

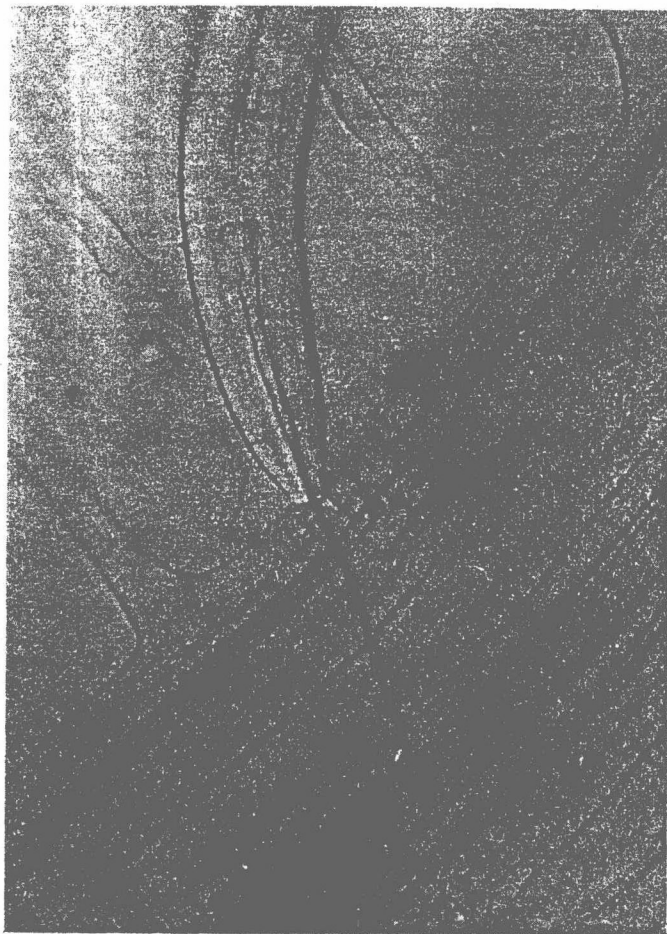
Physiology of nematodes / 2nd ed.

D. L. Lee and H. J. Atkinson.



## PHYSIOLOGY OF NEMATODES





Frontispiece Plant-parasitic nematode (*Xiphinema*) feeding by means of its stylet upon the contents of the vascular tissue in a plant root. (Courtesy of D. L. Trudgill.)

# PHYSIOLOGY OF NEMATODES

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Second Edition

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## Preface

At one time nematodes were studied almost exclusively by plant pathologists and by parasitologists, but they are now also studied by biochemists, biophysicists, geneticists, immunologists, molecular biologists, pharmacologists and neurophysiologists, who see the nematode as an exciting and challenging animal suitable for many fields of fundamental and applied research.

During the ten years that have elapsed since the appearance of the first edition of this book, a large number of research papers and reviews and several books, which describe various aspects of the biochemistry, physiology, functional morphology and ecology of free-living and parasitic nematodes, have been published. This book is intended to be an introduction to the physiology, biochemistry and structure of nematodes for undergraduate students and their teachers, and is not intended as an exhaustive review of the subject, although it is hoped that research workers in the field may also find it useful.

The information in this book is based on many publications. Unfortunately, it has not been possible to include references to all of them; consequently many references are to books, reviews and recent papers and these will introduce the reader to the earlier, more detailed, literature. For this reason, reference to an author in the text does not imply that this author was the first to have made the observation in question.

We are indebted to Professor A. O. Anya, Dr K. S. Cheah, Dr D. J. McLaren, Mr D. R. Newall, Dr D. J. Wright and Dr K. A. Wright, who allowed us to read some of their work prior to publication, and to Mrs Marjorie Miles who typed the manuscript.

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December 1975

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H. J. Atkinson

# 1 Introduction

## 1.1 General Biology

There is a general belief among biologists that nematodes are mainly parasitic, however large numbers of free-living nematodes inhabit marine- or fresh-water-mud and the soil and several million can be found per square metre in the top few centimetres of sub-littoral mud. These free-living nematodes feed upon bacteria, fungi, decaying materials and microscopic animals. They are seldom seen, however, as most of them are less than a millimetre in length. Nematodes are widely distributed and are found in almost every type of environment.

They occur in arid deserts and at the bottom of lakes and rivers, in the waters of hot springs and in the polar seas where the temperature is constantly below the freezing point of fresh water. They were thawed out alive from Antarctic ice in the far south by members of the Shackleton Expedition. They occur at enormous depths in Alpine lakes and in the ocean. As parasites of fishes they traverse the seas; as parasites of birds they float across continents and over high mountain ranges.<sup>1</sup>

Some will survive for many months in liquid nitrogen; others live in unusual habitats such as vinegar, bill posters' paste or drip mats that have been soaked in beer. A number of species are cryptobiotic and can survive for months or years in an almost desiccated state until water becomes available, when they revive. Nematodes also feed on living plants, either as ecto- or endoparasites, and they are one of the important groups of invertebrate parasites of animals and man.

The typical nematode is spindle-shaped, unsegmented and bilaterally symmetrical (figures 1.1 and 1.2). The adoption of a parasitic life has, in most cases, done little to alter the general shape of nematodes and

... the elementary student may be forgiven at times for thinking that there is only one nematode, but that the model comes in different sizes and with a great variety of life histories.<sup>2</sup>

Respiratory and circulatory organs are absent and the excretory organs are unlike those of other invertebrates. Typical flagella have been found in the intestine of *Eudorylaimus*<sup>3</sup> but this is a rare exception as motile cilia and flagella are not normally present in nematodes. Modified flagella are present in some sense organs. The body wall is composed of a collagenous cuticle, a cellular or syncytial hypodermis, and a layer of longitudinal muscle. The alimentary tract consists of a terminal mouth, buccal cavity, pharynx (more usually called the oesophagus), intestine, rectum (cloaca in the male), and sub-terminal anus. The body cavity, in which lie the gonads, is a pseudocoelom (figures 1.1 and 1.2).



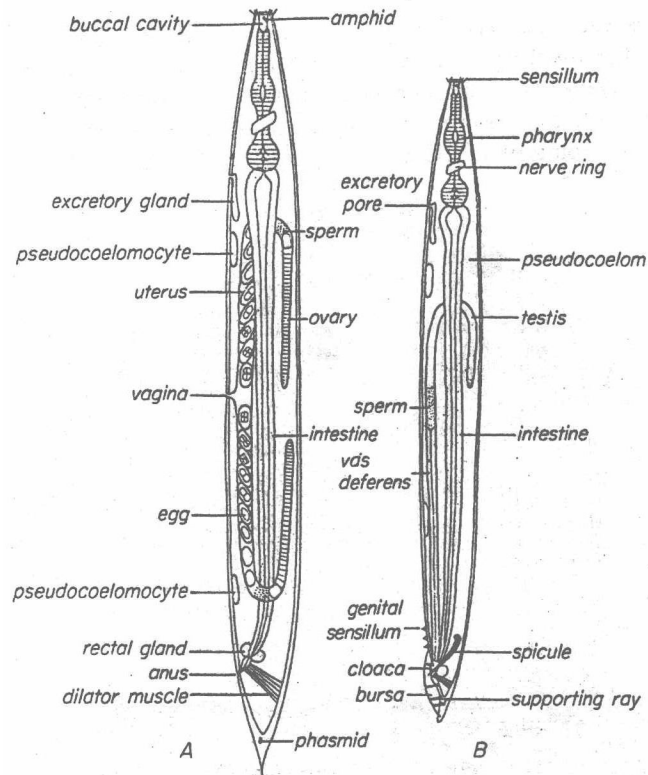


Figure 1.1 General morphology of a nematode (hypothetical). (A) female; (B) male. Lateral view.

The sexes are almost always separate. Parthogenetic females occur in some species, and in others hermaphrodites are found, the gonads first producing sperm and later producing eggs, but these are not typical. The males are usually smaller than the females and possess copulatory aids such as genital sensilla and spicules. The sperm are amoeboid and different groups of nematodes have sperm of a characteristic shape and structure (see figure 6.3).

Nematodes undergo four moults from egg to adult worm. The juvenile (often referred to as a larva in the literature) is similar to the adult nematode but differs from it in size, sometimes in mouth parts, in the lack of gonads and the absence of copulatory structures. Some nematodes moult once or twice within the egg before hatching; others retain the cuticle of the previous stage at a certain stage in the life cycle. This acts as a protective covering against adverse conditions, as in infective juveniles of various animal-parasitic species of trichostrongyles and in 'dauer' juveniles of some free-living species that are transported from place to place on the outside of insects. After the fourth moult the nematode emerges as the

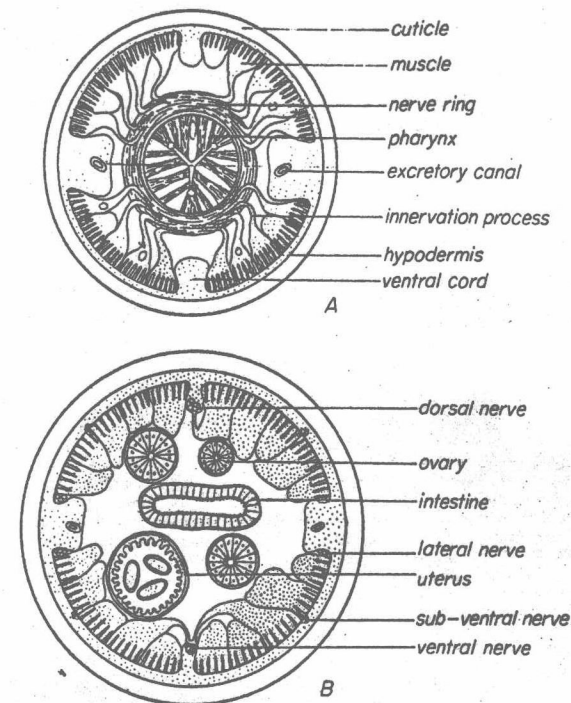


Figure 1.2 Transverse sections through the pharyngeal region (A) and the middle region (B) of a nematode.

immature adult and development of the gonads occurs. The adult nematode may continue to grow in size without further moults and in some examples (*Ascaris*, *Dracunculus*) they become very large.

## 1.2 The Organisation of the Nematode Body

### 1.2.1 Cuticle

Nematodes owe much of their success as a large and ubiquitous group to an organisation that includes a cuticle of varying complexity and considerable evolutionary plasticity. The cuticle forms the outer covering of the body but it also lines the pharynx, excretory pore, rectum, cloaca, spicule pouches, vulva, amphids and phasmids (sense organs). The stylet, teeth, hooks, spicules and certain additional copulatory structures such as the gubernaculum, are formed from toughened and hardened cuticle. The cuticle which covers the body surface is basically a three-layered structure and consists of cortical, median and basal layers (figure 2.1). This three-layered structure can be reduced to two in some



species whereas in others the three layers may be sub-divided to give more layers (see figure 2.2). The cuticle that lines the various orifices of the nematode is usually simpler in structure than the cuticle of the body wall. All cuticular structures, including the stylet, the linings of the pharynx, the rectum, the amphids and the phasmids, are shed at each moult<sup>4,5,6</sup> (see section 2.2).

### 1.2.2 Hypodermis

The hypodermis lies beneath the cuticle and may be cellular or a syncytium. It projects into the body cavity along the mid-dorsal, mid-ventral and the lateral lines to form four ridges or cords (figures 1.2 and 1.3). The lateral hypodermal

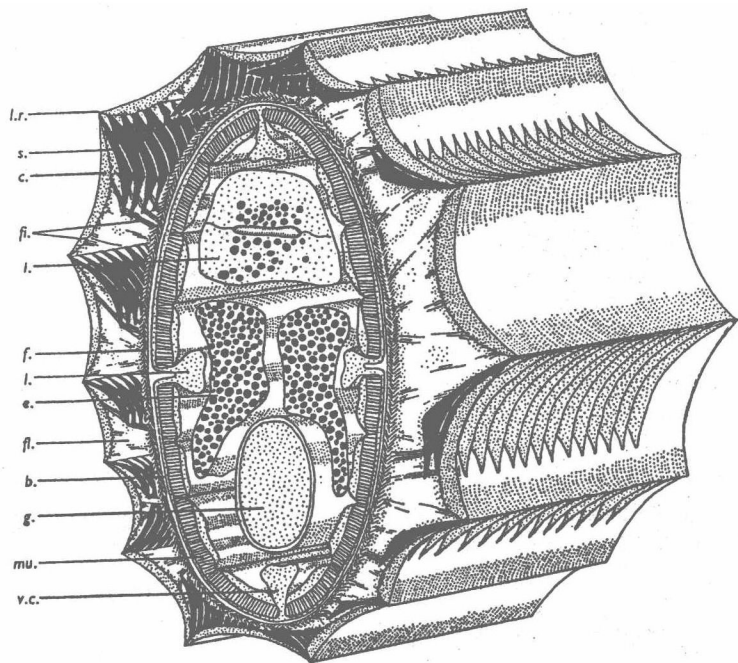


Figure 1.3 Diagram of a section taken from the middle region of *Nippostrongylus brasiliensis* (adult) to show the internal structures of the nematode and also the unusual cuticle [Lee, *Parasitology*, 55 (1965)]. *b* basal layer of cuticle; *c* cortical layer of cuticle; *e* subventral (excretory) gland; *f* fibres in basal layer of cuticle; *fl* fibrils; *fl* fluid-filled layer of cuticle; *g* gonad; *l* intestine; *l* lateral cord; *r* longitudinal ridge of cuticle; *mu* muscle; *s* strut which supports the ridge of cuticle; *v.c* ventral cord.

cords are the largest and contain the excretory canals when these are present (figure 1.2). The nuclei of the hypodermis are found only in the cords. The hypodermis contains large amounts of reserve materials (lipid, glycogen) and haemoglobin is present in some animal-parasitic nematodes. Fibres traverse the hypo-

dermis and appear to be concerned with the attachment of the muscles to the cuticle. Half-desmosomes form points of attachment between the hypodermis and the basal layer of the cuticle; fibres extend across the hypodermis from these half-desmosomes to desmosomes on the inner membrane of the hypodermis where they form points of attachment to the muscle cells (figure 1.4).

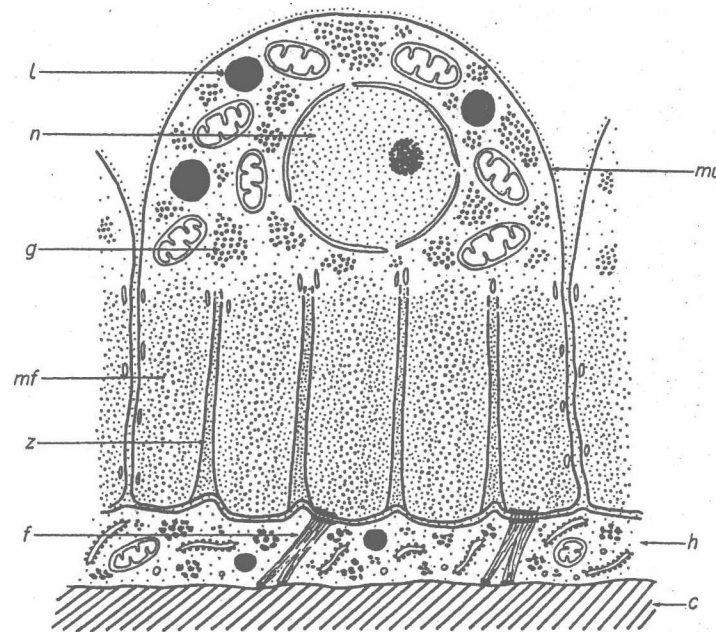


Figure 1.4 Transverse section through a muscle of the body wall to show the internal arrangement of the cell and its relationship with the hypodermis and cuticle. *c* cuticle; *f* fibres connecting muscle to cuticle; *g* glycogen; *h* hypodermis; *l* lipid; *mf* myofibrils; *mu* muscle; *n* nucleus; *z* z-band region of muscle.

### 1.2.3 Muscles

The muscles of the body wall of nematodes are unusual. They are all spindle-shaped, longitudinal muscles and the cells are divided into a contractile and a non-contractile portion (see figures 1.2 and 1.4). There are no circular muscles in the body wall. The muscles are obliquely striated (see figures 7.1 and 7.3) and the filaments of actin and myosin lie in the basal, contractile portion of the muscle cell (see section 7.1).<sup>7</sup> In some types of muscle the contractile elements also extend up the sides of the muscle cell (see figure 7.1). The remainder of the cell contains the nucleus, mitochondria, glycogen and fat. The muscles are not innervated by nerves extending to end plates on the surface of the muscle cell, as in most animals. Instead, innervation processes extend from the muscle to the neural tissue and make synaptic contact at the surface of the longitudinal nerves



or the nerve ring (figures 1.2 and 7.2). In some nematodes the muscles in each section are interconnected with each other by cytoplasmic bridges and this may be important in muscle co-ordination.<sup>4</sup> The muscles of the body wall are attached to the hypodermis by desmosomes and exert their pull on the cuticle through fibres in the hypodermis (section 1.2.2). Not all muscle cells are in the body wall of the nematodes; there are specialised muscles associated with the alimentary system and the reproductive system.

### 1.2.4 Nervous System

The nervous system consists of a ganglionated circum-pharyngeal ring with a large ventral, a smaller dorsal and two or more lateral nerves running from this nerve ring along the four hypodermal cords (see figure 7.4). Series of commissures interconnect these longitudinal nerves along the length of the nematode. Several nerves extend from the nerve ring to the anterior end of the nematode where they innervate a number of sense organs around the mouth (see figure 7.4). A few sense organs occur elsewhere on the body, particularly around the tail where they are most numerous in the cloacal region of males (figure 1.5). The pharynx

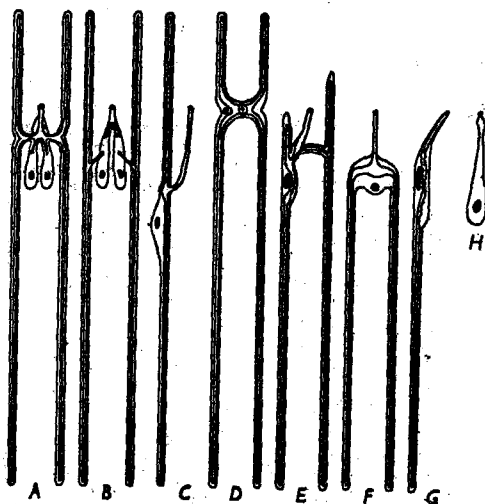


Figure 1.5 Representative types of excretory system found in nematodes. (A) Rhabditoid type, an 'H'-shaped system with two sub-ventral gland cells, the lateral canals are embedded in the lateral cords; found in *Rhabditis*, *Rhabdias* and the Strongylina in general. (B) Variant of (A), found in *Oesophagostomum*. (C) Tylenchoid type, an asymmetric system with the lateral canals and gland cell confined to one cord. (D) Oxyuroid type, an 'H'-shaped system without sub-ventral gland cells and with a shortened terminal duct. (E) Ascaroid type, a shortened 'H'-type which is almost an inverted 'U' in form. (F) Cephaloboid type. (G) Anisakid type, a reduced form related to E but with one lateral canal absent. (H) Single ventral cell type, present in *Chromadorina*, *Monhysterina* and *Enoploidea*. (After Chitwood and Chitwood.<sup>10</sup>)

is also innervated throughout its length by a pharyngeal-sympathetic nervous system.

### 1.2.5 Pseudocoelom

The body cavity of nematodes is a pseudocoelom and is filled with fluid. The pseudocoelom is of fundamental importance as its fluid bathes the internal organs and also forms the hydrostatic skeleton of the nematode. Nutrients from the intestine, oxygen from the environment, and waste products have to cross the pseudocoelom to reach the various tissues and organs. It is not surprising, therefore, to find that the pseudocoelomic fluid is a very complex solution; that of *Ascaris*, for example, contains a variety of proteins, fats, carbohydrates, enzymes, nitrogenous compounds, inorganic ions and haemoglobin.<sup>8</sup>

The pseudocoelomic fluid and body contents are always under pressure, owing to the tonicity of the body-wall musculature, and this is of great significance in the locomotion, feeding, excretion and, indeed, in the structure of nematodes.<sup>9,2</sup>

### 1.2.6 Pseudocoelomocytes

The pseudocoelomocytes (also called coelomocytes, stellate cells, athrocytes, phagocytic or giant cells) are ovoid or many-branched cells lying in the pseudocoelom (figure 1.1) and are usually two, four or six in number. Various functions have been attributed to these structures. They may be phagocytic; in *Ascaris* the pseudocoelomocytes engulf bacteria injected into the body cavity, although ink particles are not phagocytosed. Another function ascribed to these structures is the storage of insoluble waste products, but there is little evidence in support of this. The uptake and concentration of dyes, such as methylene blue and neutral red, by these cells does not necessarily indicate an excretory function because many types of animal cell take up these stains. They may be absorptive in function and remove unwanted metabolites from the body fluid in some way, or they may manufacture essential components for the body fluid. It has also been suggested that they are an oxidative centre for the nematode, but this is unlikely. Early workers believed that the pseudocoelomocytes were in continuity with the excretory system but this has been shown to be incorrect. Their physiological role in nematodes remains uncertain.<sup>10</sup>

### 1.2.7 Alimentary System

There is a mouth, surrounded by lips, a buccal cavity of varying shape, a muscular and glandular pharynx, which usually has a triradiate lumen, an intestine, rectum and anus (figures 1.1, 1.2). The pharynx is a powerful pumping organ in most species and usually contains three glands that open into the lumen at varying positions along the length of the pharynx. The intestine is lined by microvilli and



appears to be both secretory and absorptive in function. A valve or sphincter separates the intestine from the rectum or cloaca in most species. The alimentary system is described in greater detail in section 3.1.

### 1.2.8 Excretory System

The excretory system of nematodes was originally assigned this function on purely morphological grounds. It varies in structure (figure 1.6) and in some

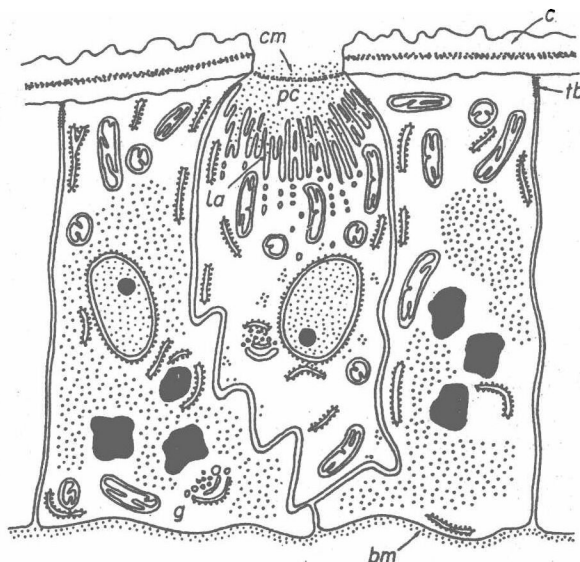


Figure 1.6 Diagram of a hypodermal gland cell and associated non-glandular cells of the lateral and ventral hypodermal cords of *Capillaria hepatica*. The hypodermal gland cell is situated below a pore in the cuticle and is surrounded by non-glandular cells. [After Wright, *J. Morph.*, 112 (1963).] *bm* basement membrane; *bl* boundary layer; *c* cuticle; *cm* cap material; *g* Golgi apparatus; *la* lamellar apparatus; *pc* pore chamber; *tb* terminal bar.

groups it appears to be completely absent. There are two basic types of so-called excretory system, glandular and tubular. The glandular system is found in many free-living nematodes. It consists of a ventral gland cell situated in the pseudo-coelom near the base of the pharynx and usually has a terminal ampulla which opens to the exterior on the ventral surface by means of a pore (figures 1.1 and 1.6H). The tubular system varies in structure in different groups of nematodes. It is a simple H-system in the oxyurids with a canal in each lateral cord. These lateral canals unite by a short duct, which opens to the exterior through a common excretory duct and pore on the ventral surface near the base of the pharynx (figure 1.6D). Some nematodes (*Nippostrongylus*) also contain a pair of sub-ventral glands (figure 1.3) that open into the canal system just behind the

excretory pore (figure 1.5A and B).<sup>11</sup> Other types of nematode possess a modified form of the canal system (figure 1.6C, E, F and G). In *Ascaris* and some other nematodes the canal system is in the form of an inverted 'U', the anterior limbs of the H-type system being absent (figure 1.6E and F).<sup>10</sup>

The lateral canals are intracellular and the whole canal system has only one nucleus. The duct of the canal lies within the elongated excretory cell and numerous vacuoles or canaliculi merge with it (figure 1.5). The glands associated with the canal system are often large (figure 1.3) and contain a number of enzymes in some animal-parasitic nematodes. They appear to have a secretory rather than an excretory function in these species (section 5.3).<sup>11,12,13</sup> There is a complete absence of flame cells in these systems but in certain nematodes pulsations of various parts of the system have been observed.

### 1.2.9 Reproductive System

The sexes are usually separate, the males frequently being smaller than the females. Males have one or two testes opening into a seminal vesicle and then into a vas deferens. This latter structure may be divided into glandular and ejaculatory parts. The vas deferens opens into a cloaca. Many male nematodes possess copulatory spicules (figures 1.1, 1.5, 6.1 and 6.2) that lie in cuticular-lined pouches connected to the cloaca. The spicules consist of hardened cuticle with a cytoplasmic core containing a nerve that runs to the tip of the spicule.<sup>14</sup> In some groups of nematode the area around the cloaca is expanded to form a copulatory bursa (see figure 6.2).

The spermatozoa of nematodes are amoeboid, but different types of spermatozoa characterise different groups of nematode. Some are round or ovoid whereas others have the superficial appearance of flagellated sperm but true flagella are not present (see figure 6.3 and section 6.1.1). Females have one or two ovaries, which open into an oviduct(s) and a uterus or uteri (see figure 6.4). The spermatozoa are usually stored in a seminal receptacle at the ovarian end of the uterus and are often closely associated with the wall of this region. Muscle fibres cover the uteri of some species and bring about movement of the contents. The uteri often end in an ovijector, which is very muscular and this, together with body movements and the high internal pressure of the body contents, serves to expel the eggs through the vagina. The eggs of nematodes vary in shape and size (see figure 6.5) but are essentially ovoid and have three main layers, the middle of which contains a chitin-protein complex. This is the only structure of nematodes that contains chitin. Some nematodes release sex-attractants and in *Heterodera* they are known to be species-specific.<sup>15,16,17</sup>

The sex ratio is density-dependent in a few nematodes, and is possibly controlled by nutritional factors. Thus, in *Mermis* the eggs develop as females or males depending on the number that are eaten by the grasshopper, a preponderance of males occurring in heavily infected grasshoppers. Similarly, sex determina-



tion in *Meloidogyne incognita* apparently depends on the abundance of food, for the proportion of the sexes varies with the intensity of infestation of the host, with more males appearing in large populations. Female juveniles of *Meloidogyne* can undergo sex reversal; if the nutritive conditions are altered they develop into adult males with two testes (normal males have one testis).<sup>15,18,19</sup>

#### 1.2.10 Hypodermal, Caudal and Rectal Glands

Hypodermal glands are present in the lateral cords of many marine nematodes. There are usually two sub-lateral rows of these unicellular glands and they are associated with pores on the surface of the nematode. Some species also possess a ventral row of these glands. Each pore on the surface of female *Chromadorina germanica* leads through a duct to one of these merocrine gland cells. A nerve cell is associated with each gland cell and a dendrite with a sensory cilium projects into the duct of the gland.<sup>20</sup> Gland-like cells occur in rows (called bacillary bands) along the length of the animal-parasitic Trichuroidea (*Trichuris*, *Capillaria*). These gland cells (figure 1.6) also open to the exterior through cuticular pores and some have sensory neurons associated with them.<sup>4,21</sup> The function of these hypodermal gland cells is not known.

Caudal gland cells (usually three) are present in many marine- and fresh-water nematodes. They produce a secretion that hardens in water and fixes the tail of the nematode to the sub-stratum. There is a high rate of production of this secretion as individuals can fix themselves to the sub-stratum many times in a short period of time. Ejaculation of the secretion is controlled by a spinneret apparatus. This consists of a retractor muscle attached to a small cone which blocks the aperture of the ducts at the tip of the tail. Retraction of the muscle withdraws the cone and ejects the secretion through the ducts and out of the aperture.<sup>22</sup> The head of the nematode can move in all directions while the tail remains fixed to the sub-stratum by means of this secretion and is of obvious value to a small organism that lives in rapidly moving currents of water.

Rectal glands lie in the pseudocoelom near the intestinal-rectal junction and open by ducts into the rectum or cloaca. There can be three to six of these glands depending on the species and sex of the nematode. The function of these glands is unknown in most nematodes. They discharge their secretion to the exterior through the rectum and anus and it could be that they are a source of sex attractant in some species. In adult female *Meloidogyne* they produce a gelatinous material, which surrounds and protects the extruded eggs at the posterior end of the swollen female.<sup>4</sup>

## 2 Cuticle, Moulting and Growth

### 2.1 The Cuticle

#### 2.1.1 Structure

The cuticle of nematodes plays an important role in their physiology. It can be a simple or a complex structure, which varies from one genus to another and may differ in juveniles and adults of the same species. It is basically a three-layered structure and usually consists of cortical, median and basal layers (figure 2.1). This three-layered pattern occurs in the juvenile stages of most nematodes but in the adult cuticle modifications often occur (figures 1.3 and 2.2).<sup>4,5,6,23</sup>

In the typical three-layered cuticle (figure 2.1) the cortical layer is covered by a membrane-like structure (we shall call it the epicuticle) which varies in thickness from 25 to 40  $\mu\text{m}$ ; it appears to consist of an outer membrane, which is similar in appearance to a triple-layered unit membrane, a middle osmiphobic layer and an inner osmiphilic layer. There is controversy about the origin of this layer. The main cortical layer is single in most juveniles and in many free-living adults, but inner and outer cortical layers occur in many nematodes.

The median layer of the cuticle (also called the matrix or homogenous layer) is a relatively structureless layer, which is composed of numerous fine fibres in most nematodes. In the marine nematode *Acanthonchus duplicatus* the median

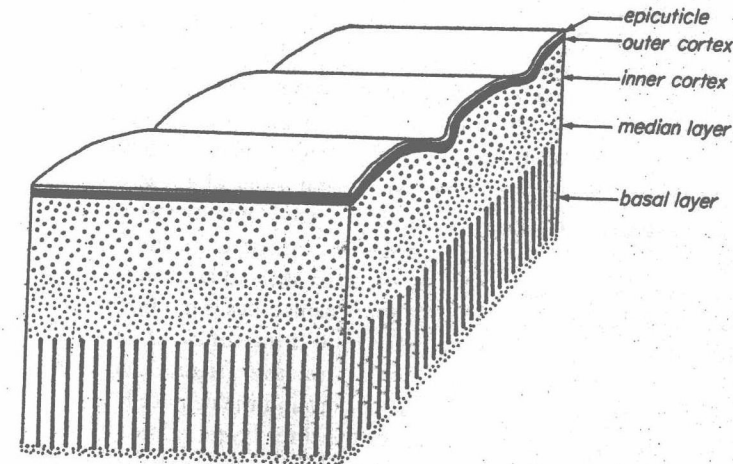


Figure 2.1 Diagram of a three-layered cuticle. This type of cuticle covers the body surface of many juvenile and adult nematodes. Modifications of the cuticle are thought to have arisen from this type.



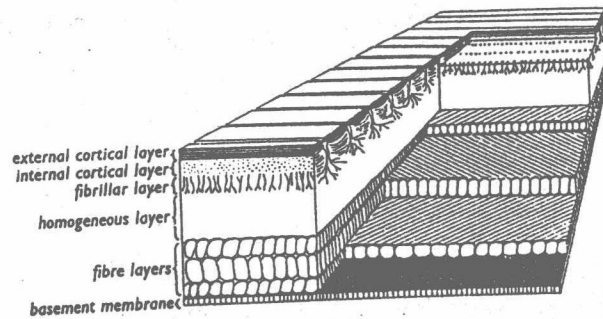


Figure 2.2 Diagram showing transverse, longitudinal and tangential sections of the cuticle of adult *Ascaris*. Note the sub-division of the basal layer into three fibre layers. [After Bird and Deutsch, *Parasitology*, 47 (1957).]

layer is traversed by dense material, which appears to be skeletal in function; pores extend from it into the hypodermis.<sup>23</sup> In adult *Nippostrongylus* this layer is filled with a red fluid containing haemoglobin. Skeletal struts or ribs lie in this fluid-filled layer and support the longitudinal ridges of the cuticle. Fibres, which exhibit the typical periodic cross-banding of collagen, traverse this fluid-filled middle layer and connect the basal layer to the cortical layer. They also suspend the skeletal struts in this layer (see figure 1.3).<sup>5,24,25</sup> The adult cuticle of several species of nematode can increase in thickness and in *Ascaris* most growth occurs in the median layer (see figure 2.9 and section 2.3).<sup>4,5,6</sup>

The basal layer varies in thickness from a very thin layer containing randomly orientated fibrils in some free-living nematodes (*Rhabditis pellio* and *Acanthonchus duplicatus*) to a thick layer containing larger fibres in a number of the larger, animal-parasitic nematodes (figures 2.1 and 2.2). The basal layer of many juveniles and of the adults of several free-living species, has regularly arranged striations with a spacing of about 20 nm. These regular spacings indicate that this layer consists of a tough, almost crystalline protein, which has very close linkages between the molecules.<sup>4,6,26</sup> It is probable that this is one of the three natural forms of fibrous collagen. The other two natural forms of collagen are an unstriated form and a striated form, which has a spacing of about 64 nm. The unstriated layers of nematode cuticle are probably the unstriated form of collagen.<sup>27</sup> This striated basal layer is possibly the main skeletal layer in the cuticle of these nematodes. The basal layer of some nematodes is modified to form two or three fibre layers. A good example of this is the cuticle of adult *Ascaris*, which has three fibre layers present in the basal layer (figure 2.2). Each fibre layer consists of large fibres that run in spirals around the nematode at about 75° to the longitudinal axis. The middle of the three layers crosses the other two at an angle of 135° to form a lattice that alters its angles as the nematode moves (see section 8.1).<sup>2</sup> Most of the larger nematodes that possess fibre layers have only two (*Oxyuris*, *Nippostrongylus*). These fibres are an important component of the cuticle in larger nematodes.<sup>4,5,9</sup>

The cuticles of the second stage juveniles and males of *Heterodera* species are similar to the basic plan described for juvenile nematodes (figure 2.1). The cuticle of gravid females differs from the basic pattern in ways that suit their swollen form, their sedentary habit and their eventual roles as protective cysts. The three layers of males and juveniles are supplemented by an extra fibrous layer in *H. schachtii* and *H. rostochiensis* and also by a fifth layer in *H. rostochiensis*. The fibres in this fifth layer are arranged helicoidally, as in the chitin of insect endocuticle, but they consist of collagen in the nematode. Chitin fibres in insect endocuticle lie in a matrix of cross-linked protein of low modulus and strength. This system has a large strength-to-weight ratio, good tensile, flexural and compressive strength and is much stronger than either of the two compounds alone. If fibres of material of high tensile strength and elasticity are orientated in a matrix of weak tensile strength and elasticity, the strength of the two-phase system is further increased. A helicoidal arrangement of the fibres gives an isotropic structure, but once it is formed it can no longer expand. The fibres in the fifth layer of *H. rostochiensis* fit this pattern and it has been suggested that such an isotropic layer would favour a spherical structure, in response to increased internal pressure as the female swells.<sup>27</sup> A somewhat similar structure is present in the innermost layer of the cuticle of female *Mermis nigrescens*<sup>6</sup> and may be formed in a similar manner to the endocuticle of insects and the innermost layer of female *Heterodera rostochiensis*. The cortical layer of adult female *Mermis nigrescens* cuticle is traversed by canals that appear to open by pores on to the surface of the nematode.<sup>6</sup>

Females of *Bradynema*, which live in the haemocoel of the mushroom fly, have no cuticle but the hypodermis has a microvillous surface and this forms the outer covering of the nematode.<sup>28</sup>

The cuticle may have characteristic longitudinal and transverse ridges and there may also be annulations, spines, punctations, or inflated areas. Most of these modifications involve the cortical layer only but, in some species, the median layer may also be involved. Lateral extensions of the cuticle, called lateral alae, are present in many nematodes. These may be stiffened, fin-like structures or blunt ridges of cuticle and may play an important role in locomotion. The fin-like alae are mainly found in animal-parasitic nematodes that are free-swimming in gut contents or other fluids and are topographically dorsal and ventral in position when the worm is swimming. They may help to stabilise the worm and may also allow greater thrust against the fluid environment during swimming, as occurs with the dorsal and ventral fins of eels. The blunt, lateral alae are found in most nematodes that live in soil or mud, including the free-living juveniles of animal-parasitic species. As most nematodes move on their sides, that is, with their lateral surfaces topographically dorsal and ventral, the blunt, lateral alae of a side will be against the sub-stratum. This will give a broad area of contact between the nematode and the sub-stratum, thus allowing greater purchase against the sub-stratum during movement.<sup>24</sup> Superficial annulations of the cuticle of nematodes (figure 2.1) allow easier bending of the nema-



tode during locomotion. Adult *Nippostrongylus* possess longitudinal ridges in the cuticle (see figure 1.3) and they appear to assist coiling of this nematode. The ridges also abrade the intestinal mucosa of the host and may thus assist in the feeding process.<sup>24</sup> No doubt the various modifications of the cuticle of nematodes can all be related to a specific function or functions.

### 2.1.2 Composition

The basic component of the cuticle of nematodes is a form of collagen associated with hyaluronic acid, chondroitin sulphate, acid mucopolysaccharide, and small amounts of lipid. In vertebrate tropocollagens the basic, triple-helical pattern is developed through interaction of three separate polypeptide chains. The triple helix of collagen from *Ascaris* cuticle is different, as it is formed when sub-unit polypeptide chains, of molar mass 60 000, fold back upon themselves to form a collagen-type triple helix. These sub-units are held together by a disulphide bond.<sup>29</sup> Cuticular collagen of *Ascaris* contains amino acids that differ in their concentrations from those contained in vertebrate collagen, elastin, fibroin, resilin and keratin (table 2.1), but is digested by bacterial collagenase. Another structural protein called cuticlin is present in the cuticle of *Ascaris*. It contains large amounts of proline and alanine and relatively low amounts of glycine and basic amino acids; it does not give the characteristic X-ray diffraction pattern of collagen nor is it susceptible to collagenase. This cuticlin may be located in the cortical layers of the cuticle.<sup>30</sup> There is also some evidence that the outer cortical layer is a collagenous layer that has been modified by the presence of disulphide and sulphhydryl groups to form a keratin.<sup>4,5</sup> Polyphenol quinone

Table 2.1 Comparison of cuticlin from the cuticle of *Ascaris* with other structural proteins (Fujimoto and Kanaya<sup>30</sup>)

Amino acid	Cuticlin ( <i>Ascaris</i> )	Collagen ( <i>Ascaris</i> cuticle)	Elastin (bovine)	Fibroin ( <i>Bombyx</i> )	Resilin (locust)	Keratin (wool)
Residues/1000 total amino acids						
Glycine	150	274	330	445	376	85
Small <sup>a</sup>	317	346	441	739	487	146
Imino acids <sup>b</sup>	301	312	221	3	79	85
Basic <sup>c</sup>	36	82	88	9	45	104
Acidic <sup>d</sup>	146	136	117	23	152	173
Half-cystine	24	27	0	0	0	106
Hydroxyproline	0	16	98	0	0	0

<sup>a</sup> glycine + alanine

<sup>b</sup> proline + hydroxyproline

<sup>c</sup> lysine + hydroxylysine + histidine + arginine

<sup>d</sup> aspartic acid + glutamic acid

tanning occurs in the cuticle of swollen female *Heterodera rostochiensis*. The cuticle of the swollen female becomes tanned by the action of polyphenol oxidase to produce a tough, resistant cyst that encloses the eggs after the death of the female.<sup>31</sup> Esterase enzymes and haemoglobin have been detected in the cuticle of adult *Nippostrongylus*; esterase is present in the median layer of *Ascaris* cuticle; and polyphenol oxidase is present in the cuticle of *Heterodera*. <sup>14</sup>C-proline is incorporated into the cuticle of adult *Ascaris* and of juvenile *Nippostrongylus* and this, together with the presence of enzymes demonstrates that the cuticle is metabolically active and not an inert covering.<sup>4,5,32,33</sup>

## 2.2 Moulting and Ecdysis

### 2.2.1 Introduction

Most nematodes moult four times during their development and certain stages in the process of moulting are apparently controlled by neuroendocrine secretions as in arthropods.

Moulting occurs in four main steps:

- (1) The stimulus is received and brings about the discharge of neurosecretory material that initiates step 2.
- (2) The old cuticle separates from the hypodermis.
- (3) The new cuticle forms between the hypodermis and the old cuticle, with or without absorption of parts of the old cuticle.
- (4) Rupture and ecdysis of the old cuticle, or its remains, occur allowing escape of the juvenile or young adult worm.

During moulting the cuticle that covers the body of the nematode, together with the cuticular linings of the amphids, phasmids, buccal cavity, pharynx, excretory pore, and the rectum are shed. During moulting in plant-parasitic tylenchids the basal part of the stylet dissolves and the head is disengaged from the anterior part of the old stylet, which is shed with the old cuticle.

### 2.2.2 The Stimulus

In most nematodes the identity of the stimulus that initiates moulting is not known. In free-living forms it is possible that growth to a certain size triggers moulting, or it could be controlled by a biological clock, such as the gradual loading, followed by sudden discharge, of neurosecretory cells. In some plant- and animal-parasitic nematodes it can be initiated, at least for one moult, when the juvenile moves from a free-living to a parasitic existence. For example, host root exudates stimulate the fourth-stage juvenile of *Paratylenchus nanus* to moult. Movement from one environment to another within the host may also initiate moulting (for example, migration of *Nippostrongylus* from the lungs to the intestine). The infective-stage juvenile of several animal-parasitic species and the third-stage juvenile (dauer juvenile) of some free-living nematodes (*Rhabditis*



*dubia*, which lives in cow dung) retain the cuticle of the second-stage juvenile as a sheath; ingestion by a suitable host, in the case of the animal-parasitic species, or transfer by flies to freshly deposited dung, in the case of *R. dubia*, provides the stimulus for exsheathment and the continuation of development. This, however, is a stimulus that brings about ecdysis and does not initiate moulting (see section 2.2.5).<sup>4,6,34,35</sup>

### 2.2.3 Formation of the New Cuticle and Ecdysis

The changes that occur during moulting seem to vary from species to species and, as moulting has been studied in relatively few species, it is difficult to generalise. Some nematodes shed the old cuticle almost intact whereas others break down part of the old cuticle and shed only the outermost layer or layers.

During moulting the old cuticle separates from the outer hypodermal membrane and thus, indirectly, loosens its attachment to the muscles. This explains why some nematodes, such as *Aporcellaimellus*, become immobile prior to and during moulting.<sup>36</sup> Leucine aminopeptidase increases in concentration in the hypodermis of *Xiphinema index* between moults and reaches a peak just before moulting commences. The concentration of the enzyme falls once moulting begins and, simultaneously, the cuticle becomes loosened from the hypodermis.<sup>37</sup> This suggests that the enzyme is released into the area between the cuticle and the hypodermis and attacks the cuticle, thus bringing about its detachment from the hypodermis. This enzyme may also be present in the exsheathing fluid of *Haemonchus contortus* (see section 2.2.5).<sup>35</sup> In some nematodes (*Meloidogyne javanica*) the hypodermis becomes more granular and the amount of RNA increases at an early stage in the formation of the new cuticle; in others (the final moult of *Nippostrongylus*) this does not occur until later in the formation of the new cuticle. This is associated with the formation of material for the new cuticle.

Globular moulting bodies appear in the hypodermis of *Hemicycliophora arenaria* at the beginning of the moult and are thought to be associated with the formation of cuticular protein. The new cuticle is formed in a series of layers, which gradually increase in thickness and complexity. The edge of the new cuticle (the epicuticle) forms at the outer surface of the hypodermis as a thin, membrane-like layer. This epicuticle may be a condensation of new cuticular material or it may be the hypodermal membrane, which has become transformed and underlain by a new hypodermal membrane. The cuticle is laid down between this membrane-like epicuticle and the hypodermal membrane.<sup>38</sup>

In *Meloidogyne javanica* the newly formed outer cortex increases in thickness together with the new inner cortical layer and basal layer as shown in figure 2.3. The new cuticle is laid down in folds, allowing rapid elongation of the nematode after ecdysis. In this nematode the gap between the old cuticle and the new becomes filled with granular material and the inner layers of the old cuticle begin to break down (figure 2.3). The old cuticle is thought to be absorbed

through regions in the newly formed cuticle where the internal cortex has not formed, until only the outer cortex is left and is eventually ecdysed.<sup>4</sup>

During the first moult of *Heterodera schachtii*, which occurs in the egg, strong contractions bring about detachment of the thin cuticle at both ends of the juvenile. This shed cuticle is then completely hydrolysed.<sup>39</sup> The first moult in *Ascaris* occurs in the egg and here also there is almost complete hydrolysis of the old cuticle.<sup>4</sup>

Cuticle formation of the body wall and of the stomodaeum are synchronised in *Aporcellaimellus*, the new stylet being formed at the same time as the new cuticle of the body wall. In this nematode, projections of the hypodermis

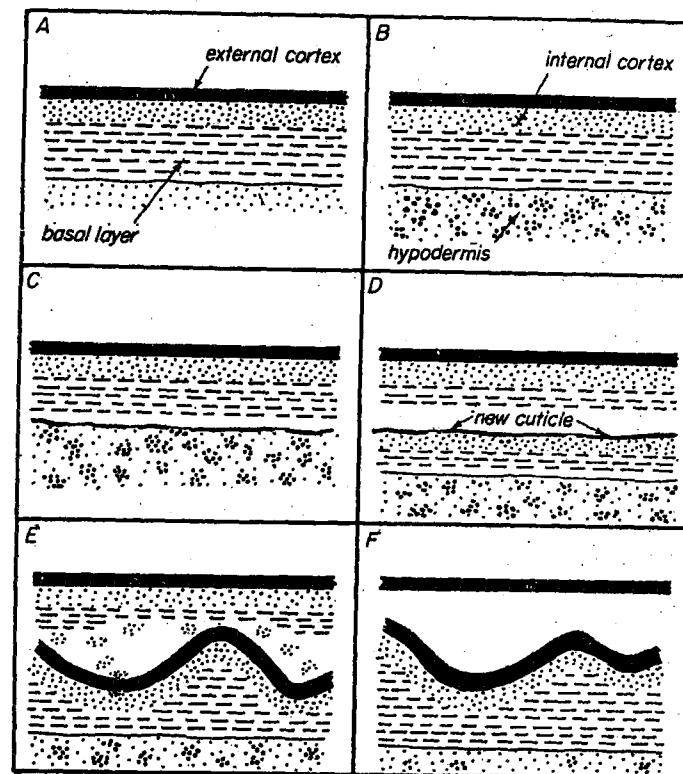


Figure 2.3 Diagram of the changes that take place in the cuticle of *Meloidogyne javanica* during moulting. (A) normal cuticle; (B) thickening of the hypodermis, together with an increase in the number of ribosomes, occurs; (C) the old cuticle has separated from the hypodermis and the new external cortical layer has begun to form on top of the hypodermis; (D) dissolution of the inner layers of the old cuticle has begun while the new cuticle begins to form the three basic layers and gradually increases in thickness; (E) inner layers of the old cuticle are increasingly dissolved (and resorbed?) while the new cuticle increases in thickness and becomes convoluted; (F) only the external cortical layer of the old cuticle remains and the new cuticle is almost fully formed. The old cuticle is then shed and the nematode extends itself, thus flattening the convolutions in the new cuticle. (After Bird.<sup>4</sup>)



extend into the newly forming cuticle but retract from it later in the moult to leave a lacunar system in the basal fibre layers. Differentiation of the cuticle into distinct zones occurs once secretion of the new cuticle is completed. There is no dissolution of cuticle.<sup>36</sup>

The cuticle of *Hemicycliophora arenaria* is unusual as it consists of an outer cuticular sheath and a more normal cuticle. Both are formed when the nematode moults and should be considered as two parts of the same cuticle. Moulting commences with separation of the old cuticle from the hypodermis. The new sheath is then formed at the surface of the hypodermis. This is followed by formation of the new inner cuticle. The old sheath and inner cuticle then break down and may be absorbed by the nematode. Breakdown and reformation of the muscles was observed at each moult in this nematode.<sup>38</sup>

Ecdysis occurs before the formation of the new cuticle has been completed in *Turbatrix aceti*. Rapid secretion and folding of the new cuticle, followed by quick elongation of the nematode occurs after ecdysis. The old cuticle appears to be shed almost intact but the striated layer becomes amorphous in appearance.<sup>6</sup> During moulting in *Panagrellus silusiae* the cuticle is gradually discarded, except during the final moult to produce the adult male, when the cuticle splits and is shed in one or two pieces. At the final moult to produce the adult female the new cuticle folds extensively before ecdysis is complete, but this is the only occasion on which this occurs in this nematode. Breakdown of the old cuticle does not occur.<sup>40</sup>

The fourth-stage juvenile and the adult of *Nippostrongylus* have a complex cuticle (see figure 1.3). The cortex is folded into longitudinal ridges and struts extend into the apex of these ridges. These struts lie in a fluid-filled middle layer. Fibrils of collagen extend across the middle layer from the basal layer to the cortex and to the struts. Formation of this cuticle is controlled partly by the hypodermis and partly by the cuticle itself (see figure 2.5). At the final moult the old cuticle separates from the hypodermis (figure 2.4A), another membrane appears to form beneath the outer hypodermal membrane, which becomes part of the epicuticle, and cuticular material is laid down between these two membranes (figure 2.4B). The new cuticle is folded into regular annuli with two to three of these annuli for each single annulus of the old cuticle. The cuticle becomes more folded and hypodermal cytoplasm extends into these folds (figure 2.4C and D), but after a time they withdraw from the annulations leaving behind an 'M'-shaped ring of material, which girds the nematode in each annulus (figure 2.4E). The area between the 'M'-shaped structures and the hypodermis rapidly enlarges and develops into the two-fibre layers of the new cuticle (figure 2.4F). Once this has occurred there is rapid deposition of material above the 'M'-shaped structures and growth of this area of the cuticle (figure 2.4G). The 'M'-shaped structures, which seem to play a part in controlling the formation of the two parts of the cuticle, then gradually flatten out and disappear, while the outer part of the cuticle condenses into the cortex and the struts, leaving a fluid-filled cavity above the fibre layers (figure 2.4H). It is at this stage

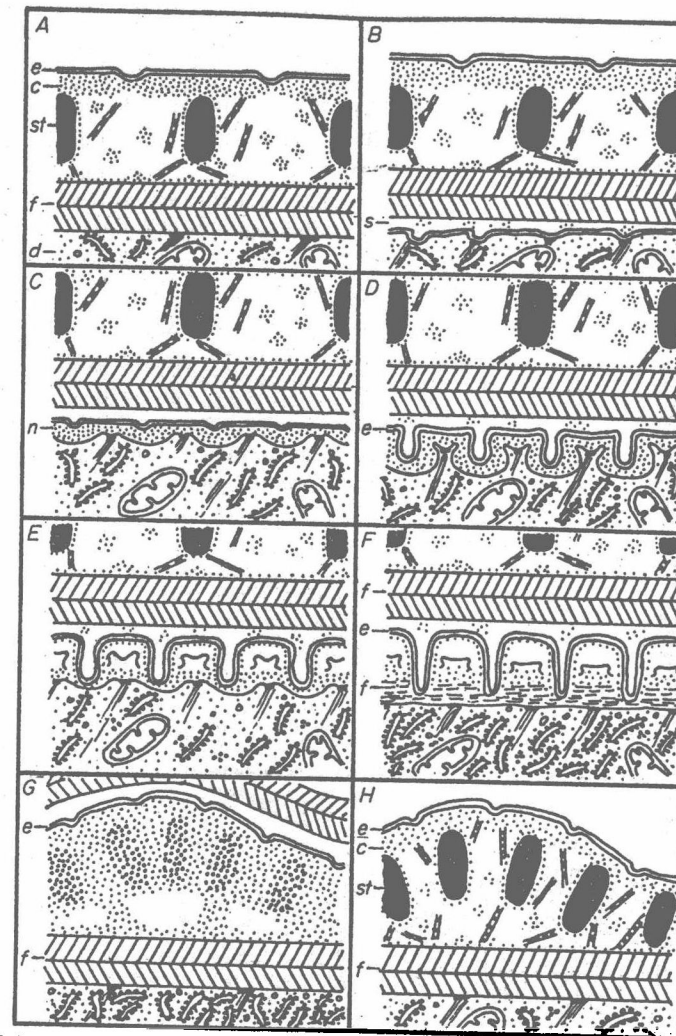


Figure 2.4 Diagram to show the stages in the formation of the adult cuticle during the fourth moult of *Nippostrongylus brasiliensis* (see figure 1.3 for a diagram of the cuticle). (A) and (B) separation of the juvenile cuticle from the hypodermis; (B) and (C) early stages in the formation of the adult cuticle, it is thought to be laid down between a new hypodermal membrane and the old hypodermal membrane which becomes part of the epicuticle; (D) the new cuticle becomes more extensively folded and the hypodermis extends into each fold; (E) the cuticle is much more extensively folded and formation of the basal (fibre) layer has begun, the hypodermis has begun to retract from the folds leaving 'M'-shaped structures in each fold, there is rapid increase in the amount of granular endoplasmic reticulum in the hypodermis at this stage; (F) later stage than (E) to show the fibre layers almost completely formed; (G) after the stage shown in (F) there is a rapid increase in thickness of the outer part of the cuticle, the 'M'-shaped structures stay close to the fibre layers and seem to play an important part in separating the fibre layers from the rest of the cuticle, the struts are shown at an early stage in their formation and the fluid-filled layer has begun to appear; (H) the adult cuticle shortly after ecdysis, the new cuticle rapidly elongates causing the folds of the superficial annulations to flatten, the struts are in the final stages of formation, collagen fibres extend from the outer to the inner layers of the cuticle and to the struts, there is reduction in the amount of granular endoplasmic reticulum in the hypodermis. There is no dissolution of the old cuticle which is shed almost intact. (After Lee.<sup>25</sup>)

c cortex; e epicuticle; f fibre layers; h hypodermis; n new cuticle.



that the old cuticle is shed more or less intact. The ridges are formed when the hypodermis pushes into the space between the hypodermis and the old cuticle, deposits new cuticle and then retreats back to the normal position. The muscles of the body wall are attached to the inner hypodermal membrane at desmosome-type junctions and fibres run from these to anchor at half-desmosome-type thickenings at the hypodermis—cuticle junction. These half-desmosomes separate from the old cuticle when moulting commences but very quickly anchor to the new cuticle—hypodermal membrane (figure 2.4A–D). Thus the muscles can exert leverage on the new cuticle very soon after the hold on the old cuticle has been lost.<sup>25</sup> Control of the formation of the various layers and of the struts in the cuticle of *Nippostrongylus* poses an intriguing biophysical problem which remains to be solved but formation of the collagen may be similar to that in fibroblasts (figure 2.5). Moulting in *Nematospiroides dubius* appears to be very similar to that in *Nippostrongylus*.<sup>41</sup>

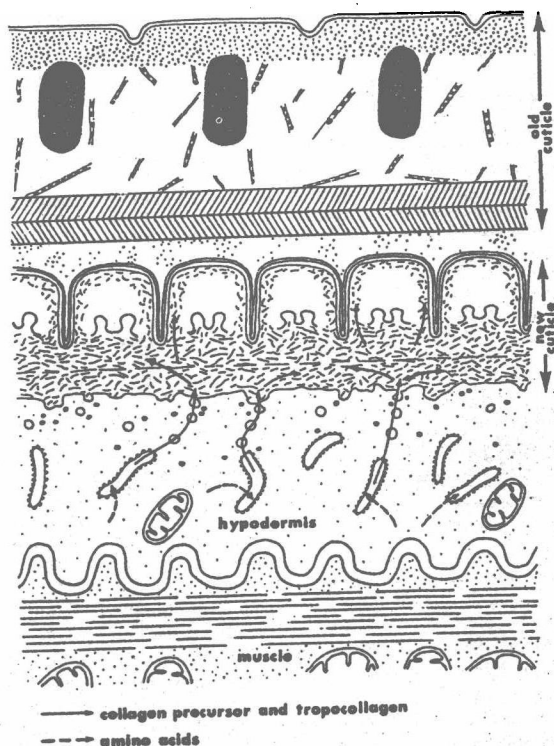


Figure 2.5 Diagram to explain how the new cuticle of *Nippostrongylus brasiliensis* (see figure 2.4) may be formed from amino acids (based on fibroblast production of collagen). Amino acids enter the granular endoplasmic reticulum in the hypodermis and are made into collagen precursors on polyribosomes. The collagen precursor is then carried to the outer hypodermal membrane in small vesicles and released into the new cuticle. In the cuticle the collagen precursor or tropocollagen becomes transported to, and orientated in, the various layers of the cuticle. (After Lee.<sup>25</sup>)

Several other nematodes do not dissolve the old cuticle during moulting; these include *Turbatrix aceti*, *Panagrellus silusiae*, *Aphelenchus avenae*, *Iirschmaniella gracilis*, *Aporcellaimellus* spp.<sup>38,40</sup> On the other hand, several nematodes do dissolve the cuticle during moulting. The inner layers of the moulted cuticle of *Trichinella spiralis* undergo a gradual breakdown during moulting.<sup>42</sup> The basal lamella degenerates first, followed by the striated layer, but the cortical layers are not affected. It is not known if this degeneration is brought about by moulting fluid secreted by the nematode or by digestive enzymes of this host. Nor is it known if the dissolved, cuticular material is absorbed by the nematode. The reasons for these different methods of moulting and ecdysis probably lie in the different environments of the different species. For example, the first-stage juvenile of *Ascaris* moults in the egg, where space and nutrients are at a premium. It is to the advantage of the juvenile to reduce the thickness of the cast cuticle as this will allow it more space to develop within the egg. The egg is a closed system and the developing juvenile relies entirely upon its food reserves until it reaches another host; it is, therefore, to its advantage to recycle the proteins obtained from absorption of the old cuticle. Similarly, *Meloidogyne javanica* moults after it has attached itself to the root of the host plant. Reduction in thickness of the old cuticle will make ecdysis and growth easier for this semi-sessile nematode. On the other hand, the fourth-stage juvenile and adult of *Nippostrongylus* live between the villi of the host's intestine and are liable to be carried out of the host in a stream of gut contents if they are unable to maintain their position in the mucosa. It is, therefore, to the advantage of the fourth-stage juvenile to moult as quickly as possible and dissolution of the old cuticle could be a hindrance in this respect. The nematode also has a plentiful supply of food and so can afford to discard the proteins of the old cuticle. The later juvenile stages of *Trichinella* also live against the mucosa of the intestine, but breakdown of the cast cuticle does occur in this species.<sup>42</sup> Nematodes that live a relatively free existence, either in the soil or in the alimentary tract of other animals, can wriggle free from their old cuticle much more easily than those nematodes that live in confined environments. It is, therefore, not surprising to find that some of these 'free' nematodes cast most of the old cuticle intact, whereas nematodes in confined environments tend to breakdown and may absorb most of the old cuticle. Obviously more examples of moulting and ecdysis in nematodes from different environments are needed before any generalisations can be accepted.

#### 2.2.4 Neurosecretory Control of Moulting and Ecdysis

There is a correlation between neurosecretory activity and the process of moulting in some nematodes, but most of the neurosecretory activity that has been studied is associated with ecdysis. Ecdysis of the fourth-stage cuticle in *Phocanema decipens* is accompanied by synthesis of leucine aminopeptidase in the excretory gland and release of the enzyme through the excretory pore into the gap between