HISTORICAL REVIEW

If we look back into the history of chemotherapy, we find that during the past two decades most of the work has been carried out on the development of anthelmintic drugs. In 1909 Paul Ehrlich laid down the foundation of the chemotherapy of parasites and proposed that the inhibition of enzymes that were crucial to the parasites but not to the host, might be the basis of a rational approach to the chemotherapy of the parasites. Since then, many chemicals have been screened for their chemotherapeutic effect and the work has been reviewed by many workers (Bando, 1951; Mansour, 1957; Norton and Beer, 1957; Tiner, 1958; Guilhon, 1968; Boray, 1969; Natoff, 1969; Boray, 1970; Standen, 1970; Coles et al., 1974; Oakley, 1978; Prichard, 1978a; Armour, 1983).

Today numerous anthelmintic drugs are available which are effective against helminth parasites, but their side effects to the host could not be ruled out. The biochemical mode of action of such anthelmintics have also been worked out and reviewed extensively by many workers (Saz and Bueding, 1966; Annabaeva et al., 1975; Sheth, 1975; Van den Bossche, 1976; Coles, 1977; Pouplard, 1977; Rew, 1978; Striebel, 1978; Behm and Bryant, 1979; Van den Bossche, 1980; Gutteridge, 1982; Rathaur et al., 1982; Sood and Kaur, 1982; Rew et al.,
Among the various groups of compounds that have been tested so far; the benzimidazoles, salicylanilides and organophosphorus compounds are found to be quite effective against helminth parasites.

In the present thesis, four anthelmintics were selected in order to work out their mode of actions at the molecular level, namely, mebendazole and fenbendazole from the benzimidazole group, oxyclozanide representing the salicylanilide group and metrifonate from the organophosphorus group. The chemical structures and the solubility of these compounds are shown in the Figure 2.

Benzimidazole compounds:

The benzimidazoles were introduced as a group of safe and effective broad spectrum anthelmintics with the discovery of thiabendazole by the Merck groups in 1961. Smith Kline and French introduced parbendazole in 1967, and since then the international pharmaceutical industry have brought a number of benzimidazole compounds like cambendazole, mebendazole, oxibendazole, thiophanate, fenbendazole, albendazole and oxfendazole onto the market.

Mebendazole: Mebendazole (Methyl-5-benzoyl benzimidazole-2-carbamate) was introduced as a new potent anthelmintic compound
by Janssen Pharmaceutica in 1971 in the form of product No. R17635. In vivo efficacy and therapeutic action of mebendazole against a number of helminths was reported by Chaia and Cunha (1971), Brugmans et al. (1971) and Banerjee et al. (1971). Van den Bossche (1972) reported for the first time the biochemical effects of mebendazole on a number of nematodes and cestodes both in vivo as well as in vitro studies. Since then a number of biochemical effects of this drug have been reported in nematodes and cestodes, which are concerned mainly with the inhibition of glucose uptake and/or transport; depletion in endogenous glycogen content; inhibition of mitochondrial phosphorylation, ATP synthesis and turnover of adenine nucleotides; inhibition of colchicine binding to the partially purified tubulin in a competitive manner (Van den Bossche, 1976, 1978, 1980; Van den Bossche and de Nollin, 1973; de Nollin and Van den Bossche, 1973; Bryant et al., 1976; Cornish and Bryant, 1976; Simpkin and Coles, 1976; Coles and McNeillie, 1977; Rahman et al., 1977; Rahman and Bryant, 1977; Kammerer and Miller, 1981; Kohler and Bachman, 1980, 1981; Ahmad, 1981; Ahmad et al., 1984a). Biochemical changes caused by mebendazole in helminth parasites have also been investigated histochemically in order to support the biochemical studies. The ultrastructural studies also revealed that this drug interacts with the tubulin of the teguments and absorptive surfaces of the helminths, resulting into the disappearance of
cytoplasmic microtubules (Thienpont et al., 1973; de Nollin and van den Bossche, 1973; de Nollin et al., 1974; Borgers and de Nollin, 1975; Borgers et al., 1975; Lumsden, 1975; Verheyen et al., 1976; Van den Bossche, 1979; Comely, 1980; Laclette et al., 1981). In vitro trials of mebendazole have also shown inhibitory actions in the egg development, increase in the rate of egg output, inhibition of the intestinal secretion and also inhibition of AChE secretion in helminths (Banerjee et al., 1971; Banerjee and Prakash, 1972; Atkinson et al., 1980; Watts et al., 1982; Ahmad et al., 1984b). Many field trials and in vivo experiments reveal that mebendazole has a broad spectrum effect against helminth parasites in both domestic and wild animals (Kelly et al., 1975; Fernando and Denham, 1976; Sujatha et al., 1976; Ambu et al., 1982; Bhopale et al., 1982).

**Fenbendazole:**

Fenbendazole (methyl-\(^{5}\)-(phenyl-thio)-benzimidazole-2-carbamate) a highly potent anthelmintic, was synthesized in the research laboratories of Hoechst AG, in 1971. Duwel (1976) and Tiefenbach (1976) summarized the results obtained upto 1975 on the trials of fenbendazole in a wide range of animal species. Efficacy of fenbendazole against helminths of pigs, horses, camels and other field and laboratory animals have been reported by many workers Fernando and Denham (1976), Sujatha
Duwel (1976) reported the mode of action of fenbendazole causing disorders in glucose absorption, inhibition of incorporation of glucose into glycogen and also the breakdown of glycogen, increased function in excretory canals and nervous system showed by historadiography, disorder of energy metabolism and neurotoxic effects in *Ascaris suum*. McCraken and Taylor (1978) also reported such biochemical effects of fenbendazole on *Hymenolepis diminuta*. Ovicidal and larvicidal activity have also been reported for fenbendazole in various helminths. Coles and McNeillie (1977) found *in vitro* ovicidal action on eggs of *Nematodirus spathiger*. Duwel (1979), designed *in vitro* experiments to demonstrate an ovicidal and larvicidal effect of fenbendazole on various trichostrongyles of sheep and cattle, and noticed 99% reduction in the infectivity of these nematode eggs. He (loc. cit.) also reported that high dose of fenbendazole produce ovicidal effect against *Fasciola hepatica* in sheep. The drug also affects the eggs within the flukes as well as eggs in the sheep's gall bladder.

**Salicylanilides.**

Among the salicylanilides, "Diaphene" and "Hilomid" were
possibly the first compound used as flukicides (Lienert, 1963; Boray et al., 1965). Salicylanilides and substituted phenols show variable activity against helminths, but are usually most active against blood-sucking worms (Stampa and Terblanche, 1961; Horak, 1962, 1964; Boray, 1969; Sinclair and Prichard, 1975). These compounds generally bind with the plasma proteins and cause metabolic disorder in the parasites. Lee (1973) reported that fasciolicidal activity of salicylanilides in sheep was dependent on the extent to which they persist in the plasma. Salicylanilides and substituted phenols are also potent uncouplers of oxidative phosphorylation (Williamson and Metcalf, 1967). Scheibel et al. (1968) demonstrated in vitro uncoupling of oxidative phosphorylation in tapeworms due to niclosamide. Corbett and Goose (1971), Van den Bossche (1972) and Cornish and Bryant (1976) reported that oxyclozanide, nitrooxynil, nitroscanate and rafoxanide uncouple oxidative phosphorylation in *F. hepatica* and *A. lumbricoides* in vitro. Further Cornish et al. (1977) and Prichard (1978b) suggested evidence of uncoupling during in vivo trials.

**Oxyclozanide.**

Oxyclozanide is one of the most potent salicylanilide compound active against trematodes and cestodes. The activity of oxyclozanide was reported in U.K. in 1966 (Broome and Jones, 1966). They found that oxyclozanide is absorbed and binds to plasma protein.
Numerous successful field trials have been reported for the efficacy of this drug against *F. hepatica*, *H. diminuta*, *H. microstoma*, *Stilesia hepatica* and few amphistomes (Broome and Jones, 1966; Walley, 1966; Harrow, 1969; Foreyt and Todd, 1973; Hopkins et al., 1973; Corba et al., 1976; Georgiev and Gruev, 1979). Some *in vitro* studies have also been accomplished on the biochemical and metabolic effects, resulting into the inhibition of malate dehydrogenase of *F. hepatica*, *F. gigantica*, *Fasciolopsis buski* and *Paramphistomum explanatum* (Lwin and Probert, 1975; Coles et al., 1980 and Probert et al., 1981) and uncoupling of oxidative phosphorylation (Corbett and Goose, 1971). Further, Edwards et al. (1981) also reported effect of oxyclozanide on the metabolism of *F. hepatica*.

**Organophosphorus compounds:**

Organophosphates have been used extensively as insecticides since late 1930's. The organophosphates napthalophos, trichlorphon, haloxon, dichlorvos and metrifonate are widely used as anthelmintics in the control of helminths of both clinical and veterinary importance. Their mode of action is primarily attributed to the inhibition of many enzymes including acetylcholinesterase, which is usually reversible (Prichard, 1978a).

**Metrifonate.** (o, o-dimethyl-2,2,2-trichloro-1-hydroxyethyl
phosphonate) is a water soluble organophosphorus compound. It is very unstable in aqueous solutions and gets converted into dichlorvos (0,0-dimethyl-2-2-dichlorovinyl phosphate) (DDVP) as reported by Holmstedt et al. (1978) and Reiner et al. (1978). The biodegradation of DDVP is well established (Wright et al., 1979). The rearrangement of metrifonate to DDVP occurs both in vitro (Barthel et al., 1955; Lorenz et al., 1955) and in vivo (Nordgren et al., 1980). Metrifonate was introduced as a drug in the treatment of schistosomiasis in 1960 (Lebrun and Cerf, 1960). Inhibition of acetylcholinesterase (AChE) by metrifonate in Metastrongylus apri, Paramphistomum microbothrium, A. suum, Neoascaris vitulorum and schistosomes, have been reported by Bueding et al. (1972), Reiner et al. (1978 and 1979). However, Reiner et al. (1978) suggested that actually DDVP and not metrifonate is the potential inhibitor of AChE and the rate of conversion from metrifonate to DDVP can be calculated from the time course of enzyme inhibition. Metrifonate induces AChE inhibition in Schistosoma haematobium and S. mansoni adults during in vitro studies (Denham and Holdsworth, 1971). The mode of action of metrifonate in S. mansoni was also reported by Bloom (1981). He (loc. cit.) reported a reduction of 20% of AChE activity from the control values and movement of S. mansoni from their sites in the mesenteric plexus to the liver, the classic "hepatic shift". Semeyn et al. (1982) reported for a decreased electrical activity from the surface of S. mansoni.
due to $10^{-6}$M metrifonate. In the presence of this drug, the motor activity of adult *S. japonicum* has been recorded by Terada et al. (1982) and during *in vitro* studies a flaccid paralysis in *S. mansoni* has also been reported (Mellin et al., 1983). Metrifonate has also been tried for the treatment of onchocerciasis (Awadzi and Gilles, 1980).

The foregoing review of the literature, indicates that all the above mentioned compounds show anthelmintic activity in one way or the other. Different parameters have been used by the various investigators for the assessment of their efficacy. It is evident from the review, that most of these studies are the results of the *in vivo* trials. Only few reports are available on the biochemical effects, ultrastructural damages or electrophysiological changes on the surface of the parasites. Such *in vitro* studies provide a detailed information about the mode of action of a particular drug which ultimately forms the basis of *in vivo* trials. However, such *in vitro* studies involve heavy expenses and require much sophisticated expertise for the screening of a single compound. Therefore, there is an obvious need to design experiments which are inexpensive, time saving and reliable in order to develop the standard parameters for assessing the mode of action and efficacy of a particular compound in *in vitro* conditions.

The easiest approach to investigate the anthelmintic
efficacy of a compound against helminth parasites is to examine the in vitro survival of the worms in presence of the compound. Baldwin (1943) devised an in vitro method to study the survival of helminths in presence of a drug. Since then, a number of studies have been carried out using this parameter to determine the efficacy of a particular drug. Visual observations for the motility and survival of Fasciola sp. and Schistosoma sp. in presence of various compounds has been tested by Tomosky et al. (1974), Coles (1975), Abrahams et al. (1976) and Henry et al. (1976). Recently a number of drugs like avermectin B1a, praziquantel and niclosamide were also tested in many helminth parasites for their motility and survival by visual observations (Sano et al., 1981a,b, 1982; Terada et al., 1982; Gupta and Katiyar, 1983). Further, Probert et al. (1981) studied visually the mortality in in vitro conditions in presence of 5 flukicides including oxyclozanide in three trematodes of veterinary importance namely F. gigantica, Fasciolopsis buski and Paramphistomum explanatum. Another drug diamfenetide also caused paralysis to F. hepatica as observed visually by Rew et al. (1983). Oxyclozanide is the only drug which has been tested for amphistomes, while other drugs understudy, have been remained completely neglected.

Further, the in vitro motility of helminths have also been recorded mechanically, in order to find out the effect of
drugs on their motility qualitatively as well as quantitatively. One of such in vitro recording was made graphically on a kymograph on the worm *Macracanthorhynchus birudinaceus* (Rebello and Rico, 1926). The first available report on the kymographic recording of the motility of *F. hepatica* in presence of drugs, was reported by Chance and Mansour (1949, 1953), Mansour (1957). Some more sensitive methods, for small worms like schistosomes, were also devised for motility recording, like ultrasonic methods (Brown et al., 1973, 1978), photoelectric device (Hillman and Senft, 1973; Senft and Hillman, 1973; Jewsbury et al., 1977; Hillman, 1979), or using suction electrodes (Fetterer et al., 1977, 1978, 1980; Pax et al., 1978; Fetterer et al., 1981). In addition to these methods, various types of transducers like isotonic and isometric transducers were also used for recording the motility of the helminth parasites (Sano et al., 1981, 1982; Terada et al., 1982; Fairweather et al., 1983, 1984) besides the schistosomes. Recently, Rew et al. (1983) used a conventional organ bath system to record the motility of *F. hepatica*, and also reported the effect of diamfenetide on it's motility. He (loc. cit.) observed that the drug diamfenetide caused paralysis to the worms which was irreversible and dose dependent, involving an increase in muscular tension and decrease in contraction amplitude. These methods provide a good opportunity to record the contraction and relaxation of the worms exposed to different anthelmintic drugs.
A number of drugs are known today, which affect the motility of the worms. Such motility recordings under **in vitro** conditions, seem to be an effective, quick and economic method for screening a large number of drugs, whether or not their mode of actions are related to the surface action potential or motility of the worms.

It is well known that the paramphistomes have a well developed posterior sucker or acetabulum, which acts as a powerful organ of attachment. Anthelmintics which cause paralysis or affect the motility of the worms by disturbing their neuromuscular systems, should also be capable of detaching the worms from their host tissues by affecting their holdfast organs. Rahman et al. (1977) treated infected sheep with mebendazole and reported that the number of *Haemonchus contortus*, *Moniezia expansa* and *F. hepatica* which detached from gastric and intestinal mucosa or bile duct walls respectively, increased with time from treatment. Chevis (1980) reported for a rapid detachment of *M. expansa* within six hours after treatment, while *Haemonchus contortus* required a longer interval from treatment (18 hours) and *F. hepatica* took even longer period of time, i.e., 30 hours after treatment in which only 29.0% worms were free.

The time taken by two anthelmintics, levamisole and fenbendazole, differed by a few hours, to detach *H. contortus*
from abomasal mucosa (Briscoe and Coles, 1980), where levamisole achieved 95% detachment in about one hour. Incidentally, no literature is available on the detachment of amphistomes, in vivo or in vitro, from their host's tissue, in presence of anthelmintics.

In recent years, the electron microscope has also been used as a powerful tool to study the actual site of the drug action in the parasites ultrastructurally and the topographical damages caused to the worms by the anthelmintic drugs. The surface topography of the amphistomes has been studied only in a few species like Bilatorchis papillogenitalis, Orthocoelium indonesiense, Cotylophoron okapi, C. congoense (Eduardo, 1980 a,b,c) and Gastrothylax crumenifer, Paramphistomum epiclitum, Calicophoron papillosum and C. calicophorum (Tandon and Maitra, 1981 and 1982), and they reported for the occurrence of ridges and papillae with and without pits over their general body surfaces and also discussed the functional roles of these structures with their modifications. Erasmus (1970) pointed out through scanning electron microscopy (SEM) the physiological importance of the tegumental surface of digenetic trematodes in the host-parasite relationship, but no reference is available on the effects of anthelmintics on the topographical damages produced in the amphistomes. However, effect of anthelmintics on the surface topography of other digenetic trematodes, cestodes and nematodes have been reported by various workers.
Among the trematodes, effect of praziquantel on *S. mansoni*, *D. dendriticum* and *F. hepatica* (Becker et al., 1980), effect of oxamniquine on *S. mansoni* (Kohn et al., 1982; Bricker et al., 1983), effect of praziquantel on human trematodes *Clonorchis sinensis*, *Metagonimus yokogawai*, *Opisthorchis viverrini*, *Paragonimus westermani* and *S. japonicum* (Mehlhorn et al., 1983) were studied and all trematodes exposed to the drugs showed tegumental alterations. Rew et al. (1983) scanned the effect of diamfenetide in vitro on *F. hepatica* and reported for wide curled body shape and vesiculated ventral surface. Among the cestodes, *Taenia taeniaeformis* was scanned after treating the mice with mebendazole, surface alterations with lesions were reported by Verheyen et al. (1978). Adult *Echinococcus granulosus* treated with praziquantel in vitro showed high deformations in the body surfaces and shape (Conder et al., 1981). Probert et al. (1982) reported topographical damages in *M. expansa* treated with oxfendazole and praziquantel in vitro. Such topographical studies related with the effect of anthelmintics on helminths, especially on amphistomes, require attention so as to assess the mode of action of the anthelmintics with the damages produced on the worms surface which forms an important physiological interface in host-parasite relationship.

The ultrastructural studies on helminths, have revealed that the tegument is metabolically active and plays an important role in transmembranosis of sugars, amino-acids and other
substances (Lumsden, 1975; Erasmus, 1977; Halton, 1982; Smyth and Halton, 1983; Threadgold, 1984). The use of radiolabelled substances in the biological and physiological studies of helminths, have provided a conclusive evidence that most of the helminth's tegument play a vital role in uptake mechanisms. Further, by the use of this technique, it is also possible to suggest the actual path of uptake, site of incorporation and synthesis of glycogen and protein etc. from the labelled nutrients in parasites (Hanna, 1975, 1976, 1980; Specian and Lumsden, 1981).

The anthelmintics which are known to inhibit the uptake processes in helminths (Van den Bossche, 1976; 1980) have not been tested yet to ascertain their inhibitory action by using radiolabelled nutrients. However, Pappas (1971), used the standard inhibitors dinitrophenyl phosphate (DNP) and iodoacetate to demonstrate the inhibition of tritiated arginine uptake in *Haematolechus medioplexus*, *in vitro*. Such studies, play an important role to assess the mode of action of drugs which inhibit uptake processes of the helminths.

von Brand (1973) suggested that most drugs interfere with the enzyme systems by inhibiting them and thus interfere with the metabolic processes. Such studies could be of immense value in knowing the effect of anthelmintic drugs at the enzyme level and their mode of actions at molecular level.
in the helminth parasites. It is now an established fact that carbohydrates play a major role in the energy metabolism of the helminths, which require a continuous supply of energy (ATP) to motivate the complex metabolic processes, essential for their normal growth and development. Hence, it is the carbohydrate metabolism that has been more intensively studied in the helminths. Various enzymes of this metabolic pathway have been identified and reported in a number of helminths by histochemical as well as biochemical techniques (for ref. see Smyth, 1966, 1969; von Brand, 1973, 1979; Barrett, 1980; Ward, 1982; Smyth and Halton, 1983; Lloyd and Barrett, 1983).

Studying the effect of anthelmintics on the level of these enzymes both histochemically as well as biochemically, can reveal the actual site, degree and mode of the drug action in helminths. However, such chemotherapeutical studies have mostly been carried out on the nematodes, while cestodes and trematodes have remained somewhat neglected (Van den Bossche, 1976). In vitro inhibition of acid and alkaline phosphatases, adenosine triphosphatase, glucose-6-phosphatase and 5' nucleotidase was reported due to mebendazole in Avitellina lahorea Cotugnia digonopora and Ascaridia galli (Ahmad, 1981; Ahmad et al., 1984a). Mebendazole has no effect on the hexokinase activity of the helminths, while the enzymes phosphorylase and phosphoglucomutase are affected by this drug, which ultimately causes the depletion in glycogen content and inhibits glucose
uptake (Van den Bossche, 1972; Ahmad, 1981). Another drug oxyclozanide, under study, as pointed out earlier, specifically inhibits malate dehydrogenase (MDH) which is one of the key enzymes of carbohydrate metabolism. The inhibition of this enzyme results into the blocking of the energy production in helminths (von Brand, 1973, 1979; Durrani et al., 1983; Smyth and Halton, 1983).

Metrifonate in helminth parasites is known to inhibit their AChE. However, AChE has been reported from a number of helminth parasites (von Brand, 1973, 1979; Durrani et al., 1983; Smyth and Halton, 1983). It has also been reported that AChE is secreted by the helminths in vitro and its physiological significance in vivo, has also been discussed by Schwabe et al., (1961), Barker et al., (1966), Sanderson (1972), Nizami et al., (1977), Watts et al. (1982), Gunn and Probert (1983). Effect of various inhibitors on AChE of helminths have been reported by a number of workers (Bueding et al., 1972; Probert and Durrani, 1977; Gunn and Probert, 1981; Martin, 1981; Rapson et al., 1981; Sharpe and Lee, 1981; Watts et al., 1982; Durrani et al., 1982 and 1983).

It is evident from the foregoing review of the literature that, the mode of action of various anthelmintic have been studied by a number of workers, but no generalizations can be made on the basis of the present status of our knowledge,
because it is often stated that a particular drug may have multiple modes of action. Also the same anthelmintic may behave differently in the taxonomically different group of parasites, thus pointing the necessity of studying each member separately for discovering the mode of actions of various anthelmintics and the possibility of their broad-spectrum efficacy.

During the course of this study it was proposed to investigate the effect of mebendazole, fenbendazole, metrifonate and oxyclozanide on the various metabolic activities of *G. explanatum* and *S. globipunctata* belonging to two different taxonomic groups, inhabiting two different hosts and habitats.

It is hoped that whatever little work has been accomplished by the present investigator, would stimulate further research work on comparative chemotherapeutic studies of helminths, in order to investigate the molecular basis of the drug action.