INTRODUCTION

In the realm of modern biochemistry the rapid strides towards developing an understanding of messenger/regulator functions of calcium in health and disease have been phenomenal, overshadowed perhaps only by the more visible advancements in the areas of molecular genetics and immunology.

The versatility of the calcium messenger system in physiology and pathology can be understood from the transduction of extracellular events into intracellular areas and vice-versa. This could be possible through functions as diverse as inositol lipids and phosphates, diacyl glycerol and protein kinases, intricately modulated homeostasis and fluxes, intracellular compartmentation and metabolic regulation besides many
other specific vital functions (Rasmussen et al., 1990; Pounds and Rosen, 1988).

In many of these processes, structural and functional modulations of the plasma membrane and organelles are involved as a common arena of interplay of free radical processes and Ca functions. The mitochondria are ideal sites for such interactions in view of the versatility of membrane functions and redox processes.

Mitochondria, Calcium and Free Radicals

By elegant multiparameter digitalised video microscopy, Lemasters et al. (1990) showed that hepatocyte mitochondria underwent steady decrease in membrane potential along with bleb formation till the onset of cell death under chemically induced hypoxia. In situ formation of O₂⁻ and interconversion products caused more serious impairment of mitochondrial energetics than when Ca²⁺ alone was used. It was also interesting that local anaesthetic dibucaine, an inhibitor of phospholipase accorded protection to the mitochondria against damage by Ca²⁺ pretreatment followed by active oxygen radical formation (Malis and Bonventre, 1986). In view of this Bonventre (1990) postulated that active oxygen species increase permeability of mitochondrial outer and inner membranes and phospholipase of outer membrane gets activated by mitochondrial Ca²⁺ influx. Lipid peroxidation (LPO) by
the radicals was suggested to make the membrane phospholipids more vulnerable to phospholipase A\textsubscript{2} (Sevanian and Kim, 1985). These data suggest calcium-active oxygen synergism on altered mitochondrial functions. Hepatocytes exposed to 95\% O\textsubscript{2}, 5\% CO\textsubscript{2} showed increased lipid peroxidation parallel to increase in cytosolic calcium (Masaki et al., 1989). Mitochondrial glutathione gets depleted during Ca\textsuperscript{2+} ionophore induced injury to isolated hepatocytes. Presence of low extracellular calcium or exogenous antioxidants like tocoherol accord protection to isolated hepatocytes against oxidative stress mediated injury (Olafsdottir et al., 1988; Pascoe and Reed, 1989).

The now classic studies of Orrenius and coworkers (Orrenius and Bellomo, 1986; Orrenius and Nicotera, 1987; DiMonte et al., 1984) suggest alterations in intracellular Ca\textsuperscript{2+} homeostasis as the cause or consequence of plasma membrane damage or cell death under oxidative injury. Direct evidence has emerged from the studies of Reed et al. (1990). Ruthenium red and lanthanum which block mitochondrial Ca\textsuperscript{2+} uniport prevented malondialdehyde formation, GSH and tocopherol loss and protein SH depletion in hepatocytes under oxidative stress. Ca\textsuperscript{2+} omission also promoted marked loss of mitochondrial transmembrane potential, which was prevented by ruthenium red, EGTA, Vitamin E and desferrioxamine (desferal). The absence of intracellular
Ca was found to promote mitochondrial calcium cycling leading to oxidative stress and cytotoxicity.

Damage to mitochondrial functional organisation under oxidative stress through the activation of non-lysosomal phospholipases and proteases by increased cytosolic Ca has also been implicated as a factor leading to cell death (Nicotera et al., 1986a; DiMonte et al., 1984). Nicotera et al. (1985) were able to correlate oxidative damage with thiol depletion (protein and non-protein) and altered Ca fluxes. Even though mitochondrial calcium homeostasis is mostly within intraorganelle sites, the work of Olafsdottir and Reed (1988) indicated that mitochondrial GSH depletion could be a critical determinant in Ca ionophore induced injury to hepatocytes. Since mitochondrial GSH is arising from the cytosolic pool, its ability to contribute towards protection against cytotoxicity indicates that membrane SH groups are involved in Ca retention (Beatrice et al., 1984; Harris and Baum, 1980). Malis and Bonventre (1986) showed that the oxygen radical mediated disturbance in Ca homeostasis in kidney mitochondria was accompanied by significant functional changes in the organelle. The negative effect of oxidative stress factors on mitochondrial transmembrane potential was abolished by blocking the Ca uniport by ruthenium red, La or EDTA (Thomas and Reed, 1988a,b) along with prevention of LPO and thiol
oxidation. Oxidative stress by paraquat (a quaternary bipyridyl herbicide, similar to methyl viologen) or N-N'‐bis(2-chloroethyl‐N-nitrosourea) (BCNU), an inhibitor of glutathione reductase also caused ATP depletion. The absence of external Ca$^{2+}$ can create oxidative stress in mitochondria. Apparently mitochondrial Ca$^{2+}$ cycling could influence oxidative cell injury as suggested by Reed et al. (1990) by a mechanism different from that of redox‐active substances. The processes involved in this model include increased oxygen radical formation as a consequence of disrupted Ca$^{2+}$ cycling in mitochondria leading to lipid peroxidation, depletion of tocopherol and GSH ultimately causing plasma membrane damage. Several studies with CCl4 indicate that mitochondrial accumulation of calcium is parallel to cell necrosis (Moore et al., 1976). However, in vitro Ca$^{2+}$ sequestration by mitochondria was unaffected by CCL4 due to absence of external Ca$^{2+}$ (Moore et al., 1990).

Regarding the mechanism as to how active oxygen radicals influence Ca$^{2+}$ homeostasis, especially in mitochondria, divergent views have been expressed. Mitochondrial Ca$^{2+}$ is a relatively minor compartment quantitatively in the vanadate‐inhibited ruthenium red‐insensitive processes in cultured renal epithelial cells. Once the non‐mitochondrial compartment is saturated only then the Ca$^{2+}$ buffering capacity of mitochondria operates (Cheung et al., 1986a). The
activation of phospholipase A by $Ca^{2+}$, leading to accumulation of arachidonic acid and the cascade of the regulatory products of its metabolism, was also shown in the kidney cells (Bonventre, 1990). The potentiation of oxygen radical injury to renal mitochondria by $Ca^{2+}$ under conditions of postischemia and toxic mitochondrial damage supports synergistic relation between mitochondrial $Ca^{2+}$ and oxidative stress. This synergistic effect between $Ca^{2+}$ and oxygen radicals on renal toxicity has been suggested by Malis and Bonventre (1986) and Bonventre (1988). Direct evidence for this was explained as a scheme of related events by Bonventre (1990), based on the observations of Thurren et al. (1987) regarding the triggering of phospholipase A.

**Implications of Calcium Functions in Toxicity with Particular Reference to Mitochondria**

Calcium is the most abundant cation and fifth most abundant element in the body. Over 90% of body calcium resides in bones and teeth enamel but the rest, $Ca^{2+}$ which might be described as mobile Ca, is found throughout the body fluids. This component takes part in a host of processes, which include muscle contraction, blood clotting, nerve excitability, intercellular communication, membrane transport of molecules, hormonal responses, exocytosis, and cell fusion, adhesion and growth (Fiskum, 1984).
Under steady state conditions, the cytosolic free $Ca^{2+}$ concentration is generally maintained at approximately $10^{-7} \text{M}$ by the active efflux i.e. $Ca^{2+}$ pumping ATPase or Na$^+\text{-}Ca^{2+}$ exchange (antiport) process and passive influx (Ca$^{2+}$ specific channels or pores) of $Ca^{2+}$ across the plasma membrane. Cellular Ca$^{2+}$ is also controlled and modulated by mechanisms other than transport across the plasma membrane. These include high affinity binding by membrane and macromolecules as well as transport by intracellular organelles.

The modulation of specific Ca functions in the etiopathogenesis of several diseases of cardiovascular (Cheung et al., 1986b), neuronal (Bondy and Kumulainen, 1988), muscular (Dayton et al., 1981) and endocrine (McConkey et al., 1988) systems has been well-established. Similarly involvement of altered Ca$^{2+}$ fluxes in toxicity under xenobiotic mediated stress is becoming increasingly clear (Moore et al., 1990b; Nicotera et al., 1986a). Many such xenobiotics manifest their effects through oxidative stress caused by native oxygen species and other free radicals (Orrenius et al., 1989,1990; Starke and Farber, 1985). In many cases this is also accompanied by changes in functional organisation of biomembranes, eventhough non-oxidative membrane damage is also encountered in toxicity (Casini et al., 1987). However, any mechanistic interrelation between oxidative damage to membranes by xenobiotics and Ca functions is not yet fully clear.
The organelle that generally possesses the greatest capacity for accumulating Ca\(^{2+}\) is the mitochondria. This happens via simple facilitated diffusion in response to the large (\(< 150 \text{ mv}\)) negative inside membrane potential generated by electron transport dependent H\(^+\) extrusion. Once Ca\(^{2+}\) is within the matrix space, it is efficiently buffered to a level of less than 0.1\% of its total concentration by reversible binding and precipitation (Fiskum, 1984). Though the massive loading of Ca\(^{2+}\) as calcium phosphate disturbs mitochondrial function only negligibly (Rossi and Lehninger, 1963), influx of Ca\(^{2+}\) across a damaged plasma membrane is an early common pathway in which cells are killed (Schanne et al., 1979). Thus mitochondria act as safety devices against toxic increases of cytosolic Ca\(^{2+}\) (Carafoli, 1987).

However, the sequence of events and their interrelations are not clear. Judah et al. (1964) proposed that for a series of hepatotoxins the pathways to cell death could be divided into two phases, the first being specific to a given hepatotoxin and the other a common pathway involving Ca\(^{2+}\) influx. Though further studies supported this hypothesis, it has become clear that the Ca\(^{2+}\) mediated final pathway is not universal (Starke et al., 1986; Farber, 1990). Shier (1985) has classified cell killing process into (i) rapid and (ii) slow mechanisms. Rapid cell killing mechanism involves dissolution or destabilization of cell membranes (Weltzien, 1979), physical disruption of
membranes (Bhakdi and Tranum-Jensen, 1983); denaturation of enzymes and structural proteins and hydrolytic, oxidative or other chemical destruction of vital cell components (Frei et al., 1985a). In nature, majority of the cell killing is by slow mechanisms damaging membranes sufficiently to let Ca$^{2+}$ leak into the cell. A wide variety of hepatotoxins manifest their toxicity by causing oxidative stress followed by a sustained increase in cytosolic Ca$^{2+}$ concentration (Orrenius et al., 1989). Chemical modification of critical thiol groups by oxidation, arylation or alkylation could cause inactivation of Ca$^{2+}$-ATPase of endoplasmic reticulum (Moore et al., 1990b) and plasma membrane (Nicotera et al., 1985). This in turn prevents sequestration by endoplasmic reticulum and Ca$^{2+}$ efflux by plasma membrane. The net result could be increase in cytosolic Ca$^{2+}$ concentration. Excessive redox cycling as a result of intramitochondrial pyridine nucleotide oxidation followed by hydrolysis could impair the ability of mitochondria to accumulate, buffer or sequester Ca$^{2+}$ under stress conditions. These events result in an impairment of intracellular calcium homeostasis as well as redistribution of Ca$^{2+}$ as a result of its mobilization from various intracellular stores (Richter and Frei, 1988). Reduced glutathione plays an important role in maintaining -SH groups in reduced state (Orrenius and Moldeus, 1984). Functionally thiol metabolism may be linked with Ca functions under health
and stress (Reed et al., 1990). The sustained increase in cytosolic calcium results in activation of certain \( \text{Ca}^{2+} \) dependent degradative catabolic enzymes like proteases (Nicotera et al., 1986a), phospholipases (Chien et al., 1979) and endonucleases (Cohen and Duke, 1984) as well as bleb formation (Nicotera et al., 1986b) which precedes cell death. Calcium and Ca-related proteins such as calmodulin play a pivotal role in the maintenance of hepatocyte cytoskeletal architecture (Cheung, 1980).

Various Ca-channel blockers like chlorpromazine, verapamil, nifedipine, D 600 (Nicotera et al., 1986a; Campbell, 1983) have accorded partial or complete protection against the onset of cytotoxicity in varied systems. Similarly, inhibition of Ca-activated nonlysosomal proteases by antipain and leupeptin (Nicotera et al., 1986a) accorded protection against hepatotoxins like cystamine, various phospholipase inhibitors like dibucaine, procaine, tetracaine, mupercaine etc. (Campbell, 1983) restored normal mitochondrial function.

Calcium related cell death may also affect genetic loci. Glucocorticoid hormone induced killing of immature thymocytes involves DNA fragmentation, chromatin degradation, nuclear condensation and nuclear blebbing. In normal physiological tissue turn over (apoptosis or programmed cell death) also these changes are involved. A Ca-activated cell endonuclease may
also have a role in cell death (McConkey et al., 1988). Thus calcium seems to be playing a very important role in the manifestation of toxic cell death.

A major manifestation in toxicity of chemicals is the peroxidative decomposition of polyunsaturated fatty acids and the ensuing structural and functional alterations in plasma membrane and organelles leading to metabolic changes. A variety of chemically different prooxidants cause Ca\(^{2+}\) release from intact mitochondria via a route which is physiologically relevant and may be regulated by protein ADP-ribosylation. When the released Ca\(^{2+}\) is excessively cycled by mitochondria, they get damaged. This leads to uncoupling, a decreased ATP supply, and a decreased ability of mitochondria to retain Ca\(^{2+}\). As a result Ca\(^{2+}\) ATPase of the endoplasmic reticulum and plasma membrane are stopped. The rising cytosolic Ca\(^{2+}\) level cannot be counter-balanced due to damage of mitochondria which under normoxic conditions act as safety devices against the increased cytosolic Ca\(^{2+}\) (Richter and Frei, 1988).

Various Pathological Conditions Affecting Structural and Functional Status of Mitochondria

Ischemic mitochondrial injury has been implicated as an important factor in the pathogenesis of irreversible ischemic cell damage (Hillered et al., 1984; Hillered, 1986), oxygen radicals being involved in this. Reperfusion of ischemic tissue halts the Ca\(^{2+}\)
induced cellular damage by supplying the substrates necessary for reenergisation of the mitochondrial transport processes (Fiskum, 1983). Mitochondrial Ca\(^{2+}\) influx and plasmalemmal Ca efflux proceed at near maximal levels soon after reperfusion is initiated followed by progressive impairment of mitochondrial ultrastructural morphology and functions such as swelling, vacuolization, cristolysis, loss of respiratory control and phosphorylation capacity (Fiskum, 1983).

**Mitochondria Under Chemical Stress**

Paracetamol intoxication in isolated mouse hepatocytes resulted in inhibition of cellular respiration as well as a lowering of cellular ATP contents and ATP/ADP ratios (Burcham and Harman, 1990). Neurotoxicant 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) and its fully oxidised metabolite- 1-methyl-4-phenylpyridinium (MPP) ion, caused a concentration and time dependent depolarization of mitochondrial membranes which followed ATP depletion and preceded cytotoxicity (Wu et al., 1990). Mitochondrial damage has been shown to be the primary cause of the MPTP induced death of nigrostraital cells in experimental Parkinson's disease with the involvement of monoamine oxidase B and NADH dehydrogenase being the site of damage (Krueger et al., 1990).
Bandy and Davison (1990) reported that the sensitivity of mitochondrial DNA to damage by mutagens predisposes mitochondria to injury on exposure of cells to genotoxins or oxidative stress. Damage to the mitochondrial genome causing mutations or loss of mitochondrial gene products, or to some nuclear genes encoding for mitochondrial membrane proteins may accelerate release of active oxygen species. Such aberrant mitochondria may contribute to cellular aging and promotion of cancer.

Mitochondrial damage has been shown to act as a mechanism of cell injury in the killing of cultured hepatocytes by tert-butyl hydroperoxide (Masaki et al., 1990). Maitotoxin, a dinoflagellate toxin caused an inordinate influx of extracellular Ca\(^{2+}\) leading to ATP depletion and irreversible cell damage (Kutty et al., 1989). The major site of increased calcium in cystic fibrosis is mitochondria. Cells from subjects with cystic fibrosis consume more oxygen than normal and respond differentially to different inhibitors of the mitochondrial function. Affected mitochondria expressed increased electron transport activity and altered kinetics of complex I (NADH dehydrogenase) along with increased total and resting energy expenditure (Shapiro, 1989). Frei et al. (1985b) reported that alloxan did not inhibit the uptake of Ca\(^{2+}\) but instead, stimulated its release from rat liver mitochondria. Oxidation of critical thiol groups seemed to be a critical event in
the alloxan induced Ca release. Doxorubicin (DOX or adriamycin) is an efficient antineoplastic agent used against human carcinomas, causes severe cardiotoxicity due to mitochondrial damage by reactive oxygen species. Since EDTA or ruthenium red inhibited this, an involvement of impaired mitochondrial Ca transport and the resultant formation of superoxide in DOX-induced cardiotoxicity is likely. In isolated rat heart mitochondria, low concentrations of adriamycin triggered a Ca dependent increase in the mitochondrial inner membrane permeability, modified mitochondrial sulphhydryl groups and oxidised mitochondrial pyridine nucleotides (Singhal et al., 1987; Chacon and Acosta, 1991). Chavez et al. (1989a) found that addition of the alcohol deterrent drug disulfiram to mitochondria induced a complete loss of accumulated Ca, accompanied by a collapse of transmembrane potential. Swelling and a diminution of NADPH/NADP+ ratio were also found. Ruthenium red reestablished normalcy and prevented the oxidation of pyridine nucleotides.

The mechanism of ethanol potentiation of aflatoxin B1 hepatotoxicity was found by Tuskulkao and Glinsukon (1990) to involve mitochondrial dysfunction. Chavez et al. (1989b) showed that Hg causes Ca release from calcium loaded kidney mitochondria through interaction with membrane thiols. Unlike the mitochondrial Ca influx as uptake which takes place through
the electric potential dependent uniport mechanism, the
mechanism of Ca\(^{2+}\) efflux or release from the
mitochondria is not clear. The possibilities are:

(a) Collapse of mitochondrial membrane potential as a
result of over activation of energy yielding
transhydrogenase reaction leading to reversal of
uniport (Beatrice et al., 1980)

(b) Ca\(^{2+}\) induced transition of mitochondrial
configuration could lead to increase in the
permeability (Howarth and Hunter, 1979; Hunter and
Howarth, 1979).

(c) Phospholipase A activation along with inhibition
of 1-acyllysophospholipid transferase resulting in
accumulation of lysophospholipids with a resultant
permeability increase (Pfeiffer et al., 1979).

(d) Formation of hydrophilic Ca channels in the
membrane (Howarth and Hunter, 1979; Hunter and
Howarth, 1979).

(e) According to Richter and Frei (1988) prooxidants
utilize glutathione and cause the oxidation and
hydrolysis of intramitochondrial NAD and release
Ca\(^{2+}\) through ADP-ribosylation of inner membrane
proteins.

The chelator EGTA induces Ca\(^{2+}\) release by reverse
uniport with H\(^{+}\) diffusing back to the matrix space
providing charge movement compensation (Riley and
Pfeiffer, 1986). Hydroperoxy eicosatetraenoic and
hydroxyeicosatetraenoic acids stimulate Ca\(^{2+}\) release
from rat liver mitochondria suggesting a role for eicosanoids in Ca regulation (Richter et al., 1987). Alterations in liver mitochondria as a consequence of CCl₄ was found by Tomasi et al. (1987) to involve formation of trichloromethyl radical. Uncoupling of oxidative phosphorylation and alterations in Ca transport also takes place as a result of CCl₄ exposure suggesting free radical induced membrane and Ca changes (Slater and Delaney, 1970).

Incubation of rat liver mitochondria with menadione in the presence of succinate and rotenone resulted in rapid depletion of glutathione and NADPH oxidation followed by Ca²⁺ release (Moore et al., 1986). Toxaphene, a polychlorinated insecticide inhibited mitochondrial Ca ATPase and Ca transport along with functional impairment of the organelle (Trottman et al., 1985). Hoke et al. (1989) reported that Tri ethyl phosphine-Gold (1) complexes (TEP-Au) induced a rapid concentration dependent collapse of the mitochondrial membrane potential which was potentiated in Ca²⁺ loaded mitochondria and was inhibited partially in the presence of ruthenium red or EGTA. TEP-Au also caused a rapid release of the sequestered Ca²⁺ from the mitochondria along with mitochondrial swelling, increased permeability of the inner membrane and the oxidation followed by hydrolysis of intra mitochondrial pyridine nucleotides. TEP-Au induced mitochondrial alterations were inhibited or reversed by exposure to the sulphydryl...
reducing agent dithiothreitol. Also the TEP-Au induced collapse of the membrane potential was restored partially by the unspecific inhibitor of phospholipases, dibucaine. This supports the multifaceted relation among SH, oxidative stress, membrane functions and Ca fluxes.

Free Radical Formation and Mitochondrial Function

There are many evidences implicating the mitochondrial electron transport chain in the generation of active oxygen species (Loschen et al., 1974; Turrens and Boveris, 1980).

Forman and Boveris (1982) have suggested that the semiquinone form of coenzyme Q (CoQ) is the major mitochondrial autooxidizable component yielding the superoxide radical. More convincing evidence supporting the notion of superoxide production and ensuing H2O2 formation from mitochondrial CoQ has been presented by Boveris et al. (1976). They found that extraction of submitochondrial particles with acetone to remove CoQ and a variety of other lipids as well as cytochrome c decreased the formation of superoxide production with succinate as substrate. Nohl (1986) observed that for the production of superoxide anion from CoQ its protonation is essential. It has been reported that aging weakens this permeability barrier progressively thereby increasing the free radical production at this site of the electron transport chain (Nohl, 1986). Leakage of electrons directly to molecular oxygen i.e. loss of
respiratory control also leads to free radical production in mitochondria (Nohl, 1986). Xanthine oxidase in the native form of xanthine dehydrogenase can be changed to xanthine oxidase under stress. It is an important physiological source of superoxide radicals (McCord and Fridovich, 1968).

**Implications of Impaired Mitochondrial Functions in the Development of Cell-Injury**

Nieminen et al. (1990) and Cannon et al. (1991) have reported that depletion of ATP is a typical feature of hypoxic and toxic injury. The importance of mitochondrial ATP formation and extracellular acidosis was evaluated in hepatocyte suspensions after different toxic treatments. Acidotic pH was protective against cell killing from all toxic treatments except for pronase, a toxic protease. Fructose, a substrate for glycolytic ATP formation, provided good protection against toxicity from cyanide, oligomycin and t-butyl hydroperoxide, menadione and cystamine (Nieminen et al., 1990). Masini et al. (1989) suggested that perturbation of liver mitochondrial Ca²⁺ homeostasis in experimental iron overload could be a possible factor in cell injury. Arnold et al. (1985) pointed out that in vitro changes in mitochondrial Ca²⁺ loading could lead to uncertainties in quantification unless ruthenium red is used to block this in vitro mitochondrial Ca²⁺ uptake during organelle isolation. With this approach Ca²⁺ accumulation during
reperfusion after ischemia along with significant mitochondrial dysfunction was observed. Experiments with sodium orthovanadate, which inhibits both the endoplasmic reticular and plasma membrane Ca ATPases and causes Ca accumulation in mitochondria and experiments with Rhodamine 123, the fluorescent dye which accumulates electrophoretically in the mitochondria also yield similar results support this view (Richlemi et al., 1989).

Trump and Berezesky (1989) have described the sequence of events in cytotoxicity as cell swelling, dilation of endoplasmic reticulum, blebbing at cell surface, condensation followed by swelling of mitochondria and clumping of nuclear chromatin. The subsequent changes include loss of the cell membrane integrity, continued swelling of mitochondria and precipitation of denatured mitochondrial proteins and calcium phosphate in the matrix.

Physiological Aspects of Mitochondrial Calcium Dynamics

McCormack and Denton (1986, 1989) have described the role of Ca ions in the regulation of intramitochondrial metabolism and energy production in rat heart. In the heart and other mammalian tissues there are 3 specific intramitochondrial dehydrogenases activated by increases in Ca. Matrix Ca may thus be a key regulator of oxidative phosphorylation by allowing
stimulated ATP production to occur without the need to
decrease the ATP/ADP ratio.

Mitochondria also act as sink or buffer of
unphysiological increases in cytosolic calcium (Fiskum, 1984). The kinetics of the mitochondrial Ca\(^{2+}\) transport
is such that the efflux of Ca\(^{2+}\) under the physiological
conditions takes place at extremely sluggish or near
negligible rates. The reason is that the Vmax. of Ca\(^{2+}\)
efflux is nearly 100 times slower than that of Ca\(^{2+}\)
influx. Besides the Ca\(^{2+}\) efflux carrier protein becomes
saturated at a very low concentration of accumulated
matrix Ca\(^{2+}\).

Use of Isolated Mitochondria in Predictive Toxicology

In view of the fact that mitochondria are the
sensitive targets of many toxins, the use of isolated
mitochondria as an in vitro test system is gaining
popularity (Knobelochn et al., 1990). The ideal
parameters are (1) the reverse electron transfer test
(2) the electron transfer test and (3) the facilitated
electron withdrawal test, using mitochondrial and
submitochondrial fractions. These tests give data in
agreement with other test systems to make them reliable
and reproducible and are considered to have several
advantages over the current testing methods being
easier, faster and less expensive to perform than whole
animal tests or cell culture analyses (Haubenstricker et
Evaluation of response of mitochondrial membrane potential to toxicants was found by Rahn et al. (1991) to be an indicator of potential cytotoxicity.

Significance of Thiols in Protection of Mitochondria against Stress

Since thiols are among the major antioxidants their homeostasis is an important factor in antistress defences. Griffith and Meister (1985) reported that the net efflux of glutathione from mitochondria is very slow which suggests that this transport mechanism functions to conserve mitochondrial glutathione during periods of cytoplasmic glutathione depletion caused by nutritional and toxicological factors. Mitochondrial glutathione in liver does not arise by intra mitochondrial synthesis, but rather from cytoplasm. GSH biosynthesis is specifically inhibited by buthionine sulfoximine (BSO), an inhibitor of \( \gamma \)-glutamyl-cysteine synthetase. The observation that mitochondrial GSH decreases more slowly than total tissue GSH after BSO administration suggested that there is a separate pool of GSH (Griffith and Meister, 1985).

Ramachandran and Bygrave (1978) reported that the SH blocker \( N \)-ethylmaleimide and parachloro mercury-benzoate added to rat liver mitochondria respiring with succinate as substrate decreased both the initial rate of \( 2^+ \) transport and the ability of mitochondria to retain \( 2^+ \). Glutathione reductase inhibitors rhein and
Nitrofurantoin were found to induce Ca\(^{2+}\) release and inner membrane permeability changes in liver mitochondria. Both compounds caused a depletion of GSH and accumulation of GSSG in energised mitochondria (Beatrice et al., 1984).

Importance of thiol groups in mitochondrial calcium transport was suggested by Chavez et al. (1985). Addition of cadmium chloride (CdCl\(_2\)) to mitochondria induced stimulation of both state 4 respiratory rate and ATPase activity, thereby resulting in uncoupling and impairment of oxidative phosphorylation. These effects were prevented by the addition of Ruthenium Red to the incubation system. Cadmium\(^{2+}\) inhibited Ca\(^{2+}\) translocation in a competitive manner, which was reverted by dithiothreitol, a reducer of -SH groups.

**Concluding Remarks**

From the foregoing it follows that mitochondria could be extremely vulnerable to stress by active oxygen species formation leading to membrane damage and functional alterations. Mitochondria also have an efficient calcium cycle as a significant part of overall calcium homeostasis, which also undergoes stress mediated modulations. Hence there exists a possible interrelation between oxidative membrane damage and Ca cycle perturbation which is the central theme of this
dissertation. It is also evident chronologically that when the present investigation was started over 3 years back, there was very little information in this regard, with most of the data coming very recently. This necessitated simultaneous pursuit in different lines to study the problem, as the work evolved, which are presented as different chapters in this dissertation.