SUMMARY AND CONCLUSION
Filariasis, which has been a major health problem of tropical and subtropical regions of the world has gained national importance because of intensive industrial development and thus the migration of population from endemic to non endemic areas. 'Elephantiasis' which is caused due to immunopathological reactions of the parasite with the host, is not fatal, but it causes considerable disability and social stigma. Till date, no effective drug has been developed for curing the disease. The knowledge about the biochemistry of the filarial parasites is not sufficient to develop any effective compound for curing the disease. Study of the cellular and metabolic machinery of the parasites can prove to be a fruitful target for antifilarial chemotherapy.

The investigations which have been done so far on the filarial worms so far, show that there is anaerobic metabolism of carbohydrates for energy generation and there is consumption of limiting amounts of oxygen (when available). The oxygen that these filarial parasites (homolactate fermentors) require is only for the oxidation of pyruvate to acetate and CO$_2$ which provides the extra energy required for the survival of the parasite.

The oxygen uptake studies (in relation to substrates and inhibitors) and the investigation of the haemoproteins which have a defensive mechanism against activated form of oxygen and in the disposition of drugs can prove a good chemotherapeutic target. PEP-succinate pathway enzymes and their sensitivity to the anti-filarials can also provide useful biochemical leads for the develop-
ment of new drugs. The purification and characterization of the regulatory terminal branchpoint enzyme (PEPCK) can also prove useful as this enzyme channels the carbon (to succinate as end product) in the generation of energy in helminths.

Due to the non availability of human pathogens in sufficient amounts, *Setaria cervi* the bovine filarial worm, resembling human parasite in microfilarial periodicity and antigenic pattern has been taken for biochemical studies.

Some preliminary experiments on oxygen uptake were undertaken using *S. cervi* (whole/cut worms) as well as its different body parts (cuticle/intestine/genital tract). Then subcellular fractions including mitochondria like particles (MLP) were studied. Subsequently the E-QO₂ studies on MLP were done to elucidate the nature of electron transport chain.

Sodium azide (1 mM) affects the motility of *S. cervi* soon after the addition suggesting a role for alternative oxidases or inhibition of catalase. Negative effect of cyanide at 1 mM on the motility of the parasite also suggested the presence of cyanide insensitive pathway in this filarial worm. Recognised inhibitors like sodium arsenite, salicyl hydroxamic acid (SHAM) and amytal did not exhibit damaging effect. A comparison of the endogenous O₂ uptake by female, male and mf stage indicated that E-QO₂ for female, whole worm was higher than for uteri-free female *S. cervi*. EQO₂ for male is lower than that for female *S. cervi*. Mf stage also consumes considerable oxygen.
Glucose, a substrate for the glycolytic pathway in *S. cervi* did not enhance O\(_2\) uptake showing that the parasite has a glucose independent respiratory pathway. Incubation of worms in Ringers medium in presence and absence of glucose for six hours did not show any difference in the E-QO\(_2\) values or their susceptibility to KCN. *S. cervi* thus possesses considerable reserves of substrate supporting oxygen uptake.

Earlier studies indicated in this laboratory had indicated that a few filaricidal drugs (suramin, DEC, centperazine and CDRI compound 72/70) exhibited inhibitory effects on the enzymes of energy generating pathways in *S. cervi*. However these drugs did not significantly effect the E-QO\(_2\) of *S. cervi*. Slow permeability of drugs through cuticular layers and very short duration of incubation could be responsible for the lack of any observable effect. The effect of varying concentration of KCN (1, 2, 5 mM) on respiratory pathway of female worm showed that E-QO\(_2\) was inhibited by 49-72%. Since large size of the worms (4-6 cm) may lead to variable results for sometimes it gets entangled to electrode of the reaction chamber cut segments (1 cm size) of *S. cervi* were also used for seeing the effect of cyanide and a few substrate like glucose succinate and fumarate. This did not lead to any consistant or discernible pattern and hence do not offer any distinct advantage over the use of whole worms. The effect of KCN and succinate on E-QO\(_2\) values of different body parts (cuticle/genital tract/intestine) were estimated and the results indicated
that cyanide was more inhibitory on whole worms as compared to isolated genital tract or cuticle. Cuticular portion exhibited strong activation (41-60%) with succinate whereas genital tract and whole worm showed lower oxygen uptake.

The mitochondria like particles were prepared from the cuticular portion of _S. cervi_ and tested with succinate to see the type respiratory electron transport chain operating in this parasite. Though succinate enhanced oxygen uptake, no effect of cyanide was observed in MLP of cuticle.

Subcellular fractions (cell free supernatant, MLP and post mitochondrial fraction) of _S. cervi_ whole worms were also analysed. MLP showed highest E-QO₂ values, however, cyanide had no effect. Addition of fumarate, succinate, glutamate or ascorbate enhanced E-QO₂ thereby suggesting the presence of an electron transport pathway using these as substrates. On the other hand addition of some intermediates of the metabolic cycle viz., malate, pyruvate, alpha ketoglutarate, oxaloacetate as well as glycerophosphate, L-ornithine, putrescine and GABA did not show enhancement of oxygen uptake.

Comparison of the effect of sodium azide, sodium arsenite and 2,4-dinitrophenol indicates that azide was most toxic. Low concentration of 2,4-dinitrophenol showed stimulatory effect, whereas higher concentration (5 mM) exhibited inhibited which may be due to uncoupling of oxidative phosphorylation. Respiratory chain poisons (SHAM and cyanides) alone could not inhibit the electron transport but when combined with azide they showed slight inhibition.
The effect of a few RET inhibitors viz., rotenone, antimycin A (substrate dependent/substrate independent), cyanide and azide on the MLP from *S. cervi* were studied. Inhibition of rotenone established the presence of complex I but antimycin A, azide and cyanide did not show any marked effect on the respiration thereby suggesting the presence of refractory branched chain electron transport (in addition to complex II and III) in *S. cervi*, which are being mediated by alternative oxidase (like cytochrome O) present in the helminth parasites. It is also not clear as to what extent these alternative pathways contribute to the energy charge/relax homeostasis of these filarial parasites.

The various subcellular fractions were analysed for haeme as well as for the haemoproteins viz., cytochrome *b*$_5$ and cytochrome *P*$_{450}$. A typical haeme spectrum could be observed at 556 nm in all the subcellular fractions, however, there was no peak at 450 nm showing the probable absence of cytochrome *P*$_{450}$. The peak at 424 nm corresponding to cytochrome *b*$_5$ was detected in the dithionite reduced spectra of post mitochondrial as well as cytosolic fraction but was not clearly discernible in the microsomal fraction. A distinct peak at 418 nm was also observed which could not be identified so far despite many efforts made.

Subcellular localization of the enzymes of PEP-succinate pathway in *S. cervi* adults show that pyruvate kinase (PK), PEP-carboxykinase (PEPCK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme and fumarase were localized in the cytosolic fraction whereas succinate dehydrogenase and fumarate
reductase were found to be of mitochondrial origin. Among cytosolic enzyme MDH was most active exhibiting 308 units/g wet worms. Significant amounts of LDH, fumarase, PEPCK and PK were observed while the level of malic enzyme was very low. The mitochondrial pellet showed the presence of 0.34 and 0.020 units of FRD per g wet worms. Among the different filaricides screened suramin was most effective inhibiting PK and fumarase at 1 mM during in vitro incubation while centperazine and DEC exerted inhibitory effect on these enzymes at 10 times higher concentration. Maximum inhibition of PEPCK was achieved by 72/70 lowering the enzyme by 40% at 1 μM while suramin was effective at 100 times higher concentration. Levamisole, DEC and centperazine could not inhibit PEPCK at 1 mM. LDH was inhibited by suramin (10 μM),centperazine levamisole, compound 72/70 and DEC at 100 μM concentration while MDH was inhibited by suramin (10 μM) and levamisole and centperazine (100 μM). Malic enzyme was maximally inhibited by suramin (100 μM) and centperazine (1 mM). Among the mitochondrial enzymes SDH was inhibited by suramin, centperazine, compound 72/70 and levamisole at 100 μM concentration, while 1 mM centperazine was needed for exhibiting inhibitory effect on FRD.

A study of the effect of these drugs on motile worms during in vitro incubation indicated that suramin, centperazine and levamisole could exert inhibitory effect on most of the enzymes of PEP-succinate pathway. It is quite possible that the cuticle of the worms is serving as a barrier for the transport of these drugs and low amounts are crossing the cuticle barrier.
Phosphoenol pyruvate carboxykinase (PEPCK) was partially purified using a combined procedure of (NH₄)₂SO₄ fractionation, gel filtration through Sephadex G-25 and affinity chromatography on blue sepharose CL-6B. Adult worms were homogenised in presence of DTT and PMSF for avoiding denaturation of the enzyme and the digestion by endoproteases. In the final stage of purification over 99% of inactive proteins were removed and 24% of original PEPCK was recovered. The enzyme was purified over 40 folds. The recovery of 117 and 164% of the enzyme in PMF and (NH₄)₂SO₄ fraction indicates the removal of an inhibitory factor during purification procedure. Tortora et al. (1985) had earlier demonstrated the existence of an inhibitor in yeast during purification of PEPCK which was identified as adenylate kinase.

The purified enzyme was very unstable and lost most of its activity when stored at -20°C in its native state. However the enzyme could be stabilised by the addition of DTT and PMSF. The enzyme was cold labile and lost 25% of its activity within a week when stored at 4°C in presence of stabilisers. In general the properties of PEPCK from bovine filarial worm did not differ markedly from the analogous enzyme from some helminths and vertebrate tissues mediating the same reaction. Optimal activity was recorded at pH 6.5. Km value was 2.0 mM with respect to PEP, the enzyme showing hyperbolic saturation curve. 0.5 mM GDP activated the enzyme while ATP lowered and FDP registered activating effect. A study of the effect of anions and cations indicate
that MnCl₂ (10 mM) and bicarbonate (50 mM) were needed for showing optimal activity whereas MgCl₂ (2 mM) showed inhibitory effect.

An overview of the findings presented above in this dissertation indicates that the S.cervi possesses a respiratory electron transport chain which deviates from the classical transport chain and not like ... the classical mammalian RET pathway. The cytochrome system lacks cytochrome P₄₅₀ which can be a good chemotherapeutic target and compounds can be developed which could be detoxified by the host but not the parasite infecting them. Further the drug resistance a major role played by cytochrome P₄₅₀ could cease in the organism. In general the properties of PEPCK from bovine filarial worm did not differ significantly from the analogous enzyme obtained from helminths and vertebrate hosts. The studies also indicate the sensitivity of some enzymes of PEP-succinate pathway to filaricidal drugs, suramin showing maximum effect.