CHAPTER V
5. GENERAL DISCUSSION

Mechanism of drug action and drug metabolism have been the least understood chapters in pharmacology. In addition, the discovery of new drugs with many fold efficacy against hitherto unconquered diseases set fresh targets for the scientists to achieve. Antileprosy drugs like rifampicin and clofazimine are such drugs which need intensive probing. The present study analyses some of the pharmacophysiological and pharmacobiochemical properties of rifampicin and clofazimine in mice tissue.

Investigation on analysis of clofazimine persistence reveals that the adipose tissue had the maximum deposition in 30 days of treatment. It indicates the lipophilic nature of the drug which is confirmed by the absorbance properties (Chapter 2). It further depicts dynamic changes in its deposition in different tissues. The finding that differential tissue deposition with time can be related to the mobilisation of clofazimine (cf. Sec. 3.3.1) corroborates the earlier reports of Desikan and Balkrishnan (1976). The drug mobilisation in turn has been related to the number and rate of migration of macrophages in leprosy patients (Conalty et al, 1971). These events following CLF administration culminate in the macrophage induced bactericidal action.

Study of tissue ascorbic acid content and Alkapase activity during clofazimine treatment suggests gross changes in general metabolism in presence of the drug. Consistent increase in the ascorbic acid level (at 0.1mg/day dose) may be correlated to the clofazimine content of the tissues. On the otherhand it can also be related to the metabolism of drug as
suggested by Venkatesan et al. (1979). From the data it is evident that this proposition is most likely to be true during the first 21 days of treatment when the deposition increases linearly (Fig. 3.2). Beyond this period the drug content decreases due to its rapid mobilisation, a process which can be evidenced by the change in serum Alkapase activity.

All these findings depict gross change in the metabolism in mice tissue as a result of the drug action. Nevertheless deeper understanding of the mechanism of action of the drugs can be achieved by studying its action on the energy metabolism at subcellular level. In the present investigation this has been attempted through assay of some key enzymes of the metabolic pathways related to the energy metabolism.

HMP Shunt, one of the major cytosolic oxidative pathways is represented by the enzyme G6PD whose activity is crucial for its operation and consequently the production of NADPH. The drug degradation pathway requires some inducible enzyme systems like hydroxylases and Cyt P₄₅₀ monoxygenase which require a large amount of NADPH. Therefore all drugs affect this pathway during their degradation. In other words the changes in G6PD activity reflects the degradation of the drug in the tissue. In the present investigation the change in G6PD activity suggests slow degradation of clofazimine in liver tissue. Similarly LDH which represents the anaerobic pathway of glucose oxidation is also affected by the drug. Increase in LDH activity in both liver and kidney tissues indicate possible shift to anaerobic energy production during the course of drug action. It reflects role of the drug in aerobic or mitochondrial energy metabolism.
Interaction of a drug with mitochondria would result in three possible changes. (1) Drug binds to the membrane and have an allosteric effect on the membrane function. (2) Drug crosses the membrane barrier and enters into the mitochondria to combine with the metabolites. (3) It enters into the core of the membrane to alter its functions such as premeability, electron transport and oxidative phosphorylation. The interaction of the antileprosy drugs with the membrane is most relevant for analysis due to their highly lipophilic nature, a property which has been analysed in Chapter 2 and also been reported earlier (Morrison and Marley, 1976a; Usha Deniz, 1983. Gilman and Goodman, 1985). The analyses of mitochondrial parameters such as hypo-osmotic swelling, permeability, SDH, MDH and GDH reveal that the antileprotic drugs have profound effect on the mitochondrial functioning. The changes in hypo-osmotic swelling and succinate dehydrogenase activity indicate the effect of the drugs on the functioning of the outer and the inner membrane of mitochondria. The inhibitory effect of CLF on malate dehydrogenase a matrix enzyme shows that the drug must be entering into the matrix crossing the inner membrane. The increment in GDH activity further strengthen this argument and depicts possible increase in amino acid metabolism linking to the TCA Cycle, while the decline in MDH activity gives an indication of inhibition of the enzymes of TCA cycle.

In this context the findings of Rhodes and Wilkie (1973) and Delhanty et al (1974) holds key evidence to the effect of CLF on oxidative phosphorylation. They have suggested that the drug might be acting as uncoupler to the oxidative phosphorylation along with increase in $O_2$ consumption. Present finding substantiates their views due to the fact that the drug affects the succinate dehydrogenase segment of electron transport
pathway (Complex II) and increases the rate of $O_2$ uptake (preliminary data not published). Therefore the drug acting as a competitive inhibitor for cytochrome oxidase as suggested by Rhodes and Wilkie (1973) is the most plausible explanation.

With regards to rifampicin there are reports of the inhibitory effect on SDH (Follet and Pennington 1973; Adhvaryu and Saha, 1984) which is supported by the present findings. However, whether this drug acts as uncoupler or inhibitory uncoupler to oxidative phosphorylation needs further investigation which we hope to take up in future.