Present state of knowledge on the structure and composition of the helminth cuticle has resulted from the application of electron microscopy and other modern biological techniques. Available evidences indicate that the three main groups of helminths, the platyhelminths, nematode and acanthocephala, have quite different external coverings. The cuticle of digenetic trematodes and cestodes seems to be syncytical cytoplasmic layer which is continuous with nucleated cytoplasm situated in the parenchyma underneath the musculature of the body wall. The surface of tapeworms is formed into microvilli which increases the absorptive area. The most superficial layer contains mitochondria and enzymes which are believed to be concerned with active transport of substances. Microvilli do not occur in the digenetic trematodes but vacuoles and vesicles, mitochondria and enzymes do occur
in the outermost layer of the body and these may serve the same purpose of transportation. In the Acanthocephala, pores and canals in the cuticle and body wall are presumed to be conduits for nutrient materials passing into a body which, like that of cestodes, lacks an alimentary canal. On the other hand, the cuticle of nematodes has a very complex and varied structure.

The body wall of nematodes consists of an external cuticle, a hypodermis and a single layer of longitudinal muscle cells (Fig. 2). The cuticle covers the whole of the external surface and also lines the buccal cavity, oesophagus, rectum, cloaca, vagina and excretory pores. Nematodes owe much of their success as a large and ubiquitous group to an organisation which includes a cuticle of great ultrastructural complexity and evolutionary plasticity. Much more is known about the structure and composition of cuticle of nematoda than about that of other helminths.

The literature on this subject shall be discussed under the following heads and subheads:

1. Structure
   a) Ultrastructure
   b) Histochemical
   c) Chemical composition
   d) Biochemical nature of the cuticular surface
Fig. 2. Electron micrograph of the cuticle of the anterior region of *Euchromadora vulgaris* (longitudinal section) to show the four major layers and the canals in the middle layer. 1, Outer membrane; 2, cortex; 3, middle layer formed from a series of overlapping plates; 4, a basal layer. epid, Epidermis (hypodermis); muse, muscle.
2. **Functions**
   a) Protective
   b) Immunological
   c) Transport and permeability
   d) Locomotion

1.a. **Ultrastructure**

The cuticle of nematodes is basically a three-layered structure with an outer cortex, a middle matrix and an inner basal layer (Fig. 2). These three layers, which are of varying thickness in different species, are often subdivided due to which it sometimes becomes difficult to determine their boundaries particularly in electron micrographs. Furthermore, since several of the layers of the cuticle are fibrous differing only in the orientation of the fibrils or degrees of compactness, it may even be difficult to assign any one layer as a particular basic layer. Inglis (1964a,b) considered that the three layered cuticle is modified around a canal system, believing that the punctations in the cuticle of the chromadorida and other nematodes represent canals. Though such canals are found to occur in *Ascaris lumbricoides*, *Stronglus equinus* and *Euchromadora vulgaris* (Bird, 1958a; Watson, 1965a,b), however, their occurrence may not be generalized. Presence of fibrils to a greater or lesser extent in the cuticle appears to be a common
feature for nematodes. In some nematodes fibrils are difficult to resolve, even with the electron microscope, and these layers then appear homogenous. On contrary, in some parasites fibrils are compacted basically to form two or three layers of fibres, e.g. in Ascaridoidea (Inglis, 1964a). In others (Enoplus, Dosylaimus and Mermis) the fibrils in the outer part of the matrix form fibres, while in Oxyuris equi, the fibrils form layers of fibres in the outer part of the matrix layer and of the basal layer (Bird, 1958a). This formation of fibres from the layers of fibrils is usually, but not always, associated with an increase in size of the body and may have mechanical associations in locomotion (Harris and Crofton, 1957). According to Watson (1965a) the struts in the fluid filled layer of the cuticle of adult _N. brasiliensis_ which support the longitudinal ridges of the cuticle may, originate from rod like structures similar to those found in the matrix layer of some free living marine nematodes.

1.b. Histochemical

The nematode cuticle has been traditionally viewed as an acellular exoskeleton. Chemically, its major structural elements are proteins similar in many respects to collageons, which are ubiquitous components of
extracellular connective tissues (Lee, 1966; Bird, 1971). In many cases it appears to be separated from the hypodermal cytoplasm by a membrane (Watson, 1965; Roggen et al., 1967), and is itself devoid of cellular organelles. However, the outermost boundary of the cuticle is a trilaminate layer approximately 100Å thick. This raises the possibility for the cuticle being limited by a plasma membrane, and thereby to be considered a cellular derivative (Bird, 1971). On the other hand, many insect cuticles are covered by a morphologically similar structure, referred to as "cuticulin", which per se is not a cytomembrane, but a stabilized layer of secreted lipids (Fisher, 1971). The electron microscopic studies thus support the early view of Chitwood and Chitwood (1950) that the nematode cuticle is derived internally from part of the hypodermis, rather than as an extracellular secretion therefrom. Thus the cuticle might be interpreted as a modified cellular component. According to Bonner et al. (1970), the outer boundary appears to be a plasma membrane derived from the apical hypodermal membrane.

This membrane is directly formed from cytomembranes of the endoplasmic reticulum, while the internal layers of the cuticle form by differentiation of the hypodermal cytoplasm. The process in certain respects appears to be analogous to the cytomorphosis manifested in the superficial...
layers of the mammalian epidermis, where cells originating in the basal regions undergo keratinization as they are displaced toward the surface.

The keratin synthesized within the cytoplasm of these epidermal cells eventually displaces the organelles, which atrophy, though the original plasma membrane is retained unlike the keratinized epidermal cells of mammalian skin. However, the nematode cuticle remains metabolically active, possessing enzymes, RNA, and the capability for further growth and differentiation (Lee, 1962; Anya, 1966a,b; Watson, 1965). As emphasized by Bonner et al. (1970), this is accomplished by the maintenance of an intimate relationship between the cuticle and hypodermal cytoplasm, manifested structurally in at least some nematodes by discontinuities in the cuticle-hypodermal membrane. The hypodermis of most adult parasitic nematodes is syncytical, with the nuclei located in the lateral cards. As determined by Thust (1968), the hypodermis of *Ascaris lumbricoides* is derived from the blastomeres which form a layer of discreet cellular units merge to form a syncytium. The hypodermal cytoplasm of these and other nematodes contains glycogen, lipid deposits, mitochondria, ribosomes, endoplasmic reticulum and golgi bodies (Wright, 1968; Jenkins, 1969; Hinz, 1963; Wisse and Daems, 1968; Lee, 1966b; Lee, 1970; Bonner et al., 1970; Kozek, 1971). During moulting, the
endoplasmic reticulum hypertrophies in connection with synthesis of cuticular proteins (Lee, 1970; Bonner et al., 1970).

1. c. Chemical composition

Bird (1954, 1956, 1957, 1958a,b) carried out a thorough and extensive study into the chemical composition of the cuticle of nematodes. He found that the sheath of the third stage larvae (i.e. the uncast cuticle of the second stage larvae) of Oesophagostomum, Ostertagia, Cheberila, Haemonchus and Trichostrongylus is soluble in water at 105°C. Nine amino acids were identified in hydrolysates of mixed collections of larval sheaths and in pure samples of sheaths from H. contortus. These amino acids, in order of the amounts present, were proline, hydroxy proline, aspartic acid, cysteic acid, glutamic acid, alanine, leucine, glycine and valine. Tests for cystine and tyrosine gave negative results (Bird, 1954).

Bird and Rogers (1956) found collagen in the cast sheaths of larval Trichostrongylus such as Haemonchus and, Simmonds (1958) found collagen in the cast cuticle of the fourth stage larva of Nippostronglus brasiliensis. Bird and Rogers (1956) were unable to demonstrate a tanning mechanism in the sheath of these larvae. However, Monne (1959), who described the larval cuticle of Dictyocaulus as collagenous, claimed that quinone tanning occurs in the
cuticle of these larvae and that the larval cuticle is more resistant to the action of probably proteolytic enzymes than is the adult cuticle. Savel (1955) identified thirteen amino acids in the cuticle of *A. lumbricoides*. He drew attention to the ratio of histidine, lysine, arginine (1:5:3) which is close to the ratio in many keratins (1:4:121:1:5:15) and considered that the cortex is composed of a keratin. This layer is however, soluble in hot dilute alkali, which is strong evidence against it containing keratin (Fairbairn, 1957). The cuticle of *A. lumbricoides* contains about 75% of water and small amounts of carbohydrates and lipids, as well as the predominating proteins (Fairbairn, 1956, 1957; Fairbairn and Passey, 1957). Apparently, the cuticle of nematodes is composed of a secreted collagen associated with hyaluronic acid, chondroitin sulphate containing acid mucopolysaccharides and a small amount of lipid. The collagen is usually present as fibrils which are more numerous and more closely associated with each other in some layers (the cortex and the fibre layer) than in others (the matrix layer). The outer cortex contains more sulphur than is found in other layers. This probably is due to disulfide linkages which, together with another type of chemical bond, stabilize the outer cortex and not due to keratin. Polyphenol-quinone tanning also plays
some part in stabilizing the outer cortex of nematodes but not all of them. The cuticle may contain a number of enzymes, RNA and hemoglobin and is evidently not an inert covering.

1.d. Biochemical nature of the cuticular surface

The way cells react to their immediate surroundings is in several respects dictated by surface carbohydrates. Membrane glycoproteins and glycolipids have been shown to be implicated in a wide range of phenomenon such as cell recognition, transport of solutes, hormone and drug receptors etc. (Lennarz, 1982).

Lectins, a class of carbohydrate-binding and cell-agglutinating proteins of non-immune origin, provide a powerful tool to the isolation and characterization of soluble glycoproteins and for probing cell surface sugar moities (Reisner and Sharon, 1980). However, only recently few investigators have directed their attention to this important subject in parasitic biochemistry. Hemoflagellates (Trypanosoma and Leishmania) have been well studied regarding their surface carbohydrates compared to helminth parasites. Among the latter class, only Schistosoma mansoni has received sufficient attention. Thus, Simpson and Smithers (1980) reported that adult male S. mansoni had very high affinity for concanavalin-A and Ricin communis agglutinin; while the parasite bound poorly to
wheat germ, soyabean and peanut agglutinins. This indicated the presence of glucose, mannose and N-acetyl-
glucosamine in high concentrations on the worm surface. Friedman et al. (1982) in Spirometra mansonoides
identified tegumental glycopeptides by the analysis of lectin. Glycopeptides of brush border membrane were
identified by the direct application of the following fluorescein isothiocynate-conjugated lectins to slab gels:
Concanavalin-A, wheat germ agglutinin, Ricinus communis agglutinin-120, soybean agglutinin, and Ulex europaeus
agglutinin-1. Based on the different sugar specificaties of the lectins tested, the oligosaccharide chains of tegumental glycoproteins of S.mansonoides was suggested to contain: D-mannose, D-glucose, N-acetyl-
glucosamine, N-acetylneuraminic acid, D-galactose, and N-acetyl-D-galactosamine. Investigations on the metabolic
events associated with the synthesis of glycoproteins, oligosaccharides and glycolipids in adult worms of
S.mansoni (Rumjanek and Smithers, 1978) revealed the occurrence of a membrane bound enzymatic systems which was
able to transfer mannose from guanosine diphosphate-mannose to a chloroform soluble compound, forming a lipid linked
oligosaccharide. Homogenate of this parasite also transferred glucose and galactose from their uridine
diphosphate derivatives to a lipid acceptor, in comparison
fucose and glucosamine were poorly transferred. The lipid acceptor was believed to be an intermediate in the glycosylation of the worm's glycoproteins and glycolipids. Since glucose, mannose and galactose were the major monosaccharide components of the worm's tegument, Rumjanek et al. (1979) suggested that the mechanism of glycosylation of tegumental macromolecules may occur through the glycosyl transfer system.

Surface carbohydrates of different stages of *Brugia malayi*, (the human filarial parasite) viz., microfilariae, infective larvae and adult worms were analyzed by incubating a panel of fluorescinated lectins (Kaushal et al., 1983). They found that infective larvae and adult worms did not bind significantly any of the lectins, while the microfilariae bound wheat germ agglutinin. The binding of this lectin was found to be saturable and specific, thereby showing the presence of N-acetyl-D-glucosamine on the microfilarial surface. In addition, *in vitro* released microfilariae bound concanavalin-A indicating the presence of glucose/mannose on this form of the parasite. However, similar concanavalin-A binding was not observed with *in vitro* released microfilariae. It was attributed that the masking or loss of surface components during development of microfilariae occurred *in vivo*.
Furman and Ash (1983) found that the sheath of mature *in vivo* derived *Brugia pahangi* microfilariae bound concanavalin-A and wheat germ agglutinin, thereby indicating the presence of N-acetyl glucosamine and glucose or mannose. The sheath of immature *in utero*-derived microfilariae also bound *Limulus polyphemus* agglutinin, peanut agglutinin, *Ricinus communis* agglutinin-I, and soyabean agglutinin, thus indicating the presence of the additional sugars like galactose, sialic acid and N-acetyl galactosamine. However, none of the tested fluorescinated lectins bound to either mature or immature exsheathed microfilariae of *B. pahangi*.

2. Functions

2.a. Protective

The cuticle of nematodes has several functions, notably to protect the parasite against the mechanical and chemical injury directed by the external surroundings thereby allowing the worms to regulate their internal environment. As a result, they have been able to invade almost every type of ecological niche. For this important functions, the parasites equip themselves with some special weapon e.g. Ascarid's cuticle possesses and secretes into the surroundings antienzymes in the form of trypsin- and chymotrypsin inhibitors (von Brand, 1973).
As discussed below, blood and tissue dwelling parasites absorb host derived materials, proteins in particular, to protect them against the immunosurveillance of the host (Soulsby, 1971).

2.b. Immunological

The cuticle of several nematode species are thought to be antigenic (Crandall et al., 1963; Taffs and Voller, 1963; Baratawidjaja et al., 1963) and accordingly antibody binding to the outer surface of the cuticle has also been demonstrated (Crandall and Avean, 1967). However, data presented by Hogarth-Scott (1968) suggest that immunoglobulins adsorbed by certain nematode species represent cross reacting naturally occurring antibodies, rather than nematode specific antibodies. As reviewed by Soulsby (1971), several workers have shown cuticular binding of complement as well as antibody, resulting in the adherence of host blood cells, though the relationship of these phenomena to protective immunity remains undermined. Soulsby (1971) pointed out that the attachment of host serum proteins might actually benefit the parasite in that, the coating of such material would render the cuticular surface more 'host like' and thereby masked from the host's immunosurveillance mechanisms. The surface of certain nematodes bears a density of electronegative charges (Hudson and Kitts, 1971; Soulsby
Bonner et al., 1970) which may reflect the presence of acidic glycans. Such materials are themselves, generally, weakly antigenic (Apfel and Peters, 1970). Treatment of Protostrongylus larvae with neuraminidase reduces this surface charge (Hudson and Kitts, 1971) and concomitantly facilitates attachment of host leukocytes to the cuticle. This observation suggests that the electronegative charges on the cuticular membrane may serve as a physical barrier to cell contact, in that leukocytes possess a similar surface charge, or that the neuraminic (sialic) acid residues otherwise militate against immunogenicity of the cuticular membrane (Apfel and Peters, 1970). In the last two years immunology of cuticle of helminth parasites has dramatically advanced. Now monoclonal antibodies have been used as valuable tools in characterizing the antigens of schistosomes, and in studying the role of the surface antigens of the parasite in the killing of challenge infections (Taylor and Butterworth, 1982). Recently Baschong et al. (1982) have extracted and tested the immunogenicity of cuticular antigens from female worms of Dipetalonema viteae. Surface specific antigens were obtained by proteolytic digestion with proteinase K, or therolysin or subtilisin. When Golden hamsters were injected with extracts, interesting observations were recorded. Subtilisin and
thermolysin extracts provoked antibody formation against somatic structures (e.g., gut, uterus, muscles) but not against the cuticle, whereas immunization with the proteinase-K extract induced antibodies exclusively against cuticular hypodermic structures of female and male worms.

2.c. Permeability and Transport

Nematohelminths have well defined digestive system and may ingest food material from surrounding host fluid and hence need not to depend for uptake of nutrients through the body surface. Possibly due to this reason not much attention was paid towards the transport mechanisms of nematode cuticle. Although these parasites are known for long to osmoregulate through cuticle (Pannikar and Sproston, 1963) indicating the ability to transport ions. Furthermore a size of literature supports the concept that nematode cuticle is impermeable to low molecular weight polar solutes (Pappas and Read, 1975). The subject had been a matter of great controversy till the employment of electron microscopy in parasitology. Using this techniques the cuticle of *Mermis migrans*, which was long known to be permeable to vital dyes (Chitwood and Chitwood, 1974) was later shown to possess morphological features consistent with the absorptive role of the body surface (Poiner and Hess, 1977). Since then number of reports have described absorption through the cuticular
surface. Thus, Weatherly et al. (1963) have shown that
_A. galli_ whose openings were closed with colloidin,
absorbed alanine and glucose through the cuticle. Later,
transcuticular absorption of various amino acids by
_A. suum_ was also established (Berdyeva and
Dryuchencko, 1972).

An alternative method to investigate transport
mechanism was described by Harris et al. (1972) who
demonstrated active transport of leucine through isolated
cuticle of _A. lumbricoides_. Amino acids and sugars were
reported to be transported through the gut of _A. suum_
(Read, 1966; Castro and Fairbairn, 1969) and _Trichuris
vulpis_ (Bueing et al., 1961). Movement of lipids across
the intestine of _A. suum_ had also been reported (Beams et al.,
1974). There are also reports regarding the permeability
of antihelmintics through the _Ascaris_ cuticle (Weatherly
et al., 1963).

As far as the uptake of nutrients by adult filarial
worms are concerned, presence of erythrocytes have been
demonstrated in the digestive tract of _Dirofilaria immitis_
(Maki et al., 1982) and the uptake of trypan blue by
_B. pahangi_ has been demonstrated in vivo (Howells and Chen,
1981), although in vitro _B. pahangi_ does not ingest trypan
blue or other high molecular weight substances (Chen and
Howells, 1979a). Adult _D. immitis_ similarly failed to
take-up trypan blue during \textit{in vitro} incubations (Chen and Howells, 1981a). Adult filarial worms appear to be able to take up nutrients via cuticle \textit{in vitro}, which together with the hypodermis shows structural modifications (Howells, 1980). Adult \textit{B. phangi} can take-up D-glucose, L-leucine, glycine, cycloleucine and adenosine by a transcuticular route (Chen and Howells, 1979a,b; Howells and Chen, 1981; Nduka and Howells, 1980), whilst \textit{D. immitis} has been shown to take up D-glucose and adenosine (Yanagisawa and Koyama, 1970; Chen and Howells, 1981a). The transcuticular uptake of glycine has also been demonstrated in \textit{Onchocerca gutturosa} (Howells, 1980). In addition, Chen and Howells (1981b) have shown that adult \textit{B. phangi} can take up uracil, adenine, hypoxanthine and guanosine (but not thymine, cytosine, orotate, formate, p-aminobenzoic acid or folate) during \textit{in vitro} incubations, whilst adult \textit{D. immitis} can take up uridine and uracil (Jaffe et al., 1972). Cuticular transport in \textit{B. phangi} and \textit{D. immitis} is selective, and neither nematode, for example, takes up exogenous L-glucose, sucrose or thymidine (Chen and Howells, 1979a,b; 1981a,b). The transcuticular uptake of glycine and cycloleucine by adult \textit{B. phangi} seems to take place by diffusion (Nduka and Howells, 1980). Though uptake of glucose and other monosaccharides from the incubation media has been shown by adult \textit{L. hawkinsi},
L. carinii and S. cervi, the site of uptake was not investigated (Buoding, 1949a; Anwar et al., 1975; 1978; Srivastava and Ghatak, 1974; Srivastava et al., 1968).

The microfilariae of L. carinii and S. cervi have also been shown to take-up glucose from the incubation media (Rathaur et al., 1980). Microfilariae have a non functional gut and so uptake presumably takes place across the cuticle, although there are suggestions that the microfilariae of Brugia sergenti ingest nutrients via the buccal cavity (Howells, 1980). The microfilariae of D. immitis can utilize exogenous glucose, amino acids and RNA precursors viz., uracil, uridine, adenine, adenosine but not thymidine (Jaffe and Doremus, 1970; Ando et al., 1980). Chen and Howells (1981b) have shown utilization of exogenous glycine, uracil, adenosine, hypoxanthine and guanine but not thymine, cytosine, orotate, formate, p-aminobenzoic acid or folate by B. pahangi microfilariae.

The 3rd and 4th stage larvae of B. pahangi have been shown, in vitro, to take up glucose and a range of amino acids and nucleic acid precursors, presumably via a transcuticular route (Chen and Howells, 1979a,b; 1981b).

The adult and larval stages of filarial worms all appear, at least in vitro, to be able to take-up low molecular weight nutrients via cuticle. Thus the old
concept regarding the unabsorptive role of the cuticle appears no more valid, however, many more studies on the subject are required to finally disapprove the decades old view.

2.d. Locomotions

Another important function of the cuticle is related to locomotion. The cuticle imposes a restraint on changes of bodily shape, but its structure is such that restraint produces those changes in shape that can and must be made (Clark, 1964). Its elastic properties or mechanical interactions between its substructural components providing an antagonistic force to muscular action (Harris and Crofton, 1957; Inglis, 1964; Wisse and Daems, 1968). According to recent concept many antihelmintics act against the gastrointestinal parasites, particularly Ascaris, by causing a reversible paralysis of their neuromuscular system. Similarly tetramisole also causes spastic muscle paralysis in a number of nematodes including filarial parasites. This movement enables the intestinal parasites to maintain their position against the parastatic movement of the intestine.