INTRODUCTION

In Indian economy, ruminants play an important role and the importance of cattle alone lies in the agriculture, dairy products and leather industries. Among the farmers, the quality and quantity of cattle is a symbol of social and economical status, particularly in the agrarian societies.

According to 1972 livestock census in India, there are 17.8 million cattle, 5.8 million buffaloes, 6.5 million goats and 4.0 million sheep. Thus it constitutes nearly 11% of the total livestock population of the world. The largest number of cattle (about 15.0%) are found in Uttar Pradesh, among which 21.5% are buffaloes only. The livestock population of the Indian subcontinent contributes little to the international trade in meat products, but domestically it is of great importance in providing milk, milk-products, transport, irrigation and dung for the fuel or energy production.

A recent estimate indicates that the cattle contribute Rs. 1,174.65 million to the national income. Of the total income from animal husbandry, 64.5% is contributed by milk and milk-products; cattle manure, 9.7%; fuel 9.7%; meat and meat products, 7.9%; hides and skins, 2.5%; hair and wool 1.1%. Cattle serve in transport too and 300 to 500 millions of rupees, however, are assessed to be the value of cattle labour in India. From the exports of tallow, animal castings, cattle
tail hair, horns, hooves and hoofmeal, the country gets about Rs. 6 million per year. The annual availability of raw bones is valued approximately Rs. 8 million. This data clearly indicates the importance of cattle in the national economy.

Despite its importance, the tropical livestock productivity is quite low as visualized by Pino (1981). This is only because of neglected management and poor health of the animals. Levels of productivity of existing cattle producing regions are influenced by climate, quality and quantity of available forage, management systems, genetic characteristics of the stock and last but not the least, diseases. Among the various diseases of livestock, helminth infections cause a great havoc.

Paramphistomiasis of livestock is now regarded as a disease of great economic importance. Amphistome infections in domestic ruminants are widely prevalent in India and this prevalence has a correlation with the areas known for irrigation and agriculture (as shown in Fig. 1). High mortality rates of cattle have been reported due to epizootic or sporadic outbreaks of paramphistome infections, particularly among the young stocks (Dutt, 1980). Acute paramphistomiasis in ruminants is characterized by severe diarrhoea, hepatic fibrosis, loss of weight, marked weakness and high rates of morbidity and mortality.
Fig. 1: Major outbreaks and prevalence of amphistome infection in the livestock of India and it's correlation with the areas known for irrigation and agriculture.
AREAS REPORTED FOR AMPHISTOME INFECTION AMONG LIVESTOCK AND ALSO KNOWN FOR AGRICULTURE AND IRRIGATION OUT BREAKS WITH YEARS

Fig. 1
The cestodes, also cause a great deal of pathological effects to their hosts. Severe cestode infections are fatal to the young animals and are often the principal cause of unthriftiness in animals of all ages. The helminthic disease also lowers vitality and resistance to other viral and bacterial diseases.

No information is available about the actual loss caused by these helminth infections among the livestock in India, but if we manage to control the parasitic infections, then the present estimated contribution to the national economy by the cattle alone, can be increased in many folds. Secondly with the increasing human population, the demand of animal proteins has also been increased. Therefore, it is necessary not only to increase herd numbers but also to improve the general health conditions of the livestock. This is only possible by proper management and by the control of various infections of the livestock including the paramphistomiasis, which has not yet received much attention by the parasitologists of the tropical countries.

The possible ways to control paramphistomiasis and other helminth infections, involves an integrated approach of different type of control methods.

For an effective control measure, the study of the relationship between various environmental factors determining
the frequency and distribution of the diseases is necessary. Therefore, it is very important to work out the biology of snail populations, including the factors responsible for the infection of snails with the larval stages, seasonal population dynamics of the larval and adult stages, and the factors determining the infection in the definitive hosts. This basic knowledge will help in devising an effective epidemiological control measure by interrupting the life cycle of the trematode parasites.

Controlled grazing of animals is another method for checking the prevalence of parasitic infections. This method required to manage restricted movement of the livestock over a safe area of the grazing field, in order to fence off the snail habitats and avoid access of the cattle to such habitats.

The molluscicides can also play an effective role for the control of snail populations. As a consequence of the rising costs of the synthetic molluscicides, an increasing interest is being developed in plants showing molluscicidal properties. Compounds like saponins, flavanoids, alkaloids and terpenoids have shown encouraging results. An appealing aspect of these natural plant products is that, while they may be highly toxic, they degrade very rapidly when released into the environment. The molluscicides should be sprayed at regular intervals in order to kill the snails of all ages.
This will ensure the killing of snail population of a particular pond or reservoir, without provoking much toxicity to other aquatic animals.

Quarantine is also a method where infected animals can be physically separated from healthy ones, and restraints can be placed on the movements of the infected animals as well as on the products that may have been contaminated.

The chemotherapeutic method is dependent upon the availability and use of safe and cheap therapeutic agents. This control method can only be applied when the infection rate shows a sudden rise in order to check the prevalence of infection.

Immunological control can be a perfect means of eradicating helminth infections, but the knowledge of nature and characterization of antigen and antibody resistance in amphistome infections is very meagre, hence practically nothing has been achieved in this field. Recent studies suggest that in amphistomes, secretory antigens and some surface antigens are present, and the possibility to develop the antibodies against such antigens, cannot be ruled out for the diagnostic tests and for the development of vaccines.

Considering the episodes of paramphistomes and other helminths, in the economic losses of the nation, there is an
obvious need to develop new anthelmintics which will be effective against these parasitic infections. There is a great need to know much about the mode of action of the existing anthelmintics. This will help into designing safer and more effective drugs acting on a wide range of worm species. At present the recent trend of research in medical and veterinary parasitology is to develop such anthelmintic drugs which do not cause any harm to the host but are effective against the parasites. Today pharmacologists are explaining drug action on a physiochemical basis. The basic concept is that the drugs exert their effect by interfering with some metabolic activity of parasites to cause membrane alterations, enzyme inhibition or uncoupling of energy mechanism. More effective eradication of parasites can be achieved only when physiological and biochemical information about the parasites and their relationship to the host is available. Therefore, for developing effective drugs, a clear understanding of the physiological and biochemical aspects of parasites is also necessary.

Generally, in the development of new drugs, the basic two approaches have been practiced. Firstly, the empirical approach, which involves the screening of thousands of natural and synthetic compounds. This may obviously cost millions of rupees and above all, can take a long period of time for the development of one single new compound. The second approach is the rational approach, where the advances in the detailed
knowledge of physiology and biochemistry of both parasites and their hosts is required. Such comparative studies will provide the knowledge of the differences in metabolic processes. If any metabolic step which is present in the parasite but is absent in the host's metabolism, can ultimately form a potential site for the chemotherapy of the parasite. This approach has been used for the development of new drugs, which is more toxic to parasite but least toxic to the host.

As Barrett (1981) pointed out that many biochemical differences between helminths and hosts are now known, but the rational approach to the parasite chemotherapy has yet to produce a successful compound. Possibly, in comparison with the empirical method, the rational approach has not had a sufficiently long trial. More promising, perhaps, is the semi-rational approach in which knowledge gained from the mode of action of empirically discovered drugs, is used as a basis for the synthesis of improved compounds.

The advancement and development of modern biological techniques, including transmission and scanning electron microscopy, radioisotope labelling, fluorescent antibody labelling, formation of monoclonal antibodies by the hybridoma technology, freeze-fracture techniques, in vitro culture etc., have revolutionised our understanding of the physiology of the helminths. Inspite of such modern developments, the mode of
action of the majority of the currently available anthelmintics are not known clearly. However, the desirable qualities of an ideal and potential anthelmintic for veterinary use, include a wide therapeutic index, efficacy, safety, ease of administration and low cost.

Rew (1978) pointed out the possible reasons for the non-availability of the informations regarding the definite mode of action of the anthelmintics. According to him, "sufficient quantities of worms are difficult to obtain for appropriate analyses because time and expenses make large-scale in vitro or in vivo parasite production nearly prohibitive; only a very few resistant mutants are available to aid analyses of the altered pathways." On the contrary to this, the livestock maintained in Indian conditions, harbour a large number of helminths as a natural infection round the year. Inspite of such heavy infections, the chemotherapeutical control of paramphistomes and cestodes still remain somewhat neglected. The details of the biochemical and molecular mode of action of the anthelmintics on these parasites are still in infancy.

Previous investigations which have been carried out on the mode of action of anthelmintics and the reviews of the last few years on these aspects (Van den Bossche, 1976; Coles, 1977; Behm and Bryant, 1979) suggest that the anthelmintics basically have either of the four mode of actions. Firstly, they may act
on the neuromuscular systems of the helminths by inhibiting acetylcholinesterase; blocking or mimicking acetylcholine; depolarising, hyperpolarizing and blocking the neuromuscular systems; resulting into either increase or decrease in their activity causing paralysis or death. Secondly, most of the helminths utilize the carbohydrate as a source of energy, and many anthelmintics disrupt the intermediary carbohydrate energy metabolism of helminths. They may inhibit glycolysis, glucose uptake, disrupt glycogen metabolism, or may uncouple the oxidative phosphorylation. Thirdly, they may interfere with the nucleic acid metabolism by inhibiting the synthesis of RNA. Lastly, many anthelmintics may cause the ultrastructural damages to the parasites and thus may disrupt their metabolism. Hence it appears that a compound which causes adverse effects and produce changes in the metabolism of a helminth parasite, but not in the host, is a potential anthelmintic.

The biochemical mode of actions of few anthelmintics are known with certainty, but it is well understood that most anthelmintics seem to have multiple effects. It should of course be realized that probably no drug inhibits only one process. Drugs interrupting a metabolic sequence, inhibit rarely, if ever, only a single enzyme (von Brand, 1973). The anthelmintics can have multiple effects resulting into a conspicuous damage
to the parasites, but no generalization is possible at the present state of our knowledge.

The present study is an attempt to investigate the mode of action of some common anthelmintics, in order to work out the possible site of drug action in the parasites. The studies are made on the freshly prepared homogenates of the worms as well as on the intact worms. As von Brand (1979) suggested that in order to understand the mode of action of a drug, a particular enzyme should be inhibited in the homogenates \textit{in vitro} as well as in the intact worms, since drugs having an effect on the homogenates \textit{in vitro} may react differently, or the degree of inhibition may vary in the intact worms.

The present work has been carried out on Gigantocotyle \textit{explanatum} from bile duct of buffalo and Stilesia globipunctata from small intestine of sheep and goats. These two species have been selected due to many reasons. They belong to two different hosts, two different habitats and to two different taxonomic groups. The facilities available to the author were limited and the work was carried out in a moderately equipped laboratory. Effect of some anthelmintics namely mebendazole, fenbendazole, metrifonate and oxyclozanide have been investigated on the parasites understudy. The mode of actions of these anthelmintics have been studied on physiological and biochemical basis. The two species are used as the model
parasites in order to study the influence of niche diversification and host difference on the physiological and biochemical mode of actions of the anthelmintics on two parasites belonging to two distinct taxonomic groups.

The anthelmintics belonging to benzimidazole compounds (mebendazole and fenbendazole), organophosphorus compound (m trifonate) and salicylanilide compound (oxyclozanide) have been selected for the present study.

In order to investigate the preliminary efficacy of these anthelmintics, the *in vitro* survival and motility of the worms were observed in presence of drugs with different dilutions. Effect of these drugs were also investigated on the different aspects of carbohydrate metabolism like glucose uptake and glycogen metabolism. Enzymes responsible for transmembrane and other enzymes have also been estimated in order to find out the actual site of the drug action in carbohydrate metabolism and uptake processes.

Further, in order to investigate the physiology of motility and to work out the possible role of acetylcholinesterase (AChE) secretion, different experiments were designed to ascertain their possible role by blocking AChE activity with metrifonate. The enzyme inhibition and distribution have also been confirmed by histoenzymological studies and by isozyme separation on gel by electrophoresis.
The topographical damages produced by the drugs were also studied by scanning electron microscope. All these studies have been correlated to find out the comparative efficacies of the drugs understudy and their mode of actions in the two parasites understudy.

It is hoped that the present work would stimulate and initiate further research on the comparatively less studied area of the pharmacological control of parasites. Further, it would certainly encourage the understanding of the intrinsic biochemical relationship between the metabolic activities of different helminth parasites. Lastly, it is hoped that such studies would also provide an insight to those who are interested in studying the mode of action of drugs and/or, in designing of new anthelmintics through the development of in vitro and in vivo models, to improve the chemotherapy of the helminth parasites, especially for the control of parasitic infections.