DISCUSSION

A. Surface ultrastructure (Plates I, II, III and IV):

In revealing and displaying the surface ultrastructure of natural objects, Scanning Electron Microscope (SEM) is invaluable. SEM is playing a significant role in unfolding natural diversity. It allows more precise and meaningful identification of biological population.

The cuticle is the outer covering of nematode's body with its invagination and outgrowths. The cuticle invaginates at the mouth, rectum, cloaca, vagina and excretory pore. The cuticle also invaginates at the anterior end with a pair of lateral sensory organs the amphids, and at the posterior end with a pair of sensory organs the phasmids.

By far the extremities of the body have received the primary importance yet the structures, like transverse striae and longitudinal ridges, vulval and anal apertures, excretory pore and egg, the morphology of which have received little attention from taxonomic point of view when observed under light microscope, have also been the subject of consideration under SEM studies in recent decades.
Discussion

Despite the records of Scanning Electron Microscopic studies of some nematode species from India detailed information about surface ultrastructure is still incomplete. There are lacunae in some of these cases because a number of description together with specific diagnosis fail to provide a clear understanding of the surface topography.

In the present study, surface topography of cephalic and caudal regions reveal that mouth is armed with three lips, one dorsal and two ventro-laterals. Each lip is trilobed. It is generally agreed that the cephalic structures arose from a primitive hexaradiate or six-lipped form. The six lips may have become fused or otherwise modified. Dorsal lip carries two large marginal papillae. Each ventro-lateral lip bears one large and one small papillae.

Soleim and Berland (1981) studies the morphology of *Thynnascaris adunca*. SEM revealed two oval shaped papillae on each of three lips and circular amphids on subventral lips.

Majumdar (1961) reported two papillae on each of three lips by Light Microscopic observation. But the present study records that the papillae of ventro-lateral lips are unequal in length.
Amphids have been located on the ventro-lateral lip. Teeth have been demonstrated in their three dimensional configuration. The present Scanning Electron Microscope study may be used for redescription purpose, for determining the morphological differences among related species and subspecies for the study of action of anthelmintic drugs on the surface topography.

The cuticular striations of Strongylurus bengalensis are extremely fine. According to Chitwood and Chitwood (1950) transverse markings are of two types. First is the striation, an indentation of the cortex. The area between two adjacent striae is the interstitial region. If a striation is deep enough to involve the median zone as well, it becomes annulation. The area between two annulations is an annule. These transverse markings are present in varying degrees in all nematodes and are associated with the characteristic dorsoventral undulatory movement (Bird, 1971). Lysex (1980) has studied the SEM morphology of Ascaris lumbricoides of both sexes. The arrangement of lip denticles, vulval morphology and transverse striae were studied. His observations indicate that transverse striae differ morphologically in different parts of the body.

Chakravorty (1936) while establishing the species reported the occurrence of nine pairs of caudal papillae in male, but
Majumdar (1961) reported 10 pairs. According to present study there are ten pairs of caudal papillae, seven of which are postanal and the rest three pairs are preanal.

Phasmids have been reported as rounded apertures between third and fourth pairs of postanal papillae. The variation in the number of postanal papillae may be due to intraspecific variability or Majumdar (1961) probably considered phasmids as one of the caudal papillae.

The spicule in its three dimensions have been described in this species. The structure of the spicule indicates that the outer surface is not smooth but armed with granular structures, which may have a copulatory function. The tail has a minute spike, the caudal end is abruptly truncated. Thus, the sucker and the cloaca open almost posteriorly. The spicules are subequal, in length. The data which are available from light microscopic studies (Baylis, 1936; Majumdar, 1961) are confirmed from the viewpoint of Scanning Electron Micrographs.

These observations, especially microphotography as apparent from SEM studies, clearly depicts the surface topography and components of the cephalic and caudal ends of this species of nematode. The present worm agrees with Heterakidae in
having well defined lips, preanal muscular caudal sucker in male with chitinoid boarder and absence of buccal capsule. Absence of cordons, presence of feeble lateral alae, club shaped oesophagus devoid of posterior bulb, unequal spicules and the absence of accessory piece are the characters of the genus.

B. **Histochemistry**

Histochemistry is a classical tool which is becoming more and more important in correlating structure and function in nematodes (Bird, 1971). The knowledge of chemical composition of nematodes came solely from the light microscopic histochemistry. The average ratio of thickness of cuticle to diameter of nematode is $1:34$ (Bird, 1971). The cuticle of this species is very thin and the ratio is atypical ($1:126$). It is likely that the ascarids in general have a thinner cuticle in comparison to their body diameter.

The cuticle is collagenous and has been demonstrated by Mallory's PTAH (Pl. XVI, Figs. 1 and 2). The blue tinge observed in the cortex and basal zones indicate the presence of fibrin type proteins along with collagens. Biochemical studies indicate that nematode's cuticular collagens differs markedly from
vertebrate collagens in molecular weights, assembly of modes of cross-linking (Selkirk et al., 1989). These unique features presumably represent a highly specialised modification of this class of proteins in order to suit the functional requirements of cuticle (Selkirk et al., 1989). The fibrin type of reaction in the cuticular zones of *S. bengalensis* may be due to a special category of collagen which is very similar to fibrin.

Periodic acid-Schiff reaction (Pl. XII, Fig. 1) demonstrated the hexose containing mucosubstances in the epicuticle-exocortex complex with lesser intensity in other zones. The surface antigens have been a focal point of studies on nematode parasites, partly because they are likely to be targets of protective immune response, but also because their structure and function may shed interesting light on the nature and operation of extra cellular nematode cuticle itself (Maizels et al., 1989). It has been biochemically observed that a number of collagenous and non-collagenous surface components including glycoproteins are exposed on nematode surface (Zuckerman et al., 1979; Masood et al., 1987; Maizels et al., 1989). The hexose containing mucosubstances that have been detected in greater amount in the epicuticle-exocortex complex must be representing the sugar moities of surface proteins.
Acetone - Sudan Black Method of staining for bound lipids reveals the presence of bound lipid in the cortex and median zones (Pl. XV, Figs. 1 and 2). Lipids have been detected in the epicuticle of various nematodes (Bird and Deutsch, 1957; Parshad and Guraya, 1977; Sood and Kalra, 1977). This surface lipid is an essential component of all nematode cuticles (Bird, 1984). The lipids of epicuticle are bound to proteins and form a strong lipoprotein complex comparable to cell membrane, which acts as a resistant layer as also as a selectively permeable membrane. The present observation of the presence of bound lipids corroborates the earlier findings.

From mercury-bromophenol blue staining (Pl. IX, Figs. 1 and 2) it is clear that the cuticle in general is made up of proteins. The epicuticle where the insignificant reaction is observed clearly indicates that its chemical makeup is different from the cuticle proper. The similar intensity of reaction in the epidermis and the contractile portion of muscle indicates the presence of similar amount of protein in these regions.

Red coloration of the cuticle by Van Gieson's stain also indicate the presence of collagen (Pl. VI, Figs. 1 and 2).
Verhoeff's method for elastic fibres clearly demonstrated the presence of elastin in the epicuticle - exocortex complex (Pl. VII, Fig. 1). The protein of exocortex was regarded as keratin by earlier workers (Lee, 1966; Bird, 1971). This protein differs from a typical vertebrate keratin in several characteristics, as also its resistance to collagenase activity and failure to detect hydroxyproline in the hydrolyzates suggests that it is not a typical vertebrate collagen either. Quinone tanning, in various species of nematodes makes this a resistant layer. The protein of exocortex has been named 'cuticlin' (Fujimoto and Kanaya, 1973). Chemically elastic tissue consists of mucopolysaccharides and a protein known as elastin which contains a large variety of amino acids (Copenhaver et al., 1971). Elastin differs from collagen in that it has very little hydroxyproline (Copenhaver et al., 1971). Apparently there is no histochemical method specific for 'cuticlin' and the structure stained black by Verhoeff's method might be due to the presence of 'cuticlin' whose chemical make up and configuration is possibly very similar to elastin.

Millon reaction is a classical method for the demonstration of tyrosine. This reaction is due to the presence of protein molecules of hydroxyphenyl group and the only amino acid
containing hydroxyphenyl group is tyrosine (Pearse, 1968). Bird (1957) detected 17 amino acids from the cuticle of *Ascaris lumbricoides*, tyrosine constituted 0.50 g of amino acid nitrogen per 100 g of total nitrogen. The present observation (Pl. X Fig. 1) indicates that in *Strongyluris bengalensis* the tyrosine containing proteins are present only in the epicuticle.

Ninhydrin-Schiff method (Pl. XIV, Figs. 1 and 2) demonstrated that the proteins with reactive NH$_2$ groups are present maximally in the epicuticle-exocortex and feebly in median zone and outermost layer of basal zone. Cuticular proteins have been characterised in a number of nematodes (Bird, 1984; Betschart et al, 1985; Selkirk et al, 1989) by biochemical methods and a number of collagen proteins with varying molecular weights have been detected. The present histochemical detection of protein bound NH$_2$ indicates that varied types of proteins are present in *Strongyluris bengalensis* cuticle.

Mallory's triple staining method (Pl. V, Figs. 1 and 2) reveals that the cuticle is composed of epicuticle, exocortex, endocortex and basal zone. The epicuticle being the outermost layer, is evident as a taint line, cortex is divided into exo - and endocortex and the basal zone is divisible into three layers.
Effect of laser on the cuticle of *Strongylurus bengalensis* 

(Pl. VIII, Fig. 2 and Pl. XI, Figs. 1 and 2): 

The experiment done by the laser radiation which was made incident on a fixed area of *S. bengalensis* cuticle, was designed to probe cuticular damage/change if any, through light microscopic observation. It is known that exocortex is the most stable and resistant layer of the cuticle, which is characterised by the presence of sulphur-containing groups and quinone tanning (Bird, 1971). The protein of this layer which was regarded as ketatin (Bird and Bird, 1969) has been named cuticlin (Fujimoto and Kanaya, 1973) due its unique nature. Moreover, the cuticle is covered by a very resistant lipid membrane forming the epicuticle (Bird, 1971; 1980). Whether these two layers prevented at the molecular level causing any break in its configuration leading to erosion or disruption of most externally located surface structures due to laser radiation in the present experiment, or any other factors resulting due to application of such beam which might have been acting upon have to be determined by further studies. It is apparent that the beam is absorbed mostly in the epicuticle-exocortex complex allowing very little portion of the beam to go inside endocortex layer. Thus it is concluded that if the intensity of incident laser radiation is increased then it is possible to have the idea
about the interaction of laser beam with the cuticular layers and it has been proposed to continue this work with increasing intensity of laser beam.

C. Organic constituents (Tables I - IX):

Relative to generalisation about biochemical behaviour of Zoo-parasitic nematodes paucity of informations preclude from drawing any conclusion. Prior to have a glimpse at the nematodes metabolism each organism must be examined as a biochemical entity (Saz, 1966). Occasionally the smaller size of the parasites and difficulties in obtaining them prove to be deterrent factors in analysing their components quantitatively. The higher percentage of protein in terms of wet weight (17.78%) in the cuticle of *Strongyluris bengalensis* corroborates the present histochemical demonstration as also earlier records (Bird, 1971). In parasitic nematodes where the major metabolic activities are directed towards egg production, considerable importance should be given on the total amount of protein present. The determination of protein and their fractions thereof is of interest both from the stand point of biochemistry and immunology. It is believed that gastro-intestinal nematodes bear identical proteins. Immense fecundity
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of most parasitic worms and the fast growth of larval forms obviously prove that helminth parasites synthesize protein rapidly (von Brand, 1973). Various species of parasites utilize protein to a different degree for energy production (von Brand, 1973). As the intestinal helminth parasites are known to draw raw materials for protein synthesis from their hosts, size of the parasite may be a factor directly related with the protein value (Srivastava and Gupta, 1976). In nematodes proteins are associated with the cuticle, muscles and other tissues. Cuticle of *Ascaris lumbricoides* contains a small amount of carbohydrates and lipids, but proteins are the predominating one (Fairbairn and Passey, 1957).

Relative to carbohydrates it has aptly been pointed out by Cheng (1973) that glycogen forms the chief stored material in the tissue system of nematodes. It is known that carbohydrate content of different parasitic worms varies considerably and an obvious correlation with the habitat of the species is difficult to establish (von Brand, 1973). The percentage of carbohydrate in the cuticle of *Strongylurus bengalensis* is about 2.68% in relation to presence of small amount of carbohydrate corroborates the earlier records (Bird, 1971). It is probable that a part of carbohydrate contributes towards the antigenic molecules expressed as surface carbohydrates and this nematode may have a great array of antigenic molecules.
expressed superficially. Ribonucleic acid has been detected in the cuticle of a number of nematodes (Anya, 1966; Bird, 1971; Sood and Kalra, 1977). The present estimation of feeble amount of pentose (0.74% of total carbohydrate) may be due to the presence of RNA in the cuticle. Hexose (23.50% of total carbohydrate), trehalose (12.68% of total carbohydrate) and glycogen (20.52% of total carbohydrate) may be concentrated in the epicuticle-exocortex complex as observed histochemically (PAS technique). Apart from monosaccharides, trehalose and glycogen together constitute 58.77% of total carbohydrate. Other carbohydrates (41.23%) may be present in the form of hyaluronic acid and chondroitin sulphate which are widely distributed in the connective tissues (Lee, 1966; Mahler and Cordes, 1968). The glycogen may not be present as reserve food material rather it may be present as structural component of surface glyco-proteins (Selkirk et al., 1989).

Lipids are important component of eggs and in parasitic nematodes, non-polar lipids are usually present in higher concentration than polar lipids. Lipids are also important constituents of all membranous structures. There are evidences of existing a direct relationship between carbohydrate and lipid metabolism of nematodes (Cheng, 1973). Lipid content of whole body of some parasitic helminths are available.
Discussion

Frayha and Smyth (1983) listed the total lipid and its fractions in whole body of some helminths. Dasgupta and Hazra (1983) provided informations on the lipid composition of some ascarids. It is apparent that the lipid composition of the cuticle remained largely restricted to larger ascarids (Frayha and Smith, 1983). The present estimation reveal that total lipid content of the cuticle is about 1.52% does not deviate markedly from the data recorded for Ascaris lumbricoides (0.61% Cavier et al., 1958). Biochemical estimation of cuticular cholesterol is about 0.45%. Of the total lipid present, cholesterol constitue 30.26%. The cholesterol is a key intermediate in the biosynthesis of related steroids (Mahler and Cordes, 1968) requires special attention. It is know that cholesterol form important component of cell membrane (Copenhaver et al., 1971). Whether the cholesterol is used as a lipid component of cuticular surface or as an intermediate in the biosynthesis of related steroids is not clear.

The present biochemical detection of ascorbic acid (0.03%) corresponds the histochemical findings of this vitamin in the cuticle of Aspicularis and Ascaris (Anya, 1966). This vitamin has been shown to be associated with collorgen fibrogenesis and it has been suggested that it is required for the hydroxylation of proline and lysine residues (Bird and Bird, 1969).