CHAPTER V
SUMMARY

The results of the present study have shown that the effects of anthelmintic compounds on the metabolic activities of parasites can be assessed by using different standard biochemical and physiological parameters. It is clear that a single compound may have different sites and modes of action in the metabolic pathway of a parasite. The mode of action of each anthelmintic understudy, viz., mebendazole, fenbendazole, metrifonate and oxyclozanide, is different. However, in the present study, these compounds show some differences in the relative activity, on comparative basis in the trematode and cestode, which is possibly a consequence of the differences in the metabolism of these parasites belonging to two distinct taxonomic groups. The ultimate, major effects of the anthelmintics understudy, appear to be associated with the energy yielding metabolism, nutrient uptake mechanisms and neuromuscular coordinations.

MEBENDAZOLE:

Mebendazole is a broad spectrum anthelmintic because it is effective in both the parasites understudy. The drug inhibits the glucose uptake, which is probably due to the inhibition in the acid and alkaline phosphatases and adenosine-triphosphatase of these parasites due to mebendazole, as evident
by the present biochemical and histochemical findings. However, the drug inhibits glucose uptake in \textit{S. globipunctata} at a higher level as compared to \textit{G. explanatum}.

Mebendazole also causes a depletion in the glycogen content of the parasites under study, only when glucose is present in the incubating medium. This shows that the glycogen depletion induced by mebendazole is a secondary effect which is caused due to the inhibition in glucose uptake (primary effect). Mebendazole causes significant changes in the phosphorylase and phosphoglucomutase levels of the parasites, as evident from the biochemical observations, which ultimately results into the depletion of glycogen contents. Glycogen depletion due to mebendazole occurs at a higher level in \textit{G. explanatum} as compared to \textit{S. globipunctata}.

From the autoradiographic studies, it is revealed that the tegument of \textit{G. explanatum} is also actively involved in the glucose uptake, besides the intestinal caeca. Mebendazole inhibits the tegumental glucose uptake in this amphistome and the drug possibly disrupts the microtubular apparatus of the tegument, due to which, the tritiated glucose is unable to move across the tegument, thus resulting into their blocking or accumulation at surface of the tegument.

In \textit{G. explanatum}, mebendazole causes a reduction in
survival time with the increase of drug concentration. The drug is unable to cause mortality in this parasite, but it disturbs the normal motility (in the form of amplitude and frequency) slowly and gradually. The drug also causes detachment of the trematode from the host tissue.

Mebendazole causes superficial topographical damage in *G. explanatum*. The surface annulations remain unaffected. Very few papillae are deformed or flattened, thus causing few superficial lesions. The drug also has a peeling effect on the tegumental layer. These effects probably produce alterations in the tegumental organisation which results into the inhibition of glucose uptake in *G. explanatum*.

**FENBENDAZOLE:**

Fenbendazole has a similar action as mebendazole, on the parasites understudy. The drug inhibits the glucose uptake in *S. globipunctata* at a higher level as compared to *G. explanatum*. Like mebendazole, fenbendazole also causes depletion in glycogen level at a higher level in the trematode as compared to the cestode. Fenbendazole causes glycogen depletion even in the absence of glucose. Thus, it appears that glycogen depletion in helminths due to fenbendazole, may be a direct action of this drug.

Fenbendazole is also unable to produce mortality in
G. explanatum, but causes a disturbance in the motility in a shorter interval of time as compared to mebendazole. The drug reduces the survival time with increased drug concentration and also detaches higher number of worms from host tissue as compared to mebendazole.

The topographical damages in G. explanatum are also more pronounced with fenbendazole as compared to mebendazole. Superficial lesions are formed due to abrasion of surface papillae. Majority of papillae collapse, giving a wrinkled appearance to the body surface. The ridges and furrows from the dorsal body surface disappear giving smooth appearance. These effects probably after the tegumental structure, which results in the inhibition of glucose uptake.

Since both, mebendazole and fenbendazole are the members of the same group (Benzimidazole), therefore, their mode of actions are almost similar in both parasites. However, differences in their primary and secondary effects cannot be ruled out. For glucose uptake inhibition, mebendazole is more effective than fenbendazole, while for glycogen depletion, the reverse is true in both the helminths understudy. Further, for the in vitro survival, worm detachment from the host tissue, motility and topographical damages in G. explanatum, fenbendazole is more effective as compared to mebendazole.
METRIFONATE:

Like the benzimidazole compounds, metrifonate also inhibits the glucose uptake in the helminths under study. This inhibition is at higher level in *G. explanatum* as compared to *S. globipunctata* probably because papillae are more sensitive to metrifonate than microtriches. Interestingly, it is found that these parasites start recovering their glucose uptake after the first 3 hours of incubation with metrifonate. It appears that metrifonate is unable to deteriorate microtubular systems of these helminths as reported for the benzimidazole compounds.

Metrifonate has no effect on the glycogen level of *G. explanatum*, but in *S. globipunctata* the drug causes a slight increase in the glycogen content. This variation certainly requires further studies to confirm as to why such increase in glycogen occurs due to metrifonate.

Metrifonate inhibits *in vitro* secretion of AChE in the helminths under study which confirms the hypothesis of Lee (1970) that such secretion act as a "biochemical holdfast" against the local peristalsis. This drug also inhibits total activity of the AChE in the worm's homogenates. Histochemically, it has been observed that this enzyme is also present in non-nervous tissues like tegument, which is also inhibited by metrifonate. Presence of the AChE in tegument and inhibition
of glucose uptake by metrifonate suggests and supports the hypothesis proposed by Schwabe *et al.* (1961) that AChE is involved in the permeability of the hydatid cyst wall. In addition to this, it has also been observed histochemically, that metrifonate inhibits the nonspecific esterases in the helminths. Electrophoretic studies reveal that the drug inhibits only one isozyme of AChE in *G. explanatum* and all isozymes in *S. globipunctata*. Thus, inhibition of the isozymes of AChE by metrifonate, suggests that only one isozyme in *G. explanatum* and all isozymes in *S. globipunctata* are sensitive to metrifonate and responsible for inhibition in glucose uptake and reversible flaccid paralysis.

Metrifonate in low concentrations also, paralyse *G. explanatum*. In $3.3 \times 10^{-6} M$ concentration the worms are paralysed within 10 minutes. The drug also causes increased percent detachment of *G. explanatum* from host tissue. The motility recordings reveal that as soon as the worm comes into the direct contact of the drug, the activity increases and then slowly the worm is paralysed within 8-9 minutes. The drug causes a flaccid paralysis. This further suggests that the AChE is also involved in nerve transmission in *G. explanatum*.

The topographical studies in *G. explanatum*, reveal that metrifonate causes deformation in the majority of the
surface papillae. Few of them burst, forming lesions on the lateral surfaces of the acetabulum. The drug also causes a peeling effect of the surface tegument. This study also provides an evidence that these tegumental papillae have some secretory functions, and possibly secrete AChE as suggested by Davies (1979). Such effects probably bring about the incoordination in the neuromuscular functioning and thus inhibit the release of AChE as well, which ultimately causes inhibition in glucose uptake and detachment of the worms due to reversible flaccid paralysis.

Metrifonate inhibits AChE secretion in *G. explanatum* at a higher level as compared to *S. globipunctata*, probably due to the differences in the nature of their tegument. However, further studies are required to ascertain the exact function of AChE secretion and its possible role in the transmembranosis.

**OXYCLOZANIDE:**

Oxyclozanide inhibits MDH activity significantly in *G. explanatum*, and insignificantly in *S. globipunctata*. The histochemical and electrophoretic studies also confirm this inhibition. In trematode, the drug causes paralysis soon as it comes into the direct contact of the drug. Oxyclozanide causes a spastic paralysis in *G. explanatum* which is irreversible and 100% worms detach from the host tissue. Thus,
oxyclozanide affects the MDH activity, and also probably inhibits the oxidative phosphorylation. These effects ultimately destroy the energy yielding mechanism and induce paralysis in G. explanatum. Thus it appears that oxyclozanide is basically a flukicide and can be used against amphistomes.

Among all the drugs investigated in the present study, oxyclozanide produces the most severe and pronounced topographical damages in G. explanatum. The worm is almost deshaped, and concentric wrinkles appear over the ventral body surface, and almost all papillae disappear from the body surface. Deep, crater-like lesions are formed, which expose the parenchymatous tissues as well as the internal organs. The circular folds of the tegument, in the inner lining of the acetabulum, are badly damaged, which may be a consequence for the quick detachment of the worm from the host tissue.

GENERAL CONCLUSION:

From the present investigation, it can be concluded that:

1. In G. explanatum, the four anthelmintics are effective in the order of: oxyclozanide > metrifonate > fenbendazole > mebendazole, as far as the in vitro survival, worm detachment from host tissue, motility of the worm, and the
topographical damages are concerned. The surface papillae are the potential sites for the drug action. Disruption of these papillae due to drugs are manifested in the form of various metabolic disorders in the amphistomes.

2. For glucose uptake inhibition, mebendazole is more effective than fenbendazole. Metrifonate is also effective in inhibiting glucose uptake, but only for a short period. For glycogen depletion, fenbendazole is more effective than mebendazole.

It can be concluded that mebendazole and fenbendazole can be used as a broad spectrum anthelmintics, since these compounds are effective in both the worms understudy and interfere the carbohydrate metabolism.

3. Mebendazole inhibits AcPase, AlPase and ATPase in both the worms understudy as evident by the biochemical and histochemical studies. Thus it appears that these phosphatases are involved in glucose uptake mechanism in helminths. Other enzymes PGM, G-6-Pase and phosphorylase are also affected by mebendazole, which resulted into the glycogen depletion.

4. Metrifonate inhibits AChE levels and AChE secretion in the parasites understudy. Histochemically, metrifonate
also inhibits AChE and non-specific esterases in these helminths. This inhibition of AChE was also noticed in the non-nervous tissue (tegument). Further this drug also inhibits glucose uptake indicating possible role of AChE secretion in glucose uptake mechanism besides neuromuscular. Since metrifonate inhibits only one isozyme of AChE in *G. explanatum* and all isozymes in *S. globipunctata*, therefore it suggests that only inhibited isozymes are responsible for the inhibition in glucose uptake as well as for the reversible flaccid paralysis. It can be concluded that metrifonate can be used against both trematodes and cestodes, but the drug is comparatively more effective against trematode.

5. Oxyclozanide inhibits MDH activity and ultimately uncouples the oxidative phosphorylation in the parasites. The drug is more effective in *G. explanatum* as compared to *S. globipunctata*. Thus it confirms that oxyclozanide is basically a flukicide and can be used against amphistomes.

These investigations are quite encouraging and tempt to infer that different anthelminitics have various mode of actions in different parasites belonging to different taxonomic groups. The efficacy of the drugs may also vary in different species of the same group. These differences are also possible
correlated to the microenvironments of the parasites, as well as to the pharmacokinetics and pharmacodynamics of a particular drug. Therefore, further studies are also required to ascertain these biochemical effects of the anthelmintics under in vivo conditions.