Sheep and cattle constitute one of the premier economic group, providing animal protein which is indispensable in regular staple diet of common man in our country, especially in native state of Jammu and Kashmir. The present study, which deals with the histochemistry of Paramphistomes of sheep and cattle, was undertaken from January 2004 to January 2007. Since there has been no detailed study on the histochemistry of helminths particularly Paramphistomes in Kashmir valley, the present study is a step towards bridging the gap. Sheep and cattle like other animals develop diseases due to variety of parasitic infections, resulting in huge economic losses. Fascioliasis and Paramphistomiasis rank high in significance of helminth diseases of sheep and cattle because they cause heavy mortality and morbidity in young cattle and sheep. Various species of Paramphistomes such as Paramphistomum cervi, Paramphistomum microbothrium, Paramphistomum skrajabini, Cotylophoron cotylophorum, Gastrothylax crumenifer and
Gigantocotyle explanatum have been incriminated as aetiological agents of Paramphistomiasis. In order to develop various control measures to these helminths we should have a clear understanding of host parasite relationship and a thorough understanding of the host parasite relationship can not be realized until a careful study is made on the biochemical nature of the parasite and its host. Histochemical study helps us to investigate qualitatively the biochemical pattern of different tissues in cellular architecture. The present work demonstrated the presence of general proteins, glycogen, lipids, cholinesterase, acid phosphatase, alkaline phosphatase and calcium in Cotylophoron cotylophorum collected from cattle in comparison with Paramphistomum cervi collected from sheep.

In order to have a clear understanding and background information about the work done related to the various aspects of the present study, a comprehensive survey of the literature was conducted. Literature survey revealed that most of the studies on the histochemistry of digenetic trematodes have been restricted to some model species like Fasciola hepatica and Schistosoma mansoni. Many other equally important species of trematodes like Paramphistomes from the point of their veterinary importance have been neglected. Literature survey also revealed that histochemistry is still a fledging field of research primarily so in Kashmir with no information on the histochemistry of helminths. Hence the present work entitled “Studies on the histochemistry of paramphistomes of sheep and cattle” was undertaken.

Mature worms of Cotylophoron cotylophorum and Paramphistomum cervi were collected from the rumen of cattle and sheep respectively, slaughtered at local abattoirs and analysed for demonstration of various biochemical constituents. The various histochemical methods which were followed during present endeavour include: Mercury Bromophenol Blue for general proteins, Best’s carmine for glycogen, Sudan Black B for lipids, Myristylcholine for cholinesterase, Lead Acetate for acid phosphatase,
Calcium Cobalt for alkaline phosphatase and Alizarin Red S for calcium. Paraffin sections were used for the demonstration of proteins, glycogen, lipids and calcium; while as frozen sections were used for the demonstration of enzymes.

Observations of the present study have been divided into two parts. The first part deals with the description of the two amphistome species which formed the subject of the present endeavour and the second part includes the histochemical study. Histochemical distribution of general proteins in Cotylophoron cotylophorum and Paramphistomum cervi was ubiquitous. High concentration of proteins was observed in the tegumental muscles, oral sucker, acetabulam, ovary and vitellaria. Moderate amount of proteins was observed in tegument, gut caeca and parenchyma in both the species under study. Staining reaction for glycogen revealed large amount of glycogen deposits in parenchyma, oral sucker, acetabulam and vitellaria of Cotylophoron cotylophorum as well as Paramphistomum cervi. In the present study moderate amount of glycogen was present in tegumental muscles, tunica of ovary and ovary. However glycogen was present in small amount in tegument, tunica of testes and testes in both the species of paramphistomes. Most conspicuous sites for the presence of lipids were excretory ducts and vesicles in both the species of paramphistomes. Histochemical distribution of lipids revealed few sudanophilic lipid granules in parenchyma which were more in the vicinity of the intestinal caeca and subtegumental regions. Moderate amount of lipids were present in tegument and subtegumental muscles. The staining reaction for lipids revealed weak to moderate reaction in gastrodermis, vitelline cells, suckers, ovary and testes.

Distribution in the tissues of Cotylophoron cotylophorum and Paramphistomum cervi revealed moderate activity for cholinesterase in the muscles of oral sucker, acetabulam and pharynx. Moderate to intense reaction was observed in gut and tegumental musculature. Tunica of
Abstract
testes and tunica of ovary of both these species revealed moderate activity. Weak activity of cholinesterase was recorded in tegument, parenchyma and vitellaria. Histochemical distribution of acid phosphatase revealed its positive activity in almost all the tissues of *Cotylophoron cotylophorum* and *Paramphistomum cervi*. Muscles of pharynx, its tegument as well as tegument lining the mouth and acetabulum showed intense reaction for acid phosphatase. Similar reaction for acid phosphatase was recorded in the intestinal caeca and various muscle layers of acetabulum in both the species. Vitelline cells and their secretory products were also acid phosphatase positive. Histochemical localization in the tissues of *Cotylophoron cotylophorum* and *Paramphistomum cervi* revealed substantial amount of alkaline phosphatase in almost all the tissues. Intense reaction for alkaline phosphatase was recorded in intestinal caeca, excretory vesicle, tunica of testes and ovary in both the species under study. Moderate reaction for alkaline phosphatase was recorded in oral sucker, acetabulum, vitellaria, tegument and sub tegument; while as parenchyma of both the species showed weak reaction for alkaline phosphatase. In the present study the histochemical distribution of calcium revealed moderate amount of calcium deposits in tegument, vitellaria, intestinal caeca, oral sucker and acetabulum of *Cotylophoron cotylophorum* and *Paramphistomum cervi*. Parenchyma, testes, ovary and excretory vesicles of both these species showed weak reaction for calcium.

As per our observations and research done by various workers, it is revealed that there is no significant difference in the histochemical distribution of various biochemical substances in the tissues of two amphistomes under study. This may be due to the fact that both these species are present in the same habitat i.e., rumen of the host. However differential histochemical distribution of various biochemical substances in different tissues of both the amphistomes showed considerable difference. The current study demonstrated high concentration of proteins
in those organs which are metabolically more active and are involved in synthetic activities. However moderate amount of proteins in caecal lining and contents is expected from dietary origin. Intense reaction for glycogen in the parenchyma can be explained by the fact that parenchyma of trematodes serves as a storage organ for energy reserves and trematodes mostly use glycogen as energy metabolite. Large amount of glycogen was observed in the reproductive organs during the present study. The reason for this may be that trematodes produce enormous amount of eggs and these eggs need sufficient amount of reserve food for the development of the embryo. Lipids were present in the tegument, caeca and reproductive organs, besides the excretory ducts and vesicles. Thus it was concluded that lipids are not exclusively the end products of carbohydrate metabolism in trematodes but they play their role in various metabolically active tissues and organs.

During the present study cholinesterase was localized in musculature of body wall, suckers, digestive system and reproductive system of the amphistomes under study. Presence of cholinesterase in the musculature of these organs suggested its role in contractility. Presence of alkaline phosphatase in the metabolically active tissues is explained by the fact that this enzyme is associated with the membrane transport of carbohydrates constituting the major source of energy for these parasites. Acid phosphatase was observed in the tissues which were associated with absorption, secretion and excretion and is postulated to be associated with these functions. The present study revealed the presence of calcium in those organs which are associated with higher muscular activity, which is explained by the fact that calcium plays very important role in muscle contraction.