Significant qualitative and quantitative alterations in calcium dynamics pattern occur in a wide variety of physiological situations and pathological conditions. In the toxic effects of several xenobiotics also such changes are implicated. The organelle that possess the greatest capacity for accumulating Ca$^{2+}$ is the mitochondria. It acts as a safety device against toxic increases of cytosolic Ca$^{2+}$. Active oxygen species are known to cause the peroxidative decomposition of polyunsaturated fatty acid rich membranes. Mitochondria act as a major source of intracellular active oxygen species generation. Therefore taking rat liver mitochondria as an in vitro model, experiments were carried out to explore the possible inter-relationship between active oxygen species, calcium dynamics and membrane damage.

Rat liver mitochondria were found to undergo swelling, peroxidative decomposition of lipids under in
vitro conditions involving formation of active oxygen species. Distinct disorganization of ultrastructure such as disintegration of cristae, loss of matrix material, fusion of mitochondria forming aggregates and electron opaque calcium crystals adhering mostly to mitochondrial membrane. $O^-$ mediated swelling caused a significant increase in the outer membrane marker monoamine oxidase but caused decrease in the activity of $\text{Ca}^{2+}$-ATPase and NADH cytochrome c reductase. Glutathione peroxidase and NADase were also decreased by $O^-$ formation. Supplementation with free radical scavengers such as superoxide dismutase (SOD), methionine, histidine and tryptophan accorded considerable protection to the organelle.

Since in vitro swelling of mitochondria was enhanced by in situ generation of superoxide, the influence of different antioxidants and free radical scavengers in this process are tested. Ascorbate below 10 mM acted as a pro-oxidant while higher concentrations in the presence of Fe$^{2+}$ showed antioxidant property. In the presence of $O^-$, 2 mM ascorbate enhanced swelling. Swelling was abolished by $\alpha$-tocopherol and reduced to 50% by BHT. Reduced glutathione reduced both swelling and lipid peroxidation whereas GSSG caused swelling without an effect on peroxidation. Hydrogen peroxide, cumene hydroperoxide and tert-butyl hydroperoxide caused progressive decreases in GSH and reduced niacinamide coenzyme levels. Dithiothreitol was found to abolish
this effect. Thus antioxidants reverse $\text{O}_2^-$ induced mitochondrial swelling and thiol levels are critical in maintaining mitochondrial integrity under oxidative stress.

In order to understand any role of lipid hydroperoxide formation in the superoxide induced swelling of rat liver mitochondria in vitro, the swelling by exogenously added organic hydroperoxide was studied. Tert-butyl hydroperoxide was found to cause swelling, which was inhibited by dithiothreitol, butylated hydroxytoluene, butylated hydroxy-anisole and $\alpha$-tocopherol by reducing the rapid initial phase of swelling. At the initial stage EDTA was more effective than EGTA which is more specific for Ca$^{2+}$. Desferal, o-phenanthroline and bipyridyl also reduced t-BHP induced swelling indicating requirement of iron especially in the initiation phase. Diethyl dithiocarbamate prolonged the lag phase but was less effective than iron chelators in abolishing swelling. Even though externally added GSH had no significant protective effect, depletion of endogenous thiols by diethyl maleate made mitochondria more vulnerable to oxidative stress. Spermine, a known allosteric activator of mitochondrial Ca$^{2+}$ uptake enhanced the initial and later phases of swelling. Lanthanum and ruthenium red reduced t-BHP induced swelling. Phospholipase A inhibitor, dibucaine and inhibitors of Ca$^{2+}$ activated proteases also accorded
protection to varying degrees against t-BHP induced swelling of the organelle. Thus, the in vitro mitochondrial swelling involves peroxidative changes, to initial rapid phase being influenced mainly by iron and the subsequent propagation phase mainly involving calcium.

In order to correlate the mitochondrial swelling with the peroxidative changes, the influence of Fe²⁺ /Fe³⁺ /Cu²⁺ in mitochondrial swelling and lipid peroxidation was studied. Fe²⁺ and Fe³⁺ both enhanced the O⁻ induced swelling as well as lipid peroxidation. EDTA, BHT and mannitol reduced the swelling in the presence of Fe²⁺ and SOD offered complete protection. α-tocopherol showed a biphasic response initially enhancing the swelling but later on it led to contraction. ATP, Mg²⁺ & BSA accorded complete protection against swelling. Desferal did not show any effect in the initial phase but subsequently caused reduction in swelling and also reduced the TBA reactive substance formation. 1,10-o-phenanthroline also reduced the swelling in the presence of Fe²⁺, ADP enhanced the peroxidation both in the absence and presence of Fe²⁺ and Fe³⁺. Diethyl dithiocarbamate inhibited lipid peroxidation caused by Cu²⁺ but it was less effective in the case of Fe²⁺ induced swelling.

For understanding the physiological and toxicological significance of the involvement of altered calcium function in the peroxidative membrane damage the
effect of several biological response modifiers on mitochondrial swelling was studied. Mitochondria isolated from anaesthetic ether exposed animals showed a higher magnitude of calcium induced swelling. CCl₄ which in vivo produces hepatotoxicity through free radical related processes also caused mitochondrial swelling in vitro. Histamine itself caused swelling, whereas antihistamines- phenergan and benadryl drastically reduced the calcium induced in vitro swelling. Anti-inflammatory agents aspirin and indomethacin did not affect the initial rapid phase of swelling but reduced it during the later phase. The uncouplers of oxidative phosphorylation and electron transport chain blockers such as DNP, antimycin A and rotenone reduced swelling in the presence of calcium. The respiratory inhibitors KCN and sodium azide completely abolish this swelling whereas oligomycin was ineffective. Trifluoperazine, an anti-calmodulin agent did not influence the initial phase of calcium induced swelling but the subsequent phase was reduced. Calcium ionophores calcimycin and lasalocid acid caused swelling and also showed a synergistic effect in the presence of Ca²⁺. Similarly cAMP itself caused swelling as well as enhanced the swelling by CaCl₂. In the presence of DO the swelling phenomenon was prolonged as compared to H₂O. A possible correlation among oxygen radicals, membrane integrity and calcium functions is indicated. Thus, the mechanisms in toxicity at the membrane level, can be
correlated in terms of inter-relation among calcium function, oxidant injury and antioxidant pool.