CHAPTER V

CONCLUSION

The radiomimetic drug, Busulfan is known to react with thiols causing an alteration of the function of certain proteins and the sequence of amino acids in a peptide or protein chain, which might account for the increase in water insoluble proteins in the busulfan treated but non-cataractous lenses. Gradually many enzyme systems containing thiol groups are affected thus upsetting the ionic balance and permeability during the cataractous stage of the lens.

The electrophoretic studies showing 41 and 45 K dalton bands indicate that the cytoskeletal constituents of the lens may be disorganized and disintegrated which may account for the balloon shaped cells at the cortical region as seen in the light microscopic studies. Impermeability of the metabolite formed by the reaction of busulfan and thiol groups, to the deeper regions of the lens (nuclear region) could be the reason for the intact γ-crystallins. As most of the enzyme systems are present outside the lens nuclear region, they seem to be the first ones to be altered by busulfan than the structural proteins of the lens.
It is known that DNA synthesis is not inhibited initially but the cells are prevented from entering mitosis. Thus the nucleic acid increase is reflected in the increased protein synthesis. In the cataractous condition, fragmented and distorted nuclei are observed in the light microscopic studies. Busulfan's site of action in the nucleic acids is speculated to be in the phosphate portion and the amino group of the purines and pyrimidines.

An increased uptake and utilization of glucose and high lactic acid levels in the lens suggested an increased metabolic rate in the treated, non-cataractous stages of the lens, while a contrary result in these parameters indicated failure of the energy producing mechanism during cataractogenesis.

Fructose levels and sorbitol dehydrogenase activity showed an increase in the treated, non-cataractous lenses which indicated activation of the sorbitol pathway but the sugars of this pathway are not accumulated in the cataractous lenses as in the diabetic cataracts.

Diminution of GSH could be due to the accumulation of oxidised glutathione (GSSG) whose reduction to GSH by glutathione reductase (GR) and NADPH is hampered in the cataractous lenses.

Increase in the insoluble protein bound-SH and decrease in the soluble protein bound-SH has lead to altered protein patterns causing scattering of light in the opaque lens.

An acceleration of the turnover rate of GSH metabolism is indicated by the increase in χ-glutamylcysteine synthetase (χ-GCS)
and γ-glutamyl transpeptidase (γ-GTP) activity. This increased metabolic rate suggests detoxication of the increased toxins formed by busulfan in the initial stages of the treated, non-cataractous lenses. In the cataractous lenses, the cumulative action of busulfan on GSH and γ-GCS bringing about its dethiolation could hinder the detoxifying mechanism. Decrease in GSH, a substrate of γ-GTP, inhibits the feedback mechanism thus reducing the activity of γ-GTP to the normal state in cataracts.

Detoxification of toxic elements formed in the lens initially i.e. in the treated but non-cataractous lenses could induce an increase in the GR activity. On the contrary, in the cataractous lens, the reduction in GR activity implies that busulfan's action on the SH groups of GR has altered its activity and is no longer able to keep pace with the increased toxins of the lens. This could affect both proteins and GSH leading to the accumulation of GSSG and H₂O₂, the latter being toxic to the lens.

Glutathione S-transferase (GST) activity also shows the same conclusive results as GR; an initial increase in its activity providing protection against xenobiotics and aiding in the metabolism of the drug, in the treated but non-cataractous group of lenses, while in the cataractous lenses its reduced activity nullifies the above mechanism.

A defensive response in the busulfan treated but non-cataractous lenses is indicated by an increase in the superoxide dismutase (SOD) activity. In the cataractous lenses, its reduction in activity is speculated to be due to the increase in H₂O₂ which
is known to inactivate SOD activity which in turn impairs its defence against $O_2^-$ toxicity.

Increase in succinic dehydrogenase (SDH) indicates an increase in the demand for energy in the lens epithelium. The excess of this energy might be used by other detoxification and antioxidant systems to combat with toxic elements in the lens during the treated, non-cataractous stage of the lens. Alterations in this enzyme or other enzymes associated with the citric acid cycle could inhibit SDH activity during opacification of the lens.

Although there is a slight decrease in glucose-6-phosphate dehydrogenase (G6PD) activity, it is not significant enough to hinder the generation of NADPH. In addition, the increase in GR activity and the normal levels of GSH is capable of encountering the oxidative stress posed by busulfan in the cataractous lenses. Of all the enzyme systems studied, G6PD is the only enzyme known to decrease slightly in the initial stages indicating that this enzyme might be one of the first targets of busulfan.

Increase in Total adenosine triphosphatase (ATPase) supports the fact that there is an increase in the metabolic rate of the lens probably to help trigger the defence mechanism as well as to counteract damages to the permeability of the lens and unbalancing (electrolytes) effects encountered in the treated non-cataractous lenses. The slow action of busulfan on the SH groups of this enzyme could diminish its activity thus disturbing the electrolyte balance and permeability of the lens in the cataractous stage. In addition, its substrate, ATP, may not be available
at this stage which in turn inhibits the ATPase activity.

The LM and SEM studies support the biochemical and electrophoretic studies which implies that disorganization of cytoskeletal elements, high $\text{Ca}^{2+}$ levels and hydration can cause swelling of lens fibers in the cataractous lenses while the initial causes may be pertained to the action of busulfan on DNA and RNA causing alterations in the gene information and/or direct involvement of the drug on the protein-SH groups causing structural and functional changes to the lens fibers.