable gultathione conjugate is formed by the nucleophilic attack of GSH on an electrophilic centre. These types of reactions occur with
substrates such as epoxides (metabolites of benzo)

\[
\begin{align*}
R-X + GSH & \overset{P_{450}}{\longrightarrow} R-SG + XH \quad (i) \\
\end{align*}
\]

Where \( X \) is a leaving group.

ii) A reduced substrate and glutathione disulfide (GSSG) are formed. In this reaction, an unstable R-GS intermediate is the enzymatic product (Eq. ii) which is attached nonenzymatically by a second molecule of GSH, yielding the final product and GSG (Eq.iii). Examples of substrates for this second type of reaction are organic nitrates and organic hydroperoxides.

\[
\begin{align*}
R-X + GSH & \longrightarrow R-GS + XH \quad (ii). \\
R-GS + GSH & \longrightarrow RH +GSSG \quad (iii)
\end{align*}
\]

To date no information is available in the literature on the possible alterations in GST activity in the different regions of rat brain following AlP intoxication. In the present work, it is planned to study the GST activity to evaluate the neurotoxicity of AlP.
2.26 Monomine Oxidase (MAO):

In 1972, Costa and Sandler discovered MAO. It is a flavin-containing enzyme located on the outer membrane of the mitochondria. Oxidative deamination of primary monoamines by MAO produces NH$_3$, aldehydes and H$_2$O$_2$ agents with established or potential toxicity (Cooper et al., 1978; Benedetti and Dostert, 1989).

\[
\text{MAO} \\
\text{RCH}_2\text{CH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{RCH}_2\text{CHO} + \text{H}_2\text{O}_2 + \text{NH}_3
\]

MAO is one of the major mammalian neuronal enzymes. It is active in both neurons and glial cells in the brain. MAO plays a strategic role in inactivating catecholamines that are free within the nerve terminals and not protected by the storage vesicles (Coyle and Snyder, 1981). When monoamines leak from the synaptic vesicles, MAO acts within the nerve fibre itself. The enzyme serves to oxidize some of the 5-HT, DA and NE after their release into the synaptic space in the nervous system, thus terminating their action. The concept of two functionally distinct forms of MAO has gained wide acceptance (Houslay et al., 1976 and Leung et al., 1981).
The MAO-A deaminates neurotransmitter amines such as 5-hydroxytryptamine (5-HT) and noradrenaline (NA) and is inhibited specifically by glergyline \([N\text{-methyl-N-propargyl-3\text{-(2,4, dichlorophenoxyl) propylamine}}]\). Whereas type B oxidizes benzylamine and \(\beta\) phenylethylamine and is preferentially inhibited by deprenyl phenylisopropylmethylpropylamine (Tipton and Dellacorte, 1979). Both forms deaminate substrates such as tyramine and tryptamine (Houslay et al., 1976).

The MAO-B activity increases in human brain in ageing (Robinson et al., 1972), while the MAO-A activity either increases (Shih, 1979) or remains unchanged (Fowler et al., 1980). In old rats MAO-A activity was decreased significantly in all the regions studied except in the cerebellum, where it was unchanged. On the other hand, MAO-B activity increased in all the areas studied except in the brain stem, where it decreased (Leung et al., 1981: Danh et al., 1984). This suggests that the mechanisms which alter the activities of the two forms are not related with each other. Any change in the usual amine concentration will disturb
their activities and result in convulsive seizures (Killian and Frey, 1973). Hence, it is likely that A1P also influences the monoamine concentration in the brain and might also be acting through this mechanism in producing central toxic effects.

2.27 Nucleic Acids (DNA and RNA):

Nucleic acids play important role in protein synthesis. In the brain the storage and transmission of genetic information as well as translation of this information leading to the synthesis of cellular proteins (White et al., 1978).

The tissue components such as average cell densities, dry weight/average cell and total number of cells in each brain area understanding with the help of DNA (May and Grenell, 1959). Generally cells of brain are diploid and contain a fixed quantity of DNA per cell (Heller and Elliot, 1954). The amount of DNA in white matter approximately equals that in the cortex, and regional differences in the amount of brain DNA are relatively small (Elliot and Heller, 1957). However, only cerebellum has exceptionally high amounts of DNA (May and Grenell, 1959).
The central role of DNA is information transfer between generations of somatic cells. Burger (1957) found parallel changes of DNA in growing brain. The low point for DNA is in the third decade, the time at which the brain reaches its greatest weight. As the dry weight of the brain decreases steadily from the third decade of life through the ninth, it is important to estimate if this apparent increase is a real one in terms of the total amount of DNA present in the brain. To answer this question, the weight in grams of dry matter on the basis of the average size brain for each decade of life was calculated. The data suggested that in the old brain, although there was loss of protein and lipid, there might be at the same time an increase in DNA which might be imputed in part to a proliferation of the glial elements. The increase in DNA in old age is due to two factors: an increase in pyknosis of the neurons and growth of glial elements (Burger, 1957). The decrease in DNA in the cytoplasm of brain cells (Hyden, 1955) and break down of DNA in the presence of deoxiribonuclease enzyme. From our laboratory, Tayyaba et al. (1981) reported that there was a remarkable decrease in the
DNA level in all the brain regions studied after ‘metasystox’ toxicosis.

A knowledge of RNA is very helpful in the study of the rate of protein synthesis and also in understanding the functional status of the nervous tissue (Bergen et al., 1974; Vijaya Kumar, 1987). The RNA amount in gray matter is usually higher in white matter (Mihailovic et al., 1958). The nucleolus and in the Nissl substance of the cytoplasm of nerve cells have more RNA concentration (Landstrom et al., 1941). The RNA concentration has also shown variations within different brain regions, the highest concentration being in cerebellum, hypothalamus and cerebral cortex and the lowest in medulla (May and Grenell, 1959). The breakdown of RNA takes place with the help of ribonuclease enzyme.

RNA and protein synthesis may be involved in the accrual of sensory information in the brain, thus indicating a possible approach to elucidation of brain function on a molecular basis (Hyden, 1964). Edstron (1956) and Edstron and Pigon (1958) have reported that there is a proportionality between RNA content and the
surface area of the cell body. Hyden (1964) has reported that the content of RNA neurons vary over a wide range. Increased RNA concentration due to metasystox neurotoxicity in cerebellum, brain stem, and spinal cord in the rat brain have been reported (Tayyaba et al., 1981 Sastry and Siddiqui, 1984). The available literature indicates that the effects of OP compounds on brain nucleic acids is still inadequately understood. As the brain regions show remarkable heterogeneity in nucleic acid contents, it is reasonable to investigate the neurotoxic effects of OP compounds and other pesticides on discrete brain areas. The present work deals with the effect of A1P on nucleic acids level in various regions of rat CNS.

2.28. Protein:

Proteins specific to the nervous system are of interest because they underline the developmental specialization and deifferentiation of the system’s cells. These proteins are usually assayed in terms of their biological activity, for example, as enzymes or receptors for specific ligands or, more generally, as antigens. These properties are also frequently employed as aids in
the isolation and purification of the proteins. Many of the important proteins of nerve tissues, including entities at the synapse, are glycoproteins, that is, they contain oligosaccharide side chains attached to selected aspartate, and perhaps serine and threonine, residues of their polypeptide chains (Mahler, 1978).

Protein, one of the many important biochemical components in the vertebrate brain, constitutes 40% of the dry weight (McIlwain & Bachalard, 1971). The changes in the neuronal activity are accompanied by measurable changes in macromolecules like protein in brain cells. It has also been reported that the increased neuronal activity decreases or inhibits the synthesis of proteins (Hyden and Lange, 1972). The specific neuronal functions such as conduction of action potentials, and synaptic transmission are extensively mediated by protein (Block, 1978). Recent evidences suggest the role of glycoproteins in a number of specific cell-cell interactions, including intercellular adhesion and the mechanisms governing neural histogenesis, regional brain differentiation and the specificity of neuronal associations (Margolis et al., 1975).
Takehara (1956 and 1957) mentioned the existence of a species-specific fraction and an organospecific fraction in the brain proteins. Also Caspara and Fiel (1963) described a brain specific antigen. The S-100 protein (which is heterogeneous) is distributed in all parts of the nervous system, both peripherally and centrally. It is probably a neuronal protein composing no part of the myelin sheath structure (Moore, 1965).

Proteins in the brain are in a dynamic state. Synthesis and catabolism have been intensively studied by Lajtha (1961).

Proteins are both implements and modulators of the autocatalytic and heterocatalytic system charged with genetic continuity and its expression, that is, as components of the replicative, transcriptional, and translational apparatus. Proteins destined for intracellular use, including peripheral membrane proteins and proteins residing on the cytoplasmic aspect of plasma membranes, are synthesized by polysomal arrays in the cytosol, that is, unattached to membranes. Recent studies have disclosed that many proteins are subject to the post translational modification by
controlled proteolysis of defined segments of the newly synthesized polypeptide chain (Lodish, 1976). It has been verified for protein synthesis in Aplysia, is discrete neurons and neuronal clusters with defined function (Berry, 1976 and Loh et al., 1977).

It has been evidenced that many environmental and nutritional factors may bring about the changes in the proteins McLlwain and Bachelard, 1971). The decrement in the protein concentration in various regions of CNS has been observed in the rats treated with different dosës of metasystox (Tayabba et al., 1981).

Brain, in general, has high rate of metabolic activities. It needs more proteins for expected high rate of protein turn over. This view is well correlated with the presence of large amount of cytoplasmic ribosomes, which gives large number of sites for protein synthesis (McLlwain and Bachelard, 1971). Any change in the protein concentration may influence the metabolic rate of the tissue. It requires rapid synthesis and renewal of protein. To analyse this view, in the present work, an attempt has been made to study the changes in the protein content of rat brain due to A1P treatment.
2.29 Acetylcholinesterase (AChE; Acetylcholinehydrolase):

AChE is one example of an enzyme that functions extracellularly; it is localized at functionally specialised parts of plasma membranes, such as the end-plate region of skeletal muscle. This enzyme has an attachment segment that resembles collagen in structure and composition (Lwebuga-Mukasa et al., 1976). AChE of skeletal muscle is located in neuromuscular junction, where it hydrolyzes the ACh released from the nerve terminal. Three lines of evidence indicate that AChE is associated with the basal lamina of muscle rather than being an integral part of the postsynaptic plasma membrane. First, mild chemical treatments release AChE from skeletal muscle or the electric organs of Torpedo. Tissues treated by mild enzyme hydrolysis (Massoulie et al., 1970), or extracted with high ionic strength solutions (Hall, 1972) release AChE. Second, the chemical structures are attached to a filamentous tail that resembles collagen fibrils present in the basal lamina (Taylor et al., 1977).

AChE of brain behaves as if it were assessible to quaternary substrates and inhibitors (Koelle and
Coauthor, 1963). They supposed that this fraction of the functional AChE is outward facing, and the reserve AChE is inward facing and in transit. The ability of peripheral cholinergically innervated tissues to form surplus ACh (Collier and Katz, 1971) in the presence of an anticholinesterase suggests that at least a small part of the enzyme transported by peripheral axons is still in the reserve orientation as it nears the synapses; cerebral cortex, which seems to have less of that ability, may possess only functional AChE. Most tissues contain several forms of AChE (Ulus et al., 1978; Son et al., 1979; Fasbraey, et al., 1990; and Shish, et al., 1993). These are catalytically identical glycoproteins, but they differ in molecular weight, ease of extraction and physical properties.

The most conspicuous feature of all organophosphorus compounds is their structural complementarity with the target enzyme molecule, ChE. In essence, organophosphorus compounds mimic the gross molecular shape of the natural substrate of ChE, ACh. ChE is perhaps one of the most studied enzymes in biological systems. The organophosphorus pesticides
mimic the natural substrate, ACh, by binding itself to the esteratic site of AChE, resulting in the phosphorylation of the enzyme which is inactive (Cremlyn, 1978). Parathion toxicity in rats showed reduced ChE activity (Du Bois et al., 1949). Subsequent researches have also shown that most of the OP's inhibit AChE in the brain of vertebrates after crossing the blood brain barrier (Emsley et al., 1976). Malathion also inhibited the activity of AChE in the rat brain (Paul et al., 1979). The organophosphorus compound diazinon inhibit ChE activity in the CNS and other parts of the body and induce hyperglycaemia (Dybing and Sognen, 1958 and Weiss et al., 1964) and increase the brain level of ACh (Kar and Matin, 1971), resulting in stimulatory effects, tremors and convulsions. The inactivation of AChE as result of OP poisoning results in the accumulation of ACh at nerve endings (Coppage et al., 1975; Cremlyn, 1978; Hall and Kolbe, 1980; and Fasbriey, et al. 1990). Inhibition of this enzyme by OP's is a result of firm binding of phosphate radicals of the OP's to the active sites of the enzyme (Johnson, 1976). This prevents the smooth transmission of nerve impulses across the synaptic cleft (Murphy, 1980), causing
restlessness, necrosis, tremor, ataxia, convulsions and depression of respiratory centres (Murphy, 1980). It has been demonstrated by 'in vitro' studies that the enzyme inhibited by dimethyl-p-nitrophenyl phosphate is more unstable than that inhibited by the diethyl analogue (Aldridge, 1971).

Anticholinesterase poisoning, if not too severe, can be relieved by treatment with atropine, supplemented by a suitable oxime (RCHNOH), if the poisoning is the result of a long-acting organophosphorus inhibitor. The oxime combines chemically with the phosphorus atom of inhibitor and so reactivates the enzyme. It was previously reported that diacetylmonoxime (DAM) readily crossed the blood brain barrier and was more effective than other oximes in reactivating the ChE in the brain (Holmstedt, 1959).

Krupka and Laidler (1961) were especially concerned with the structure of the active centre and the kinetics of enzyme action and inhibition. Their results provide good evidence on the active site of acetylcholinesterase and its mode of action. Investigations on the effect of OP pesticides on brain of
Schematic illustration of major characteristics of synthesis, release etc. for a postganglionic ACh containing neuron. ACh = dots, n = nicotinic receptors, AChE = acetylcholinesterase, ChAT = choline acetyltransferase, Ch = choline, AcCoA = acetyl coenzyme A, m = muscarinic receptor, - = inhibitor of release, + = stimulation of receptor.
both target and non-target animals are abundant but literature on changes in AChE activity due to sublethal concentration are scanty, particularly in rats. Hence, an attempt have been made to study the extent of AlP neurotoxicity and protective effect of vitamin E against AlP in various regions of rat CNS.

2.30 Aims and Objectives of the Present Study:

Review of literature on Aluminium phosphide indicates that most of the investigations on brain focussed less attention to its regional heterogeneity. Since each region has its own function and biochemical constituents, it is now realised that more enlightenment should come from the smaller divisions (Norton, 1980). Hence, in the present work, the rat brain has been divided into different regions i.e., cerebral hemisphere, (cerebrum) cerebellum, brain stem, and spinal cord to study the toxic effect of AlP.

AlP commercially known as Quickphos\textsuperscript{(R)} (Celphos), is one of many pesticides that is of wide use as a fumigant rodenticide in India. The literature on the effect of AlP on rat CNS is meagre and insufficient to understand the neurotoxicity of this pesticide on non-
target animals such as rat. Hence, AlP has been selected for the present study, and vitamin E was chosen as antioxidant. The main objectives of the present investigations are as follows:

1. Open Field Behaviour (OFB) study was done on different parameters: i.e. Ambulation, preening and rearing.

2. To evaluate the quantitative effect of phosphine on the various neurochemical parameters in different regions of CNS. The following parameters were studied:

i. **Cellular components**: Total lipids & cholesterol.

ii. **Free radical substances**: Lipid peroxides, lipid hydroperoxides.

iii. **Antioxidant substance**: Total -SH, Free -SH (Reduced glutathione), oxidized glutathione (GSSG).

iv. **Antioxidant enzymes**: Super oxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GSHPx) and glutathione-S-transferase (GST).
v. **Catacholaminergic** : Monoamine oxidase (MAO).

vi. **Sub Cellular components** : Nucleic acids (DNA) and RNA) and protein.

vii. **Cholenergic system** : Level of AChE activity.

2. To observe the possible protective effect of antioxidant vitamin E on various neurochemical parameters. (i) LPO and LHPO (ii) GSH, GSSG (iii) SOD, GR, GSHPx MAO and GST.

### 2.3.1 Lacunae in Knowledge

A critical literature survey revealed that:

i. Brain lipids are essential components of all cellular structures and the level of lipids is altered during chemical stress. Regional studies of lipids in the different brain parts are relatively limited in number. The effect of AIP intoxication on lipids of the various regions of the CNS is not clear.

ii. Lipid peroxidation is one of the major causes of pesticide toxicosis. It is a free radical mediated chain reaction. During chemical intoxication oxygen species are readily generated. No study could be
traced where the effect of AlP on the products of lipid peroxidation (such as lipid peroxides & lipid hydroperoxides) was considered.

iii. Reactive oxygen species or oxygen-centered radicals damage the cell. A precise nature of oxygen radicals produced in the brain & spinal cord was unclear. The precise nature of oxygen radicals produced in the brain & spinal cord was unclear. The presence of diffusible antioxidants provides protection against free radicals. Glutathione is essential for the protection of cells and protective enzymes such as SOD, GR, GSHPx and GST, are responsible for defense against free radical induced damage. There was no study traceable to evaluate the effect of AlP on these enzymes in the rat brain.

iv. Effect of passage of time on the levels of monoamine oxidase (MAO) has been studied. However, investigations related to the effect of AlP on the MAO are lacking.

v. Studies have been conducted to see the effect of senescence on the nucleic acids (DNA and RNA). DNA is central to genome and RNA is responsible
for protein synthesis. However, the effect of AlP on regional distribution of nucleic acids has not been studied in rat brain and spinal cord.

vi. Major constituents of the cellular organelles and biomembranes are proteins. Protein damage and an increased role of intracellular proteolysis by phosphine have been subjected critical study. However, we could not find any such study where the effect of AlP have been studied in CNS of rat.

vii. To estimate the level of AChE. AChE is a neurotransmitter and responsible for transmission of message. However, information on the effect of AlP on AChE level in rats was not available.

viii. Vitamin E (α-Tocopherol) inhibitor of free radicals. The effect of vitamin E on various regions of CNS in rats following AlP intoxication were not studied to date.

2.32 The Scope of the Present Study:

AlP is extensively used as a fumigant by farmers. It is especially used for the protection of seed grains from nematodes. Hypoxia has been claimed to be a
leading cause of death in cases of AlP poisoning (Chaudhury, 1994). In hypoxia death of neurons occurs within 1 to 5 minutes. It has also been reported that in hypoxic condition, brain lipid level decreases. But the effects of AlP poisoning on lipid metabolism, lipid peroxidation lipid hydroperoxidation and SOD activity have not been evaluated. It would be worth investigating whether like the organophosphate compounds, the inorganic AlP also possess any choliesterase inhibiting activity and Glutathione metabolism.

It has been reported that AlP is as toxic as KCN but no antidote is yet available against it. In proposed research to undertake indepth study of the mechanism of neurotoxicity of AlP with the view of find out a suitable antidote has been undertaken. Earlier, Tayabba et al. (1985) from this laboratory have successfully demonstrated the efficacy of vitamin E against metasystox (an organophosphate pesticide) toxicity.

The study is likely to open up new vistas regarding the effect of AlP and antioxidant, vitamin E (α-Tocopherol) on neurochemical mechanisms is different regions of the rat CNS.