**INTRODUCTION**

Different metabolic processes occurring in the bodies of living organisms are facilitated by various enzymes. Carbohydrate metabolism takes place in the presence of some hydrolytic enzymes such as acid and alkaline phosphatases. Digestion and absorption of lipids occur in the presence of esterases and lipases. Lipases hydrolyse esters of higher fatty acids and esterases hydrolyse those of shorter chain fatty acids.

Phosphatases are enzymes responsible for cleaving the sugar phosphatases. They have been found associated with structures of absorptive or excretory nature e.g. cuticle or subcuticle of cestodes and intestinal cells of nematodes and the excretory system of trematodes. Although a clear functional connection between localization of the enzymes and physiological activities of the various organs has not been proved, histochemical studies of these enzymes can provide a clue to their biological significance.
Histochemical and biochemical studies on enzyme activity have been carried out by a number of biologists such as Rogers (1947), Borgers et al. (1970), Jenkins (1970), Jenkins and Erasmus (1973). Acid and alkaline phosphatases have been demonstrated histochemically in the intestine of *Ascaris* and *Parascaris*, Yamao (1951). Rogers (1941) found esterases and lipases in the intestine of *Ascaris lumbricoides* and *Strongyulus edentatus*. These enzymes have been shown to be most prevalent in the foregut of *A. lumbricoides* by Carpenter (1953). Nimmo Smith and Keeling (1960) found esterases in the intestine of *Trichuris muris*. Histochemical evidence for lipases was obtained in the ovary, testes, and embryonated eggs of common pin worm, *Enterobius vermicularis* (Marzullo et al., 1957). Nonspecific esterases as determined histochemically are present in the cuticle, excretory canals, hypodermis, muscles, reproductive organs and intestinal cells of *Ascaris* (Lee, 1962) and Probert (1969).

In the present study, an attempt has been made to localize certain hydrolytic enzymes in the various tissues of *Trichuris muris* and *Gangulyeterakis spumosa* using histochemical tests. The enzymes detected
are acid phosphatase, alkaline phosphatase, lipase, esterase and polyphenol oxidase.

The biological significance of these enzymes in relation to their exact localization in the worms has been discussed. Results are supplemented with photomicrographs.

RESULTS

Phosphatases have been shown to be distributed widely in both the worms. Regarding the activity of acid phosphatase in both the sexes of *Trichuris muris* (Plate 11 a, b) a moderate reaction has been observed in the cuticle and a weak reaction in the hypodermis. Somatic muscles are almost negative to the staining action. The bacillary band showed a moderate reaction. The oesophagus also showed moderate reaction. An intense precipitation has been found in the brush border (luminal surface) and the intestinal cells. Ovary in general exhibited moderate reaction but the uterine wall is negative. The egg shell exhibited a moderate reaction, except, in the middle layer and the opercular plug. The embryo proper has a weak reaction but the egg cytoplasm is absolutely negative to the precipitation. Testis, seminal vesicle,
PLATE NO. 11:
GOMORI’S METHOD FOR ACID PHOSPHATASE


b. T.S. of male *T. muris*. Note the high concentration of acid phosphatase in the gutwall X 250.

c. L.S. of copulatory complex of *T. muris* for acid phosphatase activity X 150.
PLATE NO. 11: (CONTD.)

d. T.S. of female G. spumosa showing an intense reaction in the eggshells and embryos. X 150.

e. T.S. of G. spumosa through oesophagus. Note moderate reaction in the oesophageal cells X 150.
spicule and its spiny layer are all stained moderately. The sheath proper showed a very weak reaction.

In *Cangiuleterakis spumosa* (Plate 11 c, d, e) the cuticle, the lateral alae, the hypodermis gave moderate reactions. The somatic muscles showed weak reaction. A moderate reaction has been found in the oesophagus and in the intestine. Similar reaction has been noticed in the ovary, oviduct, egg shell and the embryo proper. No reaction is observed in the egg cytoplasm. Testis, spicule and the cloaca exhibited weak reaction.

As regards the alkaline phosphatase no significant differences have been obtained in the males and the females of both the species.

In *Cangiuleterakis spumosa* (Plate 12 a, b) a moderate reaction has been observed in the cuticle, lateral alae, oesophagus intestine and the rectum where as a weak reaction is noticed in the testis, vas deferens and ejaculatory duct. Ovary, oviduct, eggs and the egg shells all exhibited moderate reactions. Similar results are obtained in the two sexes of *Trichuris muris*. 
PLATE NO. 12: (GOMORI'S METHOD FOR ALKALINE PHOSPHATASE)


Lipase activity has been demonstrated in the different parts of both the worms using Tween 80 compound. In *Trichuris muris* (Plate 13 a, b). The cuticle, the hypodermis showed weak reactions. An intense reaction has been found in the intestine. The female reproductive organs in general showed moderate reaction. A greater concentration is found in the embryos and the egg shells (inner and outer layer) where as no reaction could be found in the egg short cytoplasm, egg plug and the middle chitin layer. In male worm, comparatively less reactions have been observed in the intestine and reproductive organs where as the cuticle and the hypodermis reacted weakly. In *Ganuleterakis spumosa* (Plate 13 c,d,e,f), the cuticle, the lateral alae, the hypodermis entertained moderate reaction. Similarly the intestinal cells and the luminal surface exhibited moderate reaction. The ovary and the eggs also showed moderate reaction but the uterine wall is negative to the staining reagent. The egg shell with its inner and outer layers stained moderately whereas no staining is observed in the middle layer. The male reproductive organs in general are stained weakly.
PLATE NO. 13: GOMORI'S METHOD FOR LIPASE

a. T.S. of female *T. muris* showing an intense reaction in the gut and reproductive organs. X 150.

b. T.S. of female *T. muris* through posterior region indicating the presence of lipase(i) in the gut, (ii) in the inner layer of the egg-shell and in the embryos. X 250.

f. T.S. of male *G. spumosa* showing lipase activity in the body wall. X 150.
PLATE NO. 13: (CONT'D.)

c. T.S. of G. *spumosa* through anterior part showing lipase activity in the body wall X 250.

d. T.S. of female G. *spumosa* showing moderate lipase activity in the gut and egg shells X 150.

e. T.S. of male G. *spumosa* through cloacal region. Note an intense reaction in the caudal alae X 150.
Esterase activity (Plate No. 14 a, b, c) has been demonstrated using Holt’s idoxyl acetate technique. In both the parasites the cuticle, hypodermis, musculature, and the intestinal cells exhibited moderate reaction. The male and the female reproductive organs in general exhibited weak reaction. However, the eggs and egg shells in both cases attained moderate reaction.

Polyphenol oxidase (Plate 15 a, b) has been shown to be prominently present in the egg shells (inner and outer layers) of both the worms. A weak reaction has been observed in the body wall (cuticle), reproductive organs and digestive tract. The embryo proper showed moderate reaction but the egg cytoplasm is found to have no reaction.

DISCUSSION

Phosphomonoesterases have an ubiquitous distribution in nature and vary greatly in their characteristics not only between species but between different tissues of the same species. The terms acid and alkaline phosphatase are group names and do not designate well defined entities (von Brand, eds. 1973) phosphatases have been generally detected histochemically in sites
PLATE NO. 14 : (HOLT'S INDOXYL ACETATE
TECHNIQUE FOR ESTERASE.)

a. T.S. of female *T. muris* showing esterase activity in the eggs and eggshells X 150.

b. T.S. of male *G. spumosa* for the esterase activity. Note its activity in the body wall and gut X 150.

c. T.S. of female *G. spumosa* indicating esterase activity in the eggs and eggshells. X 150.
PLATE NO. 15
CATECHOL METHOD FOR POLYPHENOL OXIDASE

a. A single egg of *T. muris* showing the presence of polyphenol oxidase in the egg shell and embryo X 600.

where absorption, secretion and excretion occur. Formerly, phosphatases were believed to be involved directly in absorption of nutrients and it has been suggested that their presence may be indicative of active transport (Lumsden, 1975). Their important role in the transport of glucose has also been suggested by Pappas and Read (1975). Several hydrolytic and proteolytic enzymes have been located on or near the apical surface of *Ascaris suum* intestinal cells (Lee, 1962, Borgers et al., 1970; Gemtner et al. 1972; Van den Bassoche, 1973 and Beames, et al. 1974). According to Borgers et al. (1970) acid phosphatase activity of the intestinal cells is localized in the lysosomes which are distributed throughout the cells. Nimmosmith and Keeling (1960) have shown biochemically, that *Trichuris muris* possesses a single phosphomonoesterase, a phosphodiesterase, an esterase but no lipase. Enzyme histochemistry of *Anisakis* sp. larvae and *Trichiella spiralis* larvae has been studied by Ruitenber (1972) and he has demonstrated acid phosphatase in the muscles and brush border of *Anisakis* sp. larvae with no alkaline phosphatase in these parts. Similarly, in *Trichinella spiralis* larvae no alkaline phosphatase was found. Cuticle in both cases did not show any
reaction for the above two enzymes, van Dietze and Kempen (1973) found the acid phosphatase but not the alkaline phosphatase in the free living nematode species Panagrellus redivivus, Rhabditis oxycerca and Meloidogyn hyapla.

Wright (1963) noted acid phosphatase activity in the hypodermal gland cells of Capillaria hepatica. Similarly, bacillary band and oesophagus of Trichuris suis displayed an intense activity (Jenkins, 1970) but alkaline phosphatase could not be detected. Recently, Saxena (1980) studied acid and alkaline phosphatases in Aspicularis pakistanika showing their presence in the cuticle, hypodermis, musculature and oesophagus.

Yanagisawa et al., (1970, 1973) made histochemical studies with two blood nematode Angiostrongylus cantonensis and Diroflaria immitis. They demonstrated acid phosphatase in high concentration in the hypodermis. Furthermore, Maki and Yanagisawa (1976, 1977) e carried out biochemical studies using intact Angiostrongylus cantonensis and suggested that the phosphatase in the body wall hydrolyses various phosphonomonoesters in the surrounding medium. This study has been further extended using cytochemical
techniques by Maki and Yanagisawa (1979) and they confirmed the localization of the phosphatase(s) in the body wall of *Angiostrongylus canyonensis*.

Cuticular phosphatases in nematodes have rarely been reported except those demonstrated by Anya (1966). Although the acid and alkaline phosphatase activity shown histochemically by Yanagisawa et al., (1970, 1973) was restricted to hypodermis, Maki and Yanagisawa (1976, 1977) revealed their occurrence in the cuticle as well. Sood and Kalra (1977) detected acid phosphatase in the cuticle, hypodermis and cytoplasmic portions of muscle cells of *Haemonchus contortus* while alkaline phosphatase was located in the hypodermis only.

Maki et al., (1977) carried out histochemical investigations on phosphatases of *Angiostrongylus canyonensis*, *Dirofilaria immitis*, *Ascaris lumbricoides* and *Trichuris muris*. In all the four nematodes the acid phosphatase activity was hardly seen in the cuticle and muscular layer. The bacillary band in *Trichuris muris* was strongly positive. A strong activity was observed in the intestinal membrane of *Ascaris lumbricoides* while the activity was weak in case of *Trichuris muris* and *Dirofilaria immitis*. 
Parshad and Guraya (1978) histochemically detected an intense activity of acid phosphatase, a moderate activity of lipase and no activity of alkaline phosphatase in the brushborder of intestinal cells of *Ascaridia galli*. They concluded that the intestinal epithelium of *Ascaridia galli* is mainly involved in the absorption and digestion of nutrients. Maki and Yanagisawa (1980) have shown acid phosphatase activity in the intestine and the reproductive organs of *Dirofilaria immitis*. Histochemical localization of acid phosphatase in the five worms viz. *Litomosoides carinii*, *Dirofilaria immitis*, *Ascaris lumbricoides*, *Trichuris muris* and *Ancylostoma caninum* has been studied recently by Maki and Yanagisawa (1980). The results obtained by them suggest a possible clue to understand the physiological significance of the enzymes in relation to their localization. They obtained positive reaction in the body wall of first two worms and negative reaction in the body wall of the intestinal nematodes i.e. *Ascaris*, *Trichuris* and *Ancylostoma* but the luminal surface of the intestine of *Ascaris lumbricoides* and *Ancylostoma caninum* was strongly positive. The bacillary band of *Trichuris muris* also gave strong reaction although it was obscure in the
intestine. They further observed that the somatic musculature of all the worms studied by them showed no acid phosphatase activity. The presence of acid phosphatase activity as demonstrated by them in the bacillary band of *Trichuris muris* supports the view of Sheffield (1963) who suggested that the bacillary band of whipworms is related to absorption, excretion and secretion. The author wishes to uphold these views with respect to *Trichuris muris*, under study.

Recently Maki and Yanagisawa (1980) gave comparative account of the distribution of acid phosphatase in the intestine and the body wall of *Setaria* sp. and the gastro-intestinal nematodes such as *Toxocara cati*, *Toxocara canis*, Physaloptera and *Ancylostoma duodenale*. They emphasised that these gastrointestinal nematodes possess a very high activity in the luminal surface of their intestine and no activity in their body wall where as in *Setaria* sp. the situation is reverse. The reproductive organs of all the above five nematodes show weak reaction, the activity in the egg shells being slightly higher.
Comparing the present results in the two worms under study with those of earlier gastrointestinal nematodes, it is observed that in general they follow the same pattern. As to the acid phosphatase in both the worms the luminal surface of intestine showed strong reactions while the body wall showed a weak reaction. Anya (1966) stated that cuticular acid phosphatase in the intestinal nematodes is involved in the formation of the cuticle together with the esterase in the cuticle. According to Yanagisawa and Koyama (1970) and Yanagisawa et al. (1970, 1973) an abundance of histochemically demonstrable phosphatase in the hypodermis is associated with the transcuticular absorption of glucose but Maki and Yanagisawa (1979) state that such absorption is unusual in the parasitic nematodes having a morphologically defined intestine. According to Maki and Yanagisawa (1980) the hydrolysis of the phosphatase ester by intact Angiostrongylus cantonensis occurs mostly in the body wall which is abundant in acid phosphatase.

From the studies of Maki and Yanagisawa it appears that the habitat of the parasites has a direct influence upon the distribution of phosphatase.
In this connection, Maki and Yanagisawa (1979) stated that the high phosphatases activity in the body wall is a characteristic of those parasites which live in the host body fluid. On the other hand the parasites dwelling in the gut of the host and possessing a morphologically defined alimentary canal show a high acid phosphatase activity in their intestine, as supplemented by the present results. An intense activity of acid phosphatase in the intestine of the two worms under study reveal its role in the digestion and absorption of nutrients, supportive to earlier study by Parshad and Guraya (1978). Maki and Yanagisawa (1980) are also of the opinion that the high acid phosphatase activity in the intestine is related to extracellular digestion, phosphorylation, secretion and excretion. The presence of acid phosphatase in the oesophageal wall of Trichuris muris suggests that the ingested material may be subjected to partial hydrolysis. Jenkins (1970) in Trichuris suis has also shown that the oesophageal wall is enzymically active.

In the present study phosphatases have been shown to be present in the cuticle and the hypodermis. The presence of enzymes in the hypodermis has been correlated
with the synthesis and secretion of cuticular proteins (Lee, 1962; Jamuar, 1966). Alkaline phosphatases are involved in secretion, absorption, calcification and formation of fibrous proteins (Sood and Kalra, 1977) but according to Maki and Yanagisawa (1979) the phosphatase whether concerned with protein synthesis, excretory and secretory mechanisms is a question to be resolved. Even in tissue nematodes although the physiological processes like absorption, secretion and excretion are known to occur through the body wall, Maki and Yanagisawa (1980) quote "The body walls of tissue nematodes rich in phosphatase carry out the above functions is a matter of conjecture".

There are no significant differences in the distribution of phosphatases in the males and the females of the present two worms. From these observations it can be concluded that both male and the female have similar metabolism as far as phosphatases are concerned. In the female reproductive system, besides ovary and the oviduct phosphatases have been detected in the egg shells and the embryos inside. Although the exact role of phosphatases in the eggs is not known, the author is of the opinion that the phosphatases might bring about hydrolysis of the
substances present in the shell membranes during the hatching process. With this regard Wharton and Jenkins (1978) in *Trichuris suis* have pointed out that the most likely point for enzymic degradation during the hatching process is the opercular plug which consists of an arrangement of chitin/protein complex, the protein fraction being lower in proportion. Although which of the enzyme(s) hydrolyzes the egg shell is not exactly known. In view of the earlier observations, the author feels that certain enzymic actions do take places to make open the opercular plug for the release of larva.

As stated earlier in the introductory part the lipases and esterases have wide range of distribution in the nematode body. Lipases have been detected in the alimentary canals of *Strongylus*, *Ascaris* and *Leidynema*. These nematodes have different methods of feeding and different foods. Nimmosmith and Keeling (1960) in their biochemical studies on some hydrolytic enzymes could not detect lipase activity in *Trichuris muris*. Likewise Jenkins (1970) in *Trichuris suis* also could not detect lipase activity histochemically. However, during the present investigation,
the author using Tween 80 compound, observed a clear granular precipitation in the intestinal cells and in the female reproductive organs including eggs and egg shells (Plate 13 a). As lipids have been found in abundance in the present worms, the lipase so detected in the cut might bring about the hydrolysis of the lipids. In case of eggs, the lipase might hydrolyze the lipid layer and lead to changes in the permeability of this layer during the hatching process. This is evidenced by the work of Rogers (1958, 1962). He explained the hatching stimulus in the eggs of *Ascaris lumbricoides* in presence of the enzymes which are capable of hydrolyzing the various layers of the egg shell. He has been able to isolate a lipase which had an optimum hydrolytic activity of 2% Tributyrin at a pH 7.2. This was thought to act as an esterase on the innermost impermeable lipid layer. Before hatching, the hydrolysis of the lipid layer is thought to lead to changes in the permeability of the egg shell (Croll, 1976).

Regarding the role of enzymes in the egg metabolism, it appears from the early literature that there is no precise information available. Lipids and carbohydrates
are stored in the eggs and utilized during their development. Lipid reserves predominate in the early stages of the eggs and in the free-living infective stages of some nematodes (Florkin and Scheer, 1969 eds.).

Faure-Fremiet (1913) believed that during embryonation the lipid decreases and the glycogen increases. From this observation he suggested the synthesis of glycogen from the lipids. Passey and Fairbairn (1957) also noted that there is a quantitatively important conversion of triglyceride to carbohydrate. In this respect, the author believes that the catabolism of lipids stored in the eggs may be facilitated by a lipase and/or esterase. The so formed carbohydrates may be subjected to hydrolysis in presence of phosphatases. The alkaline phosphatase in the eggs may be concerned with the synthesis of egg proteins. However, the author feels that further investigation is essential to understand the role of enzymes in the embryonic development of nematodes.
Localization of non-specific esterase has been recorded in the cuticular layer of *A. c. lumbricoides* and *Nippostrongylus brasiliensis* (Lee, 1962, 1975) but it was found to be absent in the cuticle of *Trichuris suis* (Jenkins, 1970). However, esterase has been traced in the hypodermis, muscles and the bacillary band. Esterases were identified close to the base of the spicules in *Meloidogyne* (Berd, 1966). Further, they appeared as thin strands along the length of the spicule in *Heterakis* and *Nippostrongylus* (Lee, 1973). The esterase located in the spicule is assumed to be cholinesterase (Croll, 1976 eds.) which is associated with the sensory function of the spicule. Lee and Alkinson (1976) have reviewed different nematodes such as *Nippostrongylus*, *Strongylus*, *Trichuris* and *A. c. lumbricoides* showing esterase activity in their alimentary canals.

In the present study, the presence of esterase in the cuticle, hypodermis and muscles indicates that these are the areas of intense metabolic activities. Its localization in the gut may be indicative of its hydrolytic function. The exact role of esterase in the eggs and egg shells is not fully understood. Probably
it may bring about changes in the permeability of lipid layer at the time of hatching. In this regard Rogers and Sommerville (1968) have reported that during hatching of *Ascaris* eggs, the juvenile, after stimulation, secretes esterase as one of the enzymes which is capable of disrupting the inner layer of the egg shell.

Localization of polyphenol oxidase has been noticed in the egg shell of the two nematodes under study. Special attention has been given to the role of polyphenol oxidase in the tanning process of the egg shell which has already been discussed under egg shell histochemistry.