Sheep and cattle, like other animals, develop diseases due to variety of parasitic infections. Fascioliasis and Paramphistomiasis rank high in significance of helminth diseases of sheep and cattle because they cause heavy mortality and morbidity in young ones. A thorough understanding of physiological aspects 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. Most of the work has been done on the effect of parasite on host's haematology and biochemistry and a very little attention has been paid to the changes in the parasite brought about by host. Histochemical studies help us to investigate qualitatively the biochemical pattern of different tissues in cellular architecture. The valuable contributors towards the development of histochemistry are those of Gomori (1952), Gurr (1958) and Pearse (1972). Reports
on the histochemical localization of biochemical constituents in helminths, particularly in paramphistomes are scanty. A brief account of literature on the histochemical localization of various biochemical components in helminths related to present study has been critically reviewed and summarized below.

Von Brand and Mercado (1961) studied glycogen histochemically in *Fasciola hepatica*. They found that glycogen granules of various sizes were present in the parenchymal cells, muscular organs like suckers and the cirrus pouch. They also found that glycogen deposition occurs only in non-contractile parts of muscle cells. Vitelline cells and uterine ova also showed large amount of glycogen deposition.

Crompton (1963) studied body wall of *Polymorphus minutus* morphologically and histochemically and concluded that lipoprotein is one of the main structural compounds. Distribution of two enzymes, non-specific esterase and alkaline phosphatase was found throughout the body of animal.

Watiz and Schardein (1964) studied histochemical localization of various components including lipid, glycogen, acid phosphatase, alkaline phosphatase and several other components in *Hymenolepis nana*, *H. diminuta*, *Hydatigera taeniaeformis* and *Dipylidium caninum*. They reported species differences in the distribution of alkaline phosphates, lipids, glycogen; while as no species differences in distribution of other substances. They reported moderate level of lipid in cuticle, uterine, testicular and ovarian structures. Larger
quantities of lipids were reported in sub-cuticular cells and parenchyma. Varying amounts of glycogen were reported in parenchyma, excretory duct lining, ovary and testes. Acid phosphatase was reported to be present in heavy amounts in cuticle and less of the enzyme activity in sub-cuticular cells. All other structures were essentially non-reactive for acid phosphates. All four species examined were reported with high alkaline phosphatase activity in cuticle and slight in parenchyma and parenchymal cells.

Schardein and Watiz (1965) studied histochemical localization of non-specific (simple) esterase and cholinesterase in four species of Cyclophyllidean cestodes, *Hymenolepis diminuta, H. nana, Dipylidium caninum* and *Hydatigera taeniaeformis*. They found that these enzymes were primarily localized in the cuticle and nervous system, but with quantitative and qualitative differences from species to species.

Bogitsh (1967) studied histochemical localization of some enzymes in cysticercoids of the two species of *Hymenolepis*. He reported that intermediate cell layers of the cysts surrounding the larvae of *Hymenolepis diminuta* and *H. microstoma* showed acid phosphatase activity and this activity was optimal at pH 5.0. Alkaline phosphatase activity was reported to be associated with tegument with pH range of 6.0-9.0. The author hypothesized that the former enzyme could be involved in the excystation of the cysticercoids. Bogitsh and Shannon (1971) demonstrated acid phosphatase activity in *Schistosoma mansoni* and *Schistosomatum*
douthitti while carrying out cytochemical and biochemical observations on the digestive tracts of digenetic trematodes. They reported acid phosphatase activities in the esophageal gland cells of Schistosoma mansoni, Schistosomatium douthitti and in gastrodermis of Schistosoma mansoni. At the electron microscope level they reported acid phosphatase activity in esophageal gland cells of both species in cytoplasmic vesicles. They also reported that acid phosphatase activity was associated with the infoldings of basal plasma membranes of esophagus and gastrodermis. They hypothesized that this enzyme was involved with membrane transport.

Fripp (1967) studied the histochemical localization of Acetylcholinesterase and pseudocholinesterase activity in adult Schistosoma haematobium, S. mansoni and S. rodhaini. The researcher observed strong esterase activity in the central nervous system of both sexes.

Halton (1967) studied the distribution of carboxylic esterase activity in adult Fasciola hepatica and three distinct esterases were localized. Acetylcholinesterase activity was found in the tegument and neuro-muscular tissues, non specific esterases in the reproductive structures and resistant type indoxyl esterase occurred in gut epithelium.

Krvavica et al. (1967) studied histochemical localization of acetylcholinesterase and butyrylcholinesterase in liver fluke (Fasciola hepatica) and its developmental stages. They reported large
amount of acetylcholinesterase in the muscles of pharynx, cirrus, the cerebral ganglion and the nerves of fluke. Acetylcholinesterase activity was demonstrated in different organs of larval stages also.

Barry and Thomas (1968) studied histochemical distribution of seventeen enzymes in adult liver fluke. According to their findings, the tegument which was thought to be responsible for absorption of food and excretion of waste materials, the gut was better equipped enzymatically of these functions. Glucose-6 phosphatase was present in small amounts and β-hydroxybutyrate dehydrogenase was completely absent which they explained by the fact that glycogen was supplied by the liver of host.

Thorpe (1968) carried out comparative histochemistry of immature and mature stages of *Fasciola hepatica* and demonstrated lipid, hydrolytic and oxidative enzymes. He reported that lipid droplets were present in caecal cells, excretory ducts and parenchymal cells of adult flukes but immature fluke contained more amount of lipid than in the adult. Mature *Fasciola hepatica* was reported to show positive reaction for alkaline phosphatase, acid phosphatase, succinic dehydrogenase, tetrazolium reductase, cytochrome oxidase, lactate dehydrogenase and glutamate dehydrogenase. However no detectable cytochrome oxidase or glutamate dehydrogenase activity was reported in immature flukes.

Shield (1969) worked on the histochemical identification of cholinesterases in tape worms - *Dipylidium caninum, Echioncoccus*
granulosus and Hydatigera taeniaeformis and studied the nervous system in these cestodes by esterase techniques.

Porter and Hall (1970) used histochemical techniques to study the glandular contents of a Cotylocercous cercaria of Plagioporus lepomis. They used histochemical techniques to demonstrate carbohydrates, proteins, lipids nucleic acids and metal ions including calcium. They reported presence of acid micro-polysaccharide, protein and sudanophilic substances (lipid) in mucoid glands located dorsally. The caudal glands were reported to contain micro-polysaccharide, protein, calcium and sudanophilic material. They also discussed the possible functions of these glands, with relation to their contents.

Davis and Bogitsh (1971a) while carrying out cytochemical and biochemical observations on the digestive tract of digenetic trematodes demonstrated Arylsulfatase (acid hydrolase) activity in the gastrodermis of Gorgoderina attenuata and Haematoloechus medioplexus. They reported that its activity is localized in the cytolysomes and micro vesicles as well as on invaginations of basal plasmalemma and membranous remnants in the lumen and its activity was operative over a pH range of 4.2 - 7.2. The same authors (1971b) carried out cytochemical and biochemical observations on the digestive tracts of digenetic trematode Gorgoderina attenuata. They reported that the gastrodermis had a basal lamina (muscular) and the luminal surface was extended as digitiform cytoplasmic extensions. Cytoplasm demonstrated an
extensive system of endoplasmic reticulum, Golgi areas, and numerous mitochondria. They also reported three types of membrane-delimited vesicles (DV) designated as DV₁, DV₂ and DV₃. According to them DV₁ and DV₂ vesicles demonstrate acid phosphatase activity and were interpreted as lysosomes and cytolysomes.

Trimble and Bailey (1971) carried out studies on histochemical localization of acid and alkaline phosphatases in *Aspidogaster conchicola* (Trematoda: Aspidobothrea). For acid phosphatase, they reported intense reaction in haptor and gut, moderate reaction in sub-tegument, pharynx, testis and eggs. Weak reaction in vitellaria, while no reaction on body tegument, tunica of testis, cirrus and uterus. For alkaline phosphatase intense reaction was reported in haptor, body tegument, sub tegument, tunica of testes, cirrus and vitellaria; moderate reactions in eggs; weak reaction in testes and no reaction in gut, pharynx and uterus.

Parshad and Guraya (1976) analyzed immature and mature stages of the sheep amphistome *Cotylophoron cotylophorum* histochemically for their lipids. They reported that excretory ducts of the immature worms were the common sites for the accumulation of neutral lipids (Triglycerides) and phospholipids which showed very sparse distribution at the corresponding sites in adult. They also reported that phospholipids and lipoproteins observed in the tegument of the adult were not seen in the immature forms. They reported that intestinal caeca of both forms showed the presence of
diffused and granular lipids which were relatively less in immature. They also discussed the significance of these differences in the lipid contents of the immature and mature forms. The same workers (1977) carried out histochemical localization of proteins, lipids, carbohydrates, acid and alkaline phosphatases in Ovarian balls of Centrorhynchus cervi (Acanthocephala). They reported intense to moderate reactions for lipids, proteins and negative reaction for glycogen in oogonia and growing oocyte. For alkaline phosphatase, negative reaction was observed in both oogonia and growing oocyte. However they reported moderate to intense reaction for acid phosphatase in growing oocyte. The same researchers (1978a) carried out morphological and histochemical studies on oocyte atresia in Centrorhynchus cervi (Acanthocephalan). They carried out histochemical localization of different tissue constituents including proteins, lipids and nucleic acids in growing and mature oocyte. They reported marked differences in nucleic acid distribution for which more intense reaction was observed in growing oocytes than mature ones. No such marked difference was observed in other constituents. The same investigators (1978b) studied phosphatases of four helminth species; Ascaridia galli, Centrorhynchus cervi, Cotylophoron cotylophorum and Raillietina cesticillus calorimetrically. They reported that the optimum pH for acid phosphatase activity was 5.4, 4.5, 4.7 and 5.0 in A. galli, C. cervi, R. cesticillus and C. cotylophorum, respectively. The optimum pH for alkaline phosphatase activity was 9.1, 9.5, 8.7 and 8.4 in A. galli, C. cervi, R. cesticillus and C. cotylophorum respectively. They also reported that
in *A. galli* and *C. cotylophorum* acid phosphatase showed more activity than alkaline phosphates where as the later was reported more active in *R. cesticillus* and *C. cervi*. The same authors (1978c) made morphological and histochemical studies on the digestive system of amphistome *Cotylophoron cotylophorum*. They reported intense reaction for proteins in caecal cells (both in apical and basal region), brush border epithelium and luminal contents. For glycogen they reported no reaction in caecal cells and brush border epithelium but moderate reaction in luminal contents. They observed moderate reaction in caecal cells, intense reaction in brush border epithelium but no reaction in luminal contents for acid phosphatase. They also reported moderate to weak reaction in caecal cells but no reaction in brush border epithelium for alkaline phosphatase.

Patil and Rodgi (1976) studied histochemical localization of non-specific esterase activity in *Paramphistomum cervi* recovered from sheep. They reported moderate esterase activity in oral sucker and that too restricted to the outer part of this organ. Acetabulum was reported to exhibit weak to moderate activity. The activity of esterase was reported to be more pronounced along the length of the caeca with slightly more intense reaction in caecal contents. Esterase activity in the parenchyma appeared in the form of small granules which were uniformly distributed throughout the tissue with slightly more granules accumulated in cells surrounding the caeca. They also reported moderate activity in epithelial layer of
testes and ovary. The vitellaria were reported to be negative for this enzyme activity.

Mandawat and Sharma (1978) histochemically demonstrated acetyl and butyryl cholinesterase in different tissue of *Paramphistomum cervi*. They reported intense activity of acetylcholinesterase in tegumental musculature, pharynx, gut musculature, tunica of testes, uterus, vitellaria and Mehli’s gland, while as moderate to weak activity in lymph channels, excretory canals, excretory bladder, and tunica of ovary. Negative reaction for acetylcholinesterase was reported in tegument, posterior sucker and gut epithelium. For butyrylcholinesterase intense reaction was reported in tegumental musculature, pharynx, gut musculature tunica of testes, uterus and vitellaria, while as moderate to weak activity in tegument, excretory canals, excretory bladder and Mehli’s gland. They reported negative activity for butyrylcholinesterase in pharynx, lymph channels, parenchyma and gut epithelium.

Gupta and Agarwal (1979) studied phosphatase system (alkaline and acid phosphatase) in *Gastrothylax crumenifer* (Trematoda). They used king and Wooton method for measuring enzyme activity. They reported maximum acid phosphatase activity in tissue extract of *Gastrothylax crumenifer* at pH 5.0 and that of alkaline phosphatase at pH 10.0.

Maki and Yanagisawa (1979) carried out a study on acid phosphatase activity demonstrated by intact *Angiostrongylus contonensis* with special references to its function in general. They
reported some monophosphate esters (glucose-1-phosphate, glucose-6-phosphate, α and β-glycerophosphate, P-nitrophenyl phosphate, adenosine-5-phosphate (AMP), guanosine-5-phosphate, cytidine-5-phosphate, uridine-5-phosphate and thymidine-5-phosphate) were hydrolyzed to varying degree, while other esters including adenosine triphosphate and adenosine diphosphate were hydrolyzed to a low or negligible degree. They reported that possible functions of phosphatases in helminths could be the hydrolysis of phosphate esters prior to absorption of the products of hydrolysis, in other words, phosphatases function as intrinsic digestive enzymes. Same investigators (1980) carried out histochemical studies on the acid phosphatase of body wall and intestine of adult filariae in comparison with other parasitic nematodes. Filariae examined were *Litomosoides carinii*, *Brugia pahangi* and *Dirofilaria immitis*; other parasitic nematodes used for comparison were *Angiostrongylus cantonensis*, *Ascaris lumbricoides* and *Trichuris muris*. They reported that four species of nematodes inhabiting host body fluid viz. three species of filarial worms and *Angiostrongylus cantonensis* had intense to moderate acid phosphatase activity in body wall, where as intestinal nematodes such as cuticle of *Ascaris lumbricoides*, *Trichuris muris* and *Ancylostomum caninum* do not showed any activity for it. They also reported intense acid phosphatase activity in the intestine of *Ascaris lumbricoides* while as weak to moderate activity of acid phosphates in the intestine of other nematodes.
Roy (1979) localized certain phosphatases by histochemical techniques in various tissues of a pigeon cestode, *Raillietina johri*. The author reported presence of acid phosphatase, alkaline phosphatase and adenosine triphosphatase (ATPase) in almost all structures (tegument, sub-tegumental muscles, sub-tegumental cells, excretory canal, testes, sperm ductules, vas deferens, cirrus sac, cirrus, ovary, reaceptaculum, seminis, vagina, vitelline gland cells, oocysts, uterus, embryonated eggs). Alkaline phosphatase activity was reported to be absent in parenchyma, spermatocytes, spermatids and spermatozoa and more intense in the tegument of mature and gravid proglottides. The same researcher (1980a) worked on the histochemical localization of non-specific esterase (NSE), acetylcholinesterase and pseudocholinesterase (ChE) in various tissue of a cestode *Raillietina* (*Raillietina*) *Johri* obtained from the intestine of pigeon. He reported localization of NSE in the rostellum, suckers, hooks, tegument, sub-tegumental muscle, excretory canal, cirrus sac, vagina and eggs. Acetylcholinesterase besides being localized in nerves was also visualized in all most all structures as in case of NSE, except hooks, excretory canal and eggs. ChE was reported to be present in nerves, vas deferens, cirrus sac and vagina. The same investigator (1980b) carried out studies on distribution of non-specific esterase (NSE), acetylcholinesterase (AchE) and pseudocholinesterase (ChE) in *Ceylonocotyle scoliocoelium* a bovine amphistome using cytochemical techniques. He reported NSE and AchE activity in almost all tissues of the parasite. He also reported that some structures like sub-tegumental
muscles, vitelline cells, prostate gland cells and secretory vesicles showed NSE activity but not AchE. On the other hand structures like sub-tegumental cells, pars muscular and oviduct exhibited AchE activity but not NSE. He also reported that ChE activity was present only in pharynx, ovary and posterior sucker. The author (1980c) studied distribution and functional significances of alkaline phosphatase and acid phosphatase, adenosine triphosphatase, 5-nucleotidase, glucose-6-phosphatase, thiamine pyrophosphatase and nucleoside diphosphatase histochemically in various tissues of a bovine amphistome Ceylonocotyle scoliocoelium. He reported intense reaction for acid phosphatase in anterior and posterior sucker, pharynx, tegument, sub-tegumental muscles, gut, tunica of testes, testes, seminal vesicles, tunica of ovary, ovary, oviduct, uterus, vitelline cells, while as moderate reaction for alkaline phosphatase in all these tissues. Both these enzymes were reported to show no reaction in sub-tegumental cells, ootype and Mehli’s gland.

Sharma and Mandawat (1979) studied the histochemical distribution of acid mucopolysaccharide in different tissues including tegument, sub-tegumentry cells, gut epithelium, parenchyma, pharynx, lining of sucker, excretory system, uterus, ovary, testis, prostate gland, Mehli’s gland, vitellaria and nervous system of Paramphistomum cervi (Trematoda). They reported that acid mucopolysaccharide activity was more intense in the organs which are concerned with secretion, like prostate gland, Mehli’s
gland, vitelline gland, sub-tegumentary cells, lining of excretory canal, uterus and gut epithelium. Sharma and Sharma (1981) carried out histochemical studies on neurosecretory cells of *Ceylonocotyle scoliocoelium*. They reported that neurosecretory cells stained intensely with mercuric bromophenol blue indicating synthetic activity of cells. Cells reacted weakly to Sudon Black B. These cells also showed positive reaction for mucopolysaccharides. For various enzymes they reported that neurosecretory cells contained significant amount of esterases, namely non specific esterase (NSE), acetylcholinesterase (AchE), and butyrylcholinesterase (BchE). Non specific alkaline and acid phosphatases were reported to be present in moderate quantities. Sharma *et al.* (1981) used histochemical techniques to study the chemical composition of Mehli’s gland secretion in *Ceylonocotyle scoliocoelium*. They reported intense reaction for glycogen in Mehli’s gland secretion. Moderate reaction was reported for lipids and proteins. Mehli’s gland secretion was reported to exhibit intense reaction for alkaline phosphatase and moderate reaction for acid phosphatase, acetylcholinesterase and butyrylcholinesterase. Sharma and Ratnu (1982) while carrying out morphological and histological architecture of the lymph system of *Orthocoelium scoliocoelium*, also studies histochemical localization of various constituents like glycogen, lipids, proteins, calcium carbonate, alkaline phosphatase, acid phosphatase, acetylcholinesterase and butyrylcholinesterase in the walls of lymphatic vessels and lymphatic fluids. Sharma and Hora (1983) used histochemical
techniques to study the chemical composition of esophageal gland secretion of *Orthocoelium scoliocoelium* and *Paramphistomum cervi*. In non-enzymatic histochemical reactions they reported negative reaction for glycogen in esophageal gland, gastrodermis and brush border. For simple lipids intense reaction was reported in esophageal gland, moderate reaction in gastrodermis and weak reaction in brush border. For non-specific proteins intense reaction was reported in esophageal gland and gastrodermis and moderate reaction in brush border. In enzymatic histochemical reactions they reported moderate activity for alkaline phosphatase in esophageal gland, gastrodermis and brush border. For acid phosphatase intense reaction was reported in esophageal gland and brush border while as moderate activity in gastrodermis. For nonspecific esterase esophageal gland and gastrodermis was reported to show intense activity and brush border was reported to show moderate activity. On the basis of histochemical studies they discussed the role of esophageal glands in the digestive physiology of these two amphistomes. Sharma and Hanna (1988) examined tegument of *Orthocoelium scoliocoelium* and *Paramphistomum cervi* using histochemical techniques and electron microscopy. On the basis of histochemical distribution of acid phosphatase, alkaline phosphatase, non-specific esterase, cholinesterase and succinate dehydrogenase at light microscope level, two distinct regions were recognized an outer and an inner zone. They reported moderate acid phosphatase activity in the distal region of subsyncytial zone and relatively strong activity in tegumental cells. For alkaline
phosphatase moderate but diffused and poorly localized reaction was reported in surface syncytium of tegument near to plasma membrane. Small reaction products were also reported in tegumental cells and musculature. For acetylcholinesterase, intense reaction in subsyncytial zone and moderate reaction in muscle tissue and tegumental cells was reported. The reaction product for non specific esterase was reported to be distributed in the subsyncytial zone of tegument, longitudinal muscle fibers and tegumental cells.

Kanwar and Kansal (1980) carried out cytochemical studies on the prostrate glands of trematodes, *Paramphistomum epiclitum* and *Paradistomoides orientalis*. They reported that pear shaped prostrate gland cells in these trematodes were so arranged that their broader ends containing nuclei, were away from the central lumen. The cytoplasm secretion granules and globules were present. They reported that these granules stained blue with Sudan black B and acid haematein tests revealing the presence of phospholipids. These granules also stained blue with mercuric bromophenol blue and therefore contain proteins.

Sathyanarayana and Anantaraman (1980) carried histochemical studies for localization of peroxidases in tissues of *Gastrothylax crumenifer* (Trematoda: Paramphistomidea). They reported very strong reaction of peroxidase in cuticular region, gastrodermal cells and ventral pouch. In parenchyma, positive
reaction was reported around digestive tract. Weakest activity was reported in oral sucker region.

Saxena (1980) studied acid and alkaline phosphatase in *Aspiculuris pakistanica* (Nematode). The author reported intense reaction for acid and alkaline phosphatase in intestine (basal, epithelial layer and bacillary layer), ovary and uterus but moderate to weak reaction in musculature, esophagus and excretory canals. Negative reaction was reported for acid phosphatase in cuticle and hypodermis but intense to moderate reaction for alkaline phosphatase in these two structures. Moderate reaction for acid phosphatase was reported in testis and ovary but no reaction for alkaline phosphatase.

Sharma and Sharma (1980) studied histochemical localization of proteins, lipids, glycogen, nucleic acids and acid phosphatases and their relative importance during spermatogenesis in germ cells of *Ceylonocotyle scoliocoelium* (Trematoda: Digenea). They reported intense to moderate reaction for proteins, lipids, and glycogen in spermatogonia, primary spermatocytes, spermatids and cytoplasmic residual mass. Intense reaction was also reported by them in all the above mentioned stages of spermatogenesis except cytoplasmic residual mass which showed weak reaction. Sharma (1984) studied histochemical localization of ATPase and succinate dehydrogenase (SDH) in various tissues of *Ceylonocotyle scoliocoelium* maintained in vitro in medium for 15 days and compared the results with normal activities of these enzymes in tissues of worms freshly
collected. He reported low activities of enzymes in the tissues of cultured flukes and suggested that it was due to low metabolic rate. He also reported that enzymes intensity was tissues specific.

Venkatanarsaiah (1981) demonstrated cholinesterase histochemically in nervous system, tegumental and sub-tegumental musculature of the haptor and in the pharyngeal bulb of the Oncomiracidium of Priceamultae (Monogenea). According to him presence of cholinesterase in the nervous system was attributed to neurotransmission.

Haque and Siddiqi (1982) worked on histochemical and electrophoretic studies on phosphatases of four species of trematodes viz. Gigantocotyle explanatum from liver and Gastrothylax crumenifer from rumen of water buffalo and Echinostoma malayanum and Fasciolopsis buski from small intestine of pig. They reported that both alkaline and acid phosphatases were present in tegument, gastrodermis, suckers, testes, ovary, eggs, vitellaria and uterus but in the parenchyma and excreatory ducts only alkaline phosphatase activity was observed by them.

Baqui and khatoon (1982) studied the histochemical changes in the adult worms caused by Suramin and Levamisole in rat-Steria cervi system and reported notable alteration in the histochemistry of parasite in respect of protein, glycogen and alkaline phosphatase.

Choubisa and Sharma (1983) histochemically demonstrated cholinesterase in the nervous system of Stregeoid metacercaria Tetracotyle lymnaei. They reported non specific esterase activity in
the entire nervous system, sub-tegumentary cells, fore body gland cells and lappets. They reported no reaction in the alimentary canal. Oral and posterior suckers were reported to reveal strong esterase activity. Adhesive organs were reported to show only weak activity and caecal cells of fully mature metacercaria revealed moderate activity.

Farooq and Farooqui (1983) studied histochemical localization of esterases in *Avitellina lahorea* (Cestoda) intestinal parasite of sheep and goats. They reported that nonspecific esterases were present in the sucker muscles, post acetabular ganglia, nerve trunks, tegument, excretory canals, cirrus sac, vagina, uterus and the inner membrane of the embryophore. Acetylcholinesterase was also reported in all the above organs including vas deferens and sperm ducts, but was absent from excretory canals and eggs. They also reported that intensity of acetylcholinesterase and acetyl thyocholinesterase was weak compared to NSE. Same authors (1984) histochemically localized non-specific and specific phosphatases in different tissues including tegument, parenchyma, nerves, testes, ovary, uterus, paruterine organ, egg pouches, eggs, muscles, cirrus sacs, vagina, excretory canals and reproductive ducts of *Avitellina lahorea*, an intestinal, parasite of sheep and goats. They reported large quantities of acid phosphatase, alkaline phosphatase and adenosine triphosphatase in almost all these organs except parenchyma, where they reported moderate amounts of acid phosphatase and no alkaline phosphatase. Reproductive
ducts were reported to show moderate amounts of alkaline phosphatase.

Gupta et al. (1983) carried out histochemical studies on the oocapt gland cells of Paramphistomes. They studied histochemical localization of various components including protein, glycogen, lipids and mucopolysaccharides in oocapt gland cells. They reported moderate amount of protein in nucleus and duct of the oocapt gland and body of the oocapt gland showed weak reaction for proteins. Strong reaction was reported for glycogen in body and duct of oocapt gland while as negative reaction for glycogen in nucleus. They also reported moderate reaction for lipids in body and duct while as negative reaction in nucleus of oocapt gland for lipids. Gupta et al. (1987a) carried out histochemical studies on egg shell formation in Paramphistomum cervi (Digenea: Paramphistomatidae). They reported that the newly formed egg shell stained lightly with mercuric bromophenol blue, ninhydrin-schiff and chloramine T-schiff, revealing the presence of proteins containing both free and bound NH₂ groups. Test for phenol and phenoloxidase were reported completely negative. The egg shell was reported to stain moderately with alkaline tetrazolium revealing the presence of both -SH groups and S-S linkages. Strong reaction was reported with performic acid Schiff and positive reaction with performic acid alcian blue revealed the presence of keratin. They also reported that egg shell do not stained positively for carbohydrates and lipids with the various histochemical tests employed in this study. Gupta et al. (1987b)
carried out studies on the histochemistry of immature, maturing and mature vitelline cells of *Paramphistomum cervi*. They carried out histochemical localization of different components like proteins, glycogen, lipids, amino acids and phenols in the above mentioned cells. For proteins they reported intense reaction in immature and maturing vitelline cells. For glycogen and lipid intense reaction was reported in all the three types of cells.

Leflore and Bass (1983) carried out observations on morphology and hydrolytic enzyme histochemistry of excysted metacercariae of *Himasthla rhigedona* (Trematoda: Echinostomatidae). They reported that reactions for alkaline phosphatase occur throughout the excretory system while as for acid phosphates in the gut, oral and ventral suckers. Reactions for non-specific esterases and cholinesterases were reported throughout nervous system, in the gut and in the oral and ventral suckers.

Gupta and Sinha (1984) carried out studies on acid and alkaline phosphatase biochemically and histochemically in *Haplorchoides ritae*. They reported acid and alkaline phosphatase activity in cuticle, oral sucker, testes, ovary uterus, vitellarium and eggs.

Haseeb *et al.* (1984) carried out histochemical lipid studies on *Schistosoma mansoni* adults maintained in situ and in vitro. They reported that males contain neutral lipid mainly in the parenchyma and tubercles, while as females contain neutral lipids in vitellaria. They also reported that neutral lipids were released from tubercles
of both paired and unpaired males maintained in vitro. Sudan black B staining for total lipids was reported positive in tubercles, parenchyma and vitellaria.

Dunn et al. (1985) carried out ultrastructure and histochemical studies on lymph system in three species of amphistome viz., *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica* from the Indian water buffalo *Bubalus bubalis*. They reported weak to moderate histochemical reactions for carbohydrates in some lymphatic vessels in all the three trematodes. For general proteins, intense reaction in *Gastrothylax* and moderate reaction in other two trematodes was reported. Moderate reaction was reported for lipids in all the three trematodes. Dunn et al. (1987a) carried out ultrastructural and histochemical studies on the foregut and gut caeca of *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica*. They used histochemical methods for the localization of carbohydrates, general proteins, lipids, acid phosphates, non specific esterases, DNA, RNA, haemoglobin, succinate dehydrogenase and adenosine triphosphatase in gut caeca (lumen, microvilli and epithelium) and esophagus and esophageal cells of these trematodes. They reported that fine structure and histochemistry of gut was similar in *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica*. Dunn et al. (1987b) carried out ultrastructural and cytochemical observations on the tegument of paramphistomes - *Gigantocotyle explanatum*, *Srivastavaia indica* and *Gastrothylax*
crumenifer. Histochemical tests for general proteins, lipids, acid phosphatases, non-specific esterases, succinic dehydrogenase, adenosine triphosphatase, DNA, RNA, haemoglobin and ferric iron on the tegument and tegumental cells of the above mentioned trematodes were performed by them. Moderate reaction for carbohydrates and proteins was reported in all the three species. For lipids moderate reaction was reported in tegumental cells. Weak to moderate reaction was reported for acid phosphatase in tegumental syncytium and tegumental cells in G. crumenifer and S. indica but no results in G. explanatum. Negative results for non-specific esterase were reported in G. crumenifer and S. indica but no results in G. explanatum. With their results, they concluded that tegument was largely protective in function and had limited absorptive potential.

Arfin and Nizami (1986) used histochemical techniques to determine chemical nature of egg shell/capsule of some Cyclophyllidean cestodes. They reported moderate to intense reaction for basic proteins in testes, ovary, vitelline glands, uterus and egg shell by using Bromophenol blue method.

Fujino and Ishii (1986) carried out comparative histochemical studies of glycosidase activity in Clonorchis sinensis, Eurytrema pancreaticum, Fasciola hepatica, Dipylidium caninum, Hymenolepis nana, Ascaris suum, Toxocara canis, Ancylostoma caninum, Trichuris vulpis and Dirofilaria immitis. They reported variation in enzyme distribution and intensity among species and also between
trematodes and nematodes and no marked positive reaction of these enzymes in cestodes.

Mishra and Tandon (1986) used histochemical techniques to visualize nervous system in *Olveria indica*, a rumen Paramphistome. On the basis of esterase localization they described complete nervous system in *Olveria inidica*.

Rajvanshi and Mali (1986) carried out studies on biochemistry and histochemistry to analyse alkaline and acid phosphatase in digenetic trematode, *Pegosomum egretti*. They reported that the optimum pH for acid phosphatase was 5.0 and for alkaline phosphatase 10.0. Histochemical localization of acid and alkaline phosphatase revealed difference in enzyme activity in various tissues, like epidermis, gut, vitellaria, eggs, ventral sucker, tunica of testes, testis, ovary and prostate gland. Intense reaction for acid phosphatase was reported in gut, vitellaria, eggs and prostate gland.

Rao and Krishna (1986) studied histochemical localization of malate dehydrogenase activity in tissues of *Gigantocotyle explanatum* (Trematoda: Digenea). They reported intense malate dehydrogenase activity in tegument, vitellaria and eggs. Moderate activity was reported in musculature, gut, nerves, excretory canal, uterus, testes and Mehli’s gland.

Saxena *et al.* (1986) studied distribution pattern of different hydrolytic enzymes including acid phosphatase and alkaline phosphatase in various body parts of *Setaria cervi*. They reported
that intestine of *S. cervi* exhibited higher levels of these enzymes than the genital tract and body wall.

Wajihullah *et al.* (1986, 1990) carried out studies on histochemical distribution of glucose phosphatase, succinic dehydrogenase, glutamate and malate dehydrogenase in *Setaria cervi, Diplocriena tricuspis* and *Oesophagostomum columbianum*.

Mackinnon (1987) carried out histochemical localization of proteins, lipids, glycogen and nucleic acids in the oogonia and oocytes in the Trichostronglid nematode *Heligmosomoides polygyrus*. He reported that small granules in cytoplasm of oocytes stained strongly for proteins and lipids. He also reported very little staining for glycogen in both oogonia and oocytes.

Breckenridge and Nathanael (1988) carried out studies on vitelline glands in the commensal temnocephalid *Paracaridinicola platei* using histochemical techniques. They used histochemical techniques to detect various tissue components like carbohydrates (glycogen), proteins, lipids and other tanning precursors. They reported the presence of tanning precursors namely protein, phenols and phenolase in the vitelline glands of *Paracaridinicola platei* (Platyhelminth).

Sukhdeo *et al.* (1988) carried out studies on the histochemical localization of acetylcholinesterase in the cerebral ganglia of *Fasciola hepatica*. They reported acetyl cholinesterase activity in the cell bodies and extra-cellularly in the neuropile of the cerebral ganglia of the adult *Fasciola hepatica*. They also reported that the
reaction product of acetylcholinesterase reaction was found around the somatic cell membranes and extracellular space between closely apposed nerve processes in the neuropile.

Kishore and Sinha (1989) carried out histopathological and histochemical observations on *Microsomanthrus coliaris* (Hymenolepididae: Eucestoda) infection in small intestine of domestic ducks. They used histochemical techniques for the detection of glycogen, proteins, lipids and mucin. They reported presence of heavy glycogen reserves in the sections of cestode and found intense PAS and bromophenol positivity in the section of cestodes. They reported negative reaction for mucin.

Kulkarni and Deshmukh (1989) studied in detail the histochemical distribution of lipids in the parasitic nematode *Trichuris muris* using Sudan black B method. They found cuticle, muscles of the body wall, esophagus, brush border of intestine, reproductive organs - testes, ovary, Oviduct and uterine wall containing eggs to be rich in lipids.

Ramakrishna *et al.* (1989) carried out studies on the demonstration of nervous system in whole mounts of *Moniezia expansa* and *Moniezia benedeni* based on the histochemical distribution of cholinesterase. Besides the presence of acetylcholinesterase and non specific esterase in nerves system, they also reported their presence in reproductive organs.

Abidi and Nizami (1991) carried out a comparative study of the protein content of ten different species of helminths including
Review of Literature

Gigantocotyle explanatum, Fasciola hepatica, Gastrothylax crumenifer, Fiscoederius elongatus, Orthocoelium scoliocoelium, Calicophoron calicophorum, C. cauliorchis, Paramphistomum epiclitum, Stilesia globipunctata and Avitellina lahorea. They found that all the amphistomes as well as other trematodes and cestodes occupying same or different habitats show wide intra-specific variations in their protein content. They suggested that these biochemical differences might be attributed either to the individual metabolic state of the parasite or due to the influence of the host physiology leading to the biochemical variations and adaptations of the parasites as in the case of pouched amphistomes.

Brennan et al. (1992) worked on ultrastructural and histochemical studies of lymph system of Gastrodiscoides hominis (Paramphistoma: Digenea). They carried out histochemical test for localization of general carbohydrates, general proteins, lipids, DNA, RNA and haemoglobin on the lymphatic fluid of the above mentioned trematode. They reported that lymph matrix contained high amounts of proteins and neutral lipids while carbohydrates were generally absent. They also reported presence of haemoglobin in the lymph system.

Mattison et al. (1992a) carried out ultrastructural and histochemical studies on the digestive tract of juvenile Paramphistomum epiclitum. They carried out histochemical tests of esophagus and intestinal caeca for localization of carbohydrates, general proteins, acid mucopolysaccharides, melanin, DNA, RNA
haemoglobin and ferric iron. They reported that esophagus displayed staining reaction similar to the tegument, while as lumen was reported to show faint evidence of amylase, susceptible carbohydrates. According to them general proteins and acid mucopolysaccharides displayed faint staining in lumen of both the esophagus and caeca. They reported faint staining for amylase resistant carbohydrates in caecal epithelium and moderate staining for general proteins and mucopolysaccharides. They also carried out enzyme histochemical tests on the esophagus and intestinal caeca of juvenile *Paramphistomum epiclitum* for various enzymes including acid phosphatase and alkaline phosphatase. Moderate activity for acid phosphatase was reported in esophagus and weak activity for alkaline phosphatase. In intestinal caeca they reported moderate enzyme activity for both acid phosphatase and alkaline phosphatase. Same investigators (1992b) carried out ultrastructural and histochemical studies on proto-nephridial system of juvenile *Paramphistomum epiclitum* and *Fischoederius elongatus*. They carried out histochemical tests on protonephridial tertiary ducts of newly excysted *Paramphistomum epiclitum* for general carbohydrates, general proteins, acid mucopolysaccharides and melanin. They reported moderate to intense reaction for carbohydrates in syncytium and lumen of tertiary ducts. For general proteins faint staining was reported in the syncytium and no reaction in lumen. They also carried out histo-enzymological tests on protonephridial ducts of juvenile *Paramphistomum epiclitum* and *Fischoederius elongatus* for various enzymes including acid and
alkaline phosphatase. They reported intense activity for alkaline phosphatase in syncytium of *Paramphistomum epiclitum* and *Fischoederius elongatus*, while no reaction for alkaline phosphatase, in the lumen of these parasites. Moderate activity for acid phosphatase was reported in syncytium of *Paramphistomum epiclitum* only and no reaction in *Fischoederius elongatus*. Same researchers (1992c) carried out ultrastructural and histochemical studies on lymph and parenchyma of juvenile paramphistomes, *Paramphistomum epiclitum* and *Fischoederius elongatus*. They reported intense staining for general proteins and only faint staining for glycogen in rumen stages of *Paramphistomum epiclitum*. Faint staining was also reported for DNA and RNA moderate to intense staining for lipid bound sulphurhydral and disulphide groups was reported. Enzyme histochemical tests were also carried out by them on lymph and parenchyma of juvenile *Paramphistomum epiclitum* and *Fischoederius elongatus* for various enzymes including acid phosphatase and alkaline phosphatase. They reported moderate reaction for acid phosphatase and alkaline phosphatase in lymph of *Paramphistomum epiclitum* and *Fischoederius elongatus* but weak activity in parenchyma of both the trematodes. Same authors (1994) carried out studies on histochemistry and ultrastructure of the tegument of juvenile paramphistomes *Paramphistomum epiclitum* and *Fischoederius elongatus*. Histochemical tests were carried out for localization of general carbohydrates proteins, acid mucopolysaccharides, melanin, DNA, RNA, haemoglobin, ferric iron and various enzymes including acid phosphatase and alkaline
phosphatase in the tegument of above mentioned trematodes. They reported moderate to intense staining for carbohydrates (amylase resistant) in the syncytium and intense in the cytons and pigment cells. For general proteins they reported faint to moderate staining in syncytium and pigment cells. They also reported intense staining for general proteins in the cytons where carbohydrates were not detected. In histo-enzymological studies they reported that phosphatases exhibited only faint activity in the syncytium and moderate activity in the tegumental cytons.

Johal and Joshi (1992) histochemically observed localization and distribution of proteins and carbohydrates in female reproductive system of Trichuris ovis, a nematode parasite of sheep. They reported that oocytes accumulate both proteins and carbohydrates during their migration down the ovary as well as in the fertilization chamber. According to them during shell formation carbohydrates were used in the formation of chitinous layer where as proteins were stored in the form of yolk. They also reported that uterine epithelium secreted proteins which were deposited in the outermost layer of the egg shell. Johal (1995) carried out studies on histochemical localization of proteins, carbohydrates lipids and nucleic acid in Oesophagostomum columbianum during oogenesis. The author reported presence of negligible amount of protein and carbohydrates in oogonia where as oocytes showed progressive increase in concentration of these two metabolites. The researcher reported that in mature ova, proteins formed the main bulk of egg
yolk while the glycogen was used in the formation of outer envelopes of the egg shell. Lipids were reported to be restricted to oolemma and nuclear region in oocytes but were incorporated in large quantity in the egg yolk of fertilized ova. Johal and Shivali (1996) carried out histochemical observations on the body wall of *Trichuris ovis*. They reported that the cuticle of *Trichuris ovis* was enveloped by a thin membranous epicuticle having carbohydrates, acid mucopolysaccharides and lipids as its main constituents. The latter two were responsible for the resistant nature of the cuticle and make it a selectively permeable entity. The protein present in the contractile part of the muscle cells was of collagenous type where as proteins along with RNA were observed in cortical layer, hypodermis and non-contractile part of the muscle cell suggesting that these layers of the body were metabolically active. Johal and Jatindar (1998) carried out histochemical study on the intestinal epithelium of *Oesophagostomum columbianum*. They used mercuric bromophenol blue (for proteins), periodic acid Schiff (for carbohydrates), Best’s carmine (for glycogen) Sudan Black B (for lipids) and Methyl green and Pyronin Y (for nucleic acids) methods for histochemical study. They found that the intestinal epithelium is rich in carbohydrates, glycogen and proteins. Lipids were reported to form main structural elements of basal lamina enclosing intestinal epithelium.

Fried *et al.* (1995) used thin layer chromatography and histochemistry to analyze neutral lipids in the intramolluscan larval
stages of *Leucochloridium variae* and in tissues of the snail host *Succinea ovalis*. In the histochemical tests they reported the presence of neutral lipid droplets in the suckers, parenchyma, and excretory system of the encysted metacercariae. The residual snail tissue was reported ORO (oil red o) negative by them.

Humphries and Fried (1996) made histochemical and histological studies on the excysted metacercarial and cercariae of *Echinostoma revolutum* and *Echinostoma trivolvis*. They found general staining of body with PAS, Alcian blue, Toluidine blue, thionine, bromophenol blue, Oil Red O, Alizarin red and silver nitrate in excysted metacercariae and cercariae of both echinostome species.

Arsac *et al.* (1997) studied histochemically alkaline phosphatase activity of *Echinococcus multilocularis*. They used Gomori's method for detection of alkaline phosphatase in different stages of the developing worm. They reported alkaline phosphatase activity in the excretory ducts of 8 to 11 day old strobila and in the tegument of mature proglottis of 16 day old worm.

Sampour (2001) studied the chemical nature of the egg shell of *Haploporus benedenii* (Haploporidae: Digenea) using histochemical tests. He found that egg shell was produced by phenolic compounds in vitelline globules within vitelline cells and consisted of a guinone-tanned protein, together with some stabilization with S-S bonds.
Humiczewska (2002) by using histochemical and cytometric methods studied the enzymes responsible for membrane transport (alkaline phosphatase, adenosine tri phosphatase and 5-nucleotidase) in developing sporocyst of *Fasciola hepatica*. He subjected tegument, parenchyma as well as the germ balls to the histochemical analysis at various periods of growth and development of sporocyst. He found that the most active metabolism occurred in the germ balls of sporocysts on 8th and 15th day of development, which was associated with intensive proliferation and subsequently differentiation of embryos with in the germ balls.

Cokugras (2003) reviewed the structure and physiological importance of butyrylcholinesterase (Cholinesterase). He reported that animal cholinesterase was widespread enzyme present in cholinergic and non-cholinergic tissues as well as in plasma and other body fluids. BChE preferentially acts on butyrylcholine, but also hydrolysates acetylcholine. Its activity was more important in scavenging of organophosphate and carbamate inhibitors, in regulating cholinergic transmission in the absence of acetylcholinesterase and in activation of some drugs such as cocaine, aspirin, amitriptyline, bambuterol and heroin.

Zurawski *et al.* (2003) carried out cytochemical studies on the neuromuscular system of the deporpa and juvenile stages of *Eudiplozoon nipponicum* (Monogenea: Diplozoidae). By using histochemical localization they demonstrated the neuronal
pathways in whole mount preparation of the unpaired diporpae and freshly paired juvenile stages of *Eudiplozoon nipponicum*.

Ghosh *et al.* (2005) carried out studies on lipid classes and fatty acid composition of a digenetic trematode, *Paramphistomum cervi* and compared it with the fatty acid composition of its host, *Capra hircus*. They found that the total lipid content of *Paramphistomum cervi* was 1.23% of the wet weight of the tissue. Percentage of lipid classes of *Paramphistomum cervi* recorded were 39.04% (neutral lipid), 21.23% (glycolipid) and 39.73% (phospholipid). Palmitic (saturated) and oleic (unsaturated) acids were predominant fatty acids in both the parasite and the host. They assumed that the parasite was completely dependent on the host for fatty acids; however parasite increased the amount of some of the fatty acids by chain elongation process.

Humiczewska and Rajski (2005) studied the effects of the presence of sporocysts, rediae and cercariae of *Fasciola hepatica* on the lipid content in the digestive gland of *Lymnaea truncatula* as well as on lipid levels in tissues of the parasites. Lipids were examined by means of histochemical and cytophotometric techniques. They found that the snail's digestive gland lipid level was almost halved in 20 days past infection, a more than 80% reduction was visible after the subsequent 40 to 60 days. They suggested that the reduction of lipids in the digestive gland of the infected snail points at mobilization of lipid’s energy reserves to compensate for the deficiency of carbohydrate used by the parasites.
They also found that parasite tissue such as tegument, pharynx, suckers and germ balls show considerable lipid contents and were metabolically active. From this they concluded that lipids were used as energy source by developmental stages of this parasite.

Kemmerling et al. (2006) studied the activity, location and molecular forms of acetylcholinesterase (AChE) in different stages of development of *Mesocestoides corti* (cestode) from larvae to adult forms of this endoparasite. They suggested that AchE is a molecular marker of nervous system in Platyhelminthes. The change in molecular forms of this enzyme and increase in its activity during development from larvae to adult worm may reflect the more complex nervous system necessary to adjust and coordinate the movement of a much biggest structure.

Literature survey reveals that histochemistry is still a fledgling field of research primarily so in Kashmir. Some work regarding histochemistry with respect to fish fauna of Kashmir has been done by Channa (1979), Raina (1983) and Tiku (1983) who have histochemically investigated the absorption of iron and lipid in few freshwater teleosts. But no information is available on the histochemistry of helminth parasites including Paramphistomes in Kashmir. Hence the present work entitled “Studies on Histochemistry of Paramphistomes of Sheep and Cattle” was undertaken in order to make a beginning of this type of a study which could prove a beacon light for future researchers.