INTRODUCTION

Large scale utilization of the massive mineral resources has been one of the sustaining factors of India's progress. During mining, transporting and processing of ores, large amounts of mineral dusts are released into the occupational and community atmosphere. Prolonged inhalation of dust polluted air causes the accumulation of noxious dust in the respiratory system leading to occupational lung disease called pneumoconiosis. In order to safeguard the health of workers prone to such occupational health hazards, it is essential to evaluate the toxicity of such dusts. Among hazardous particulate air pollutants inorganic compounds of Si such as quartz, asbestos, talc, clay and mice are the most alarming under Indian conditions. The chronic toxicity in human beings and the pathological, physiological and biochemical basis of the toxic conditions have been well studied. However, the basic chemical mechanisms involved in the biological effects for these dusts are not understood.
It is an interesting aspect for bioinorganic chemist to explore the basic mechanisms which render these chemically inert dusts more toxic to the system. There is considerable reason to believe that silicic acid dissolved out gradually from dust could be a factor responsible for pathogenicity. But how such a weak and inert substance affects biological systems especially leading to diverse biological effects like, preferential deposition of fibrous proteins, decreased gas exchange capacity, damage to biomembranes and in some cases even cancer, is not hitherto clear. Therefore, studies on bioinorganic mechanisms in lung tissue was studied in relation to the toxicity of Si containing dust. One aspect important in this respect is a central bioinorganic mechanism involving interaction of Si with proteins being responsible for some of the biological effects. If such a process can explain the molecular interactions and metabolic adaptations found in toxicity, this aspect was studied in detail.

Secondly, the presence of the dust in lung may effect the toxicity and metabolic fate of organic air pollutants. Such additive effects are important in cases where the person is exposed to multiple toxic factors such as dusts and organic solvents. For this purpose, the pulmonary response to aniline vapours has been studied in relation to Si containing dust toxicity.
STUDIES ON ANILINE HYDROXYLASE IN LUNG IN RELATION TO TOXICITY OF SILICON CONTAINING DUST

Aniline is most widely used in industry and today one of the most important aromatic compounds in the market. Environmental and occupational exposure to this compound causes diverse toxic effects such as asphyxia, liver cirrhosis, atrophy and methemoglobinemia. Hence aniline was selected for toxicological studies. Since lung is a site of entry, any toxic effect on lung tissue of aniline *per se* and the chemical transformation aniline undergoes in lung were studied. Using this system, how far Si containing dust influences the toxicity of aniline was also studied. Since the chemical changes which air-borne organic compounds undergo in lung tissue are not understood, so also the chemical characteristics of the pulmonary system are not characterized, therefore, some effects were channelised in this direction too. For these studies an experimental model for studying aniline toxicity was developed by exposing rabbits, rats and guinea pigs to 10 ml aniline vapour in a 175 liter glass chamber for 30 min. Using this system, the introduction of -OH group into aromatic nucleus caused by mono-oxygenase was characterized in detail. Aniline hydroxylase activity of normal rabbits was 30 and 15 times higher than that of rat and guinea pig suggesting this to be a more efficient model for aniline toxicity studies. Single exposure to aniline vapour for 30 min caused significant increase
in the activity of the enzyme without any increase in the protein content indicating the induction of the enzyme. Incorporation of the radioactive amino acids into microsomal proteins also confirm induction of aniline hydroxylase.

Studies with prenatal and neonatal rats showed the developmental pattern of enzymic activity which reached the adult levels by 20 days. The optimal conditions for activity assay of aniline hydroxylase in post-mitochondrial fraction and microsomes was established. By TLC and HPLC the product of the reaction was found to be p' aminophenol. Induction of aniline hydroxylase was also detected after polyacrylamide gel electrophoresis of microsomal proteins. SDS polyacrylamide gel electrophoresis followed by benzidine staining for hemoproteins showed the active cytochrome component to have molecular weight of above 54,000 daltons. CO action spectra showed an optimal peak at 450 nm and comparison with the controls indicated that additional synthesis of the existing components took place rather than formation of new components. The covalent binding of aniline by lung microsomes was established and kinetics studied. The microsomes of control animals had a higher capacity to bind with aniline than those of experimental animals.

Aniline toxicity was accompanied by a decreasing tendency of lipid peroxidation in vitro which may be due to the induction of superoxide dismutase. Lung mitochondria
was a target of aniline toxicity as evident from spectral
and electron microscopic studies indicating swelling of the
organelle. Likewise, the transport of Ca\textsuperscript{45} and its regula-
tion by cyclic nucleotides was affected in aniline toxicity.

\textit{In vitro} studies showed that aniline could be
adsorbed on dusts of quartz and asbestos and kinetics of
this process studied. Quartz dust was found to cause non-
enzymatic hydroxylation of aniline. Intratracheally injec-
ted quartz dust did not have any effect on aniline hydroxy-
lase whereas aniline itself induced the enzyme. When quartz
and aniline were given together there was no induction, thus
indicating that dust exposed lung may have less capacity to
deal with foreign organic compounds.

The above studies revealed the salient features of
the xenobiotic monoxygenase system of lung as compared to
its better studied hepatic counterparts. Its induction on
aniline exposure clearly indicated the capacity of lungs to
chemically deal with xenobiotics as a part of body defence.
The preliminary biotransformation products could be trans-
ported through blood for elimination or to specific tissues
for the target effects of aniline toxicity. It is also
interesting that in a dust laden lung this capacity is
effected adversely so that biotransformation of organic
compounds could be retarded. This indicates that combined
exposure could be a serious hazard if dust exposed workers
are exposed to organic solvents or vice versa. It was also apparent that interaction with Si could affect the pulmonary biotransformation mechanism, thus emphasizing possible bioinorganic interactions.

**BIOINORGANIC STUDIES ON SILICON-PROTEIN INTERACTION**

In order to understand the molecular mechanism responsible for the toxicity of Si containing particulate air pollutants, the interaction between silicic acid and proteins was studied since the results from the earlier section of the thesis showed that silicic acid may have an effect on the hydroxylation reaction on lung microsomes. Hence, protein-Si interaction studies could explain the metabolic changes in toxicity. Using bovine serum albumin as model, the kinetics of Si-protein interaction was studied. In *in vitro* conditions, the reaction was pH governed showing an optimal requirement for neutral pH range. The activity-time response also showed indication of a chemical reaction, binding increasing progressively with an increasing period of Si-albumin contact. The fluorescence of albumin was examined by increasing the amount of Si with a fixed amount of albumin and increasing the amount of BSA against a fixed amount of Si. Even though there was no qualitative change in the fluorescence spectrum, there was a decrease in the fluorescence indicating a possible involvement of tryptophan.
Involvement of other specific amino acid groups of albumin was indicated by the decreased efficacy of BSA-Si complex as a substrate for pure trypsin in comparison to native BSA. That Si could bind with other proteins also became evident because Si-bound trypsin was less active than native trypsin. On the basis of Sephadex G75 molecular sieving, the presence of a protein fraction binding silicic acid was detected in rat lung. Such a receptive protein for Si could be involved in specific toxic effects of Si containing dusts. Evidence for Si-binding proteins was also obtained from protein fractionation studies in rats exposed to Si dust. Thus Si-protein interaction is an important bioinorganic mechanism in Si containing dust.

CONCLUSIONS

From the above results, it follows that the introduction of hydroxyl ions into organic compounds in lung tissue mediated by microsomal monooxygenase, is an early chemical event in the biological effects of organic compounds inhaled through polluted air. The biotransformation of organic compounds in lung tissue, which is the port of entry of air pollutants, is a factor deciding the toxicity on lung cells or after transport through blood or other tissues. The capacity of lung cells to hydroxylate aniline to form p-amino phenol which is more polar and, therefore, more
easily disposable, has been characterized by the present study. The hemoprotein cytochrome P-450 involved in this also has been studied. It is interesting that the hydroxylation enzymes are induced, in response to stress by aniline. This increased activity as a part of the system to defend against the chemical stress by converting it to less toxic product is due to additional synthesis of existing biocatalysts, rather than formation of new molecular models. Under aniline toxicity, oxygen based free radicals are formed leading to membrane damage and ion transport. This could explain the chemical basis of respiratory toxicity by aniline. Silicon containing dusts could adversely affect the metabolic disposition of organic xenobiotics by lung as evident from the decreased aniline hydroxylase activity. In many occupational situations, cohorts are exposed to multiple factor such as dusts and hydrocarbons, the combined effects are often different and more severe than individual exposure. In such cases, any effect of one pollutant on the biological fate of the other could assume significance in the pathogenic mechanism. It may be pointed out that one of the reasons for asbestos induced cancer, has been attributed to the dust interfering with the metabolic disposition of polynuclear aromatic hydrocarbons. These results also suggest the possibility of direct interaction of silicic acid with biomolecules, which may be involved in the effect of dusts alone, or in presence of other toxicants, in
structural and functional molecules such as biomembranes and enzymes. Such bio-organic mechanisms are, therefore, important in the toxicity of environmental pollutants.

Direct evidence for protein biomolecule interaction has been obtained from studies on the chemical nature of protein-Si interaction. This interaction involving specific amino acid residues affects the physiochemical properties and biological functioning of the proteins, as indicated by the trypsin-Si studies. The presence of a Si-binding protein in lung tissue, indicates that particulate Si compounds on prolonged residence in lung, gradually get dissolved in body fluids and the silicic acid gets eliminated through blood and urine. This is indicative of a chemical clearance mechanism for inert inorganic dusts. The presence of protein bound Si in dust exposed animals raises the possibility that protein bound Si in serum could be an index of dust exposure and toxicity and may have diagnostic and curative value. Apart from the above implications in dust toxicity, Si-protein interaction also may be significant in the formation of silicon based stones in plants and animals, the essential nutritional role of Si in mammalian collagen function and on regulating the rheological properties of blood vessels in cardiovascular diseases and in ageing. Further, the important role of Si in diatoms and higher plants, can be explained in terms of Si-biomolecule interactions. Thus, inspite of the inert nature imposed by physical
and chemical factors, Si could have significant biological reactivity in health and disease. Therefore, bioinorganic chemistry of Si, deserves detailed attention in biomedical and environmental sciences.