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A CYTOLOGICAL STUDY OF THE HYPODERMIS OF THE PARASITIC NEMATODE, CAPILLARIA HEPATICA

by

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ABSTRACT

Both electron microscopy and light microscope histochemistry have been used to describe the cytology of the hypodermis of the parasitic nematode, *Capillaria hepatica*. The hypodermis of the mid portion of the body of this species forms a complete cellular layer around the worm and may be divided into the following regions: the multicellular lateral and ventral chords; the interchordal hypodermis, apparently formed by flat processes of the cells of the multicellular chords; and the dorsal chord, probably formed by the internal reflection of the hypodermal cell processes which form the interchordal hypodermis. The cytology of the hypodermal gland cells which occur within the multicellular chords is described in detail. The elaboration of the infolded cell membrane of the gland cell forms a region of the cell termed the lamellar apparatus. This region is associated with a pore through the cuticle, but is separated from this pore by an area termed the pore chamber which contains a gel-like material. The outer margin of this material is marked by a boundary layer while diffuse, granular cap material is associated with the outer surface of this layer. No morphological evidence has been found to suggest that the cell is glandular in function, but the structure of the lamellar apparatus would be consistent with the
suggestion that the cell may function in either osmotic or ionic regulation. Experiments in which the osmotic value of the external environment of the worms was altered offer no evidence either for or against such a function. The uptake of neutral red and colloidal gold by the hypodermis is similarly inconclusive, but shows that such materials are concentrated into vacuoles within the non-glandular hypodermal cells. Glycogen, the chief storage product of the body wall, occurs in the basal two-thirds of the non-glandular cells. The possible significance of the concentration of the most of the organelles of the non-glandular cells in the apical cytoplasm is discussed.

The structure of the six-layered cuticle, and of the somatic musculature is described, and the relationship of the hypodermis to these tissues is discussed. No evidence has been found for the existence of pore canals extending from the hypodermis into the cuticle, nor of supportive fibrils extending from the muscle cell into the hypodermis.
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INTRODUCTION

Early anatomical studies on nematodes were performed by such workers as Loos (1879), de Man (1886), Goldschmidt (1903), Martini (1916), and Rauther (1918). These studies established the basic body plan of the nematode and initiated comparative nematode morphology. Chitwood and Chitwood (1950) have reviewed most of this work and evaluated it from the phylogenetic viewpoint. Further general reviews of nematode morphology may be found in Hyman (1951) and in Thorne (1961).

The nematode body wall is an elongate, hollow spindle, enclosing a complete digestive tract, which begins at an anterior, terminal mouth and ends at a posterior, sub-terminal anus. The space between the alimentary tract and the body wall is formed by the embryonic blastocoel and hence may be termed a pseudocoelom. Within this pseudocoelom lies the simple tubular reproductive system. A nervous system is present, composed of a circumoesophageal nerve ring with longitudinal nerve trunks. All nematodes have this basic body plan.

The body wall functions in multiple roles: it serves as the semi-rigid skeletal element which imparts the characteristic shape to the species; it contains the somatic musculature responsible for locomotion; it carries within
it the longitudinal excretory tubules (in those species possessing a complex excretory system), and the longitudinal nerve cords; it possesses the various sensory structures which serve to inform the organism of the external environment; and it serves as a barrier against this external environment, allowing the organism to maintain its distinctive "milieu interieur." Especially in regard to the latter role, it is of interest to understand the intricate details of the structure of the body wall.

The body wall of the nematode characteristically consists of three layers: the hypodermis; the external non-cellular cuticle, presumably formed by the hypodermis; and the somatic musculature, responsible for the locomotion of the organism.

The hypodermis exists as a thin sheet of tissue, one cell layer in thickness, lying against the inner surface of the cuticle. In the most primitive condition, the nuclei of the hypodermal cells occur in enlarged cell bodies. These cell bodies are arranged in four longitudinal rows forming a dorsal, a ventral, and two lateral hypodermal chords. Processes of these cells are closely appressed to the cuticle between the chords. These interchordal areas are covered internally by the somatic musculature. With increasing size, especially in the parasitic species, there is a tendency in the hypodermis towards both increase in
cell size and in cell number, and in some forms towards the formation of syncytia. With these developments nuclei migrate from the hypodermal chord into the interchordal hypodermis. Syncytia occur only in one of the two subclasses of the Nematoda, the subclass Phasmidia. The hypodermal chords contain, in many members of the subclass Aphasmidia, a system of presumably glandular cells which appear to be connected to the exterior by means of a pore through the cuticle. As these occur more commonly in the lateral hypodermal chords they have been termed by Chitwood and Chitwood (1950) "lateral hypodermal glands." The hypodermal chords are considerably modified both anteriorly and posteriorly where they are associated with the sensory papillae, the chemosensory amphids and phasmids, supplementary organs of the male, and foot or cement glands of the tail.

The cuticle is a multi-layered structure consisting, in general, of a cortical layer, a matrix layer and an innermost layer or layers of obliquely arranged fibers. The relative thickness of these layers and their subdivision into more layers varies greatly among species. The proteinaceous cuticle is highly resistant to many enzymes and impermeable to large molecules but is readily penetrated by water and gases (Fairbairn, 1960).

The somatic musculature of the body wall lies against the inner surface of the interchordal hypodermis in the form
of four longitudinal muscle bands separated by the hypodermal chords. The elongate, spindle-shaped cells are oriented longitudinally in these bands. The form of the muscle cell varies depending on the number of muscle cells present. In species containing few muscle cells (meromyarian nematodes), the muscle cell is flattened against the hypodermis, the longitudinal ribbon-like myofibers being oriented perpendicular to the cell membrane adjacent to the hypodermis; this form of the muscle cell was termed platymyanrian by Schneider (1860). In contrast to this form is that found in species containing many muscle cells (polymyanarian nematodes) where the cells have an enlarged cell body on one side of the mid region of the spindle. These cells are usually flask-shaped in transverse sections taken through the cell body; the elongate, contractile region abuts the hypodermis. Within this contractile region, the myofibers are oriented perpendicular to the cell membrane of all three sides of the contractile region; this more complex form of muscle cell was termed coelomyarian by Schneider (1860). Transitional forms occur between these two types, making the classification arbitrary but still useful. The muscle cells are further characterized by the fact that innervation of the cell is by means of a process of the muscle cell which extends to connect with the longitudinal nerves within the hypodermal chords rather than by a nerve process.
Although the histology of the body wall of many nematodes has been thoroughly investigated, our knowledge of cellular detail is limited to a rather gross level of cytology by the small size of many species, and by the limits of resolution of the light microscope. Recent developments in cytology, especially the development of techniques for the examination of biological tissues with the electron microscope, have opened new approaches to understanding the function of cells through the study of their structure.

Short papers dealing with electron microscopy of the cuticle of *Ascaris lumbricoides*, and the muscle cell of *Parascaris equorum*, have been published by Bogoiavlenskii (1958) and by Hinz (1959), respectively. However, no electron microscope study attempting to integrate the structure of the hypodermis, cuticle, and muscle has yet been reported.

The present study was undertaken in order to investigate in greater detail the structure of the hypodermis of a nematode by employing both light and electron microscopy, and to consider if possible the inter-relationships between the three components of the body wall.

The nematode chosen for study, *Capillaria hepatica* (Bancroft, 1893), belongs to the subclass Aphancondria and occurs as a parasite of the liver of a wide range of mammalian hosts. It is a member of the superfamily Trichurioidea, a highly specialized group of nematodes parasitic in
vertebrates. It is one of the larger members of the genus *Capillaria* Zeder, 1800. It is readily prepared for light microscopy as it is found in the parenchyma of the liver, and hence, may easily be fixed *in situ*. The pathology of the infection has been described by Luttermoser (1938) and by Pavlov (1955, reviewed in Skrjabin *et al.* 1957). The life cycle of *C. hepatica* has recently been studied by Wright (1961).

Certain characteristics of the structure of the body wall of *C. hepatica* render the study of greater interest: the hypodermis contains large numbers of lateral hypodermal gland cells which are referred to collectively as bacillary bands; the thin cuticle shows no evidence with the light microscope of fiber layers; and the somatic musculature, coelomyarian in structure, is restricted to very narrow bands due to the extreme expansion of the lateral hypodermal chords containing the bacillary bands. The following sections shall review the extent of our present knowledge of the structure and function of the hypodermal gland cells, the cuticle, and the somatic musculature of nematodes.

**Lateral Hypodermal Glands and Bacillary Bands**

The presence of an enigmatic system of gland cells, the lateral hypodermal glands, was noted above in the hypodermis of the lateral chords of many aphasmidian nematodes.
The occurrence of these cells has been recorded in many species of free-living marine, free-living terrestrial, and parasitic nematodes. The function of these cells is completely unknown. Indeed, the cytology of the cells has not been sufficiently investigated to conclude that they are identical in the three ecological groups. Their sole resemblance is that they occur beneath a pore in the cuticle, through which their supposed product might be extruded.

The lateral hypodermal gland cells of the free-living marine nematodes have been noted by Chitwood and Chitwood (1950), Chitwood (1951), Timm (1952, 1953, 1961), and Weiser (1954, 1959). In these species (chiefly the Chromadoroidea), the pore is conspicuous and the cells easily recognized below the pore. The cells occur in four rows, two rows within each lateral hypodermal chord, and are sparsely distributed from the anterior to the posterior extremity.

The lateral hypodermal gland cells of the terrestrial species (chiefly the Dorylaimoidea) are distributed in the lateral hypodermal chords in a pattern similar to that of the free-living marine species. In most reports, reference is not made to the gland cells, although the distribution of the cuticular pores is frequently noted (Thorne, 1961; Williams, 1961; Lee, 1961). However, Stekhoven and Tuinissen (1938) have described the gland cells of Anaplectus granulosus; Chitwood and Chitwood (1950) have described
those of *Anonchus mirabilis*; and Maggenti (1961) has briefly described those of some species of the genus *Plectus*.

The lateral hypodermal gland cells obtain their greatest complexity in the two genera of parasitic nematodes, *Capillaria* and *Trichuris* (superfamily Trichuroidea). Here, a great many lateral hypodermal gland cells occur in one or more of the hypodermal chords in such concentrations that, when the surface of the cuticle is examined carefully, the cuticular pores are seen in a uniformly spaced pattern, the cells being faintly discernible below as irregular hexagons. The pores occur in a broad continuous band extending down the length of the worm. The regions of the cuticle containing the pores and the underlying hypodermis have been named bacillary bands.

The number and size of bacillary bands varies in different species. Only a single lateral bacillary band is found in the species of the genus *Trichuris*, and this band is restricted to the region of the esophagus. In the genus *Capillaria*, one, two, three, or four bacillary bands may occur. Thus, *Capillaria contorta* contains only one band, while the male of *C. hepatica* contains two, the female of *C. hepatica* contains three, and *C. meleagridis* contains four bacillary bands. Since the gland cells may occur in both the dorsal and ventral hypodermal chords as well as in the lateral chords, it is somewhat inaccurate to refer to the
cells as "lateral hypodermal glands." In subsequent descriptions they shall be referred to solely as the gland cells.

Bacillary bands were recognized by Jägerskiöld (1901) and Rauther (1918) to consist of hypodermal glands, while Müller (1929) considered the bands to consist of a system of pores or channels allowing for the transfer of food through the cuticle and hypodermis to the alimentary tract. This suggested function was discounted by Chitwood and Chitwood (1950) as it was based upon the erroneous assumption that the esophagus of trichuroids was not functional. Jägerskiöld (1901) noted the occurrence of lateral hypodermal glands in many species lacking an excretory system; the implication being that the glands might be excretory in function. This correlation is most striking in the superfamily Trichuroidea where an excretory system is lacking, and where the hypodermal glands are most highly developed. Yet, as noted by Chitwood and Chitwood (1950) a "ventral cell," the characteristic excretory system of the Aphasididae, is present along with lateral hypodermal glands in many genera (e.g. Plectus). Timm (personal communication) has suggested that lateral hypodermal glands of marine species might function in osmotic regulation. However, to date, no experiments have been performed to test these suggestions. Neither has the cytology of the glands been cri-
tically examined. Stekhoven and Tuinissen (1938) found an increase in the number of lateral hypodermal gland cells during the growth of *Anaplectus granulosus*, and Chitwood and Chitwood (1950) noted dark pigment granules in the gland cells of *Anonchus mirabilis*, while Wright (1961) has described eosinophilic striations in the gland cells of the bacillary bands of *Capillaria hepatica*.

The Cuticle

The external cuticle of nematodes has received considerable attention. The early studies on the cuticle of *Ascaris lumbricoides* by von Siebold (1848), and of *Oxyuris equi* by Martini (1916), have been greatly extended by Chitwood (1936), Brown (1950), Monné (1955, 1959), Bird (1956, 1957, 1958), Bird and Deutsch (1957), and Carbonell and Apitz (1960).

The following description of the cuticle is based largely upon that of *Ascaris lumbricoides*, the most thoroughly investigated species. However, the histochemical studies of Monné included the following animal parasites: *Ascaris lumbricoides*, *Toxocara cati*, *Ascaridia galli*, *Subulura jacchi*, *Haemonchus contortus*, *Uncinaria stenocephala*, *Chabertia ovina*, and *Echinuria uncinata*. The cuticle consists basically of an outermost cortical layer, sub-divisible into external and internal cortical layers, a matrix layer, and usually three internal fiber layers. The
external cortical layer has been shown to contain polyphenol-quinone tanned proteins and a high degree of disulfide binding (Brown, 1950; Monné, 1955; Carbonelle and Apitz, 1960). Phenol oxidase, responsible for the tanning of this layer, was demonstrated by Bird (1957). The internal cortical and matrix layers contain progressively less disulfide linkages and more sulphydryl groups (Carbonell and Apitz, 1960). The principal protein component of the matrix layer, as determined chemically by Chitwood (1937) and histochemically by Monné (1955) is elastin-like. It has been named matracin. Monné has suggested that matracin is the chief structural protein of the cuticle, being continuous throughout the matrix layers, extending outward into the cortical layers and inward between the fibers of the fiber layers. The fiber layers have been shown by chemical analyses (Chitwood, 1937), by histochemistry (Monné, 1955), and by X-ray diffraction studies (Fauré-Fremiet and Garraud, 1944) to consist of a collagen-like protein which has been named ascarocollagen. Lipids have been extracted from the cuticle of Ascaris lumbricoides and Strongylus equinus by Bird (1956). The localization of this lipid is unknown as it could not be visualized with histochemical methods. However, the studies of Trim (1949) on the penetration of drugs into nematodes suggest that the lipid may occur as a thin layer on the outside of the cuticle. Bird and Deutsch (1957) have
interpreted the osmiophilic outer layer of the cuticle of *Ascaris lumbricoides*, as seen with the electron microscope, as essentially a lipid layer. Carbohydrates isolated chemically from *A. lumbricoides* cuticle by Bird (1956) are thought to occur in combination with proteins.

Although the above description may characterize the structure of the cuticle of most large animal parasitic nematodes, it is not known if such a pattern is universal in the Nematoda. To date, few free-living species have been carefully investigated. No fiber layers were seen by Chitwood and Chitwood (1950) in the cuticle of the members of the family Chromadoridae that were examined. These authors are, nevertheless, of the opinion that fiber layers are present but not visible because of the extreme thinness of the cuticle. Monné (1955) was unable to distinguish layers of any sort in the cuticle of larvae of *Metastrongylus elongatus*, *Dictyocaulus viviparus* or *D. filaria*. It is doubtful if the structure of the cuticle layers can be adequately studied in small species by the use of the light microscope alone.

Bird (1958), using phase contrast microscopy, has described "pore canals" in the cuticle of *Ascaris lumbricoides* and postulated that such canals might facilitate the passage of phenol oxidase enzymes to the surface of the cuticle where they function in the tanning of the external layer. Lee
(1961) has reported the presence of esterase in the matrix layer of the cuticle of *Ascaris lumbricoides*. Findings similar to these have been reported from light and electron microscopy of insect cuticle (Locke, 1961).

The Somatic Musculature

Since the early studies on the muscle cell of nematodes by Schneider (1860, 1866), Bütschli (1892), Martini (1916), and others, little new information has been added. The elongate muscle cells in simple meromyarian forms may extend the entire length of the body. In larger species, where muscle cells are not so long, the myofibers of the contractile portion of the cell are said to be continuous from one cell to the next throughout the length of the body (Chitwood and Chitwood, 1950). There has been disagreement on the structure of the myofibers; Apathy (1894) considered each fiber to be composed of many smaller fibrils, while Cappe de Baillon (1911) considered the myofibers to be homogeneous structures. The fibers were described as being cross striated by Flenk (1924), but as being smooth by Roskin (1925).

Confusion also exists concerning the function of a fibrillar system originally described in *Ascaris* muscle by Apathy (1894). These fibrils are irregularly arranged, apparently radiating from the region of the nucleus, and were described as penetrating through the sarcoplasm and
muscle cell membrane to enter the hypodermis. These fibrils were considered to be neural in function by Apathy, but supportive by Schneider (1902) and Goldschmidt (1904, 1909).

The electron microscope study of the muscle cell of *Ascaris lumbricoides* reported by Hinz (1959) has again opened the question of the identity of both the supposed myofiber and supportive fibrils.
MATERIALS AND METHODS

Source of Nematodes

*Capillaria hepatica* infections were maintained in white mice, strain ICR, obtained from Dublin Laboratories, Dublin, Virginia. Mice were maintained on a diet of Purina Laboratory Chow and water.

Anaesthetized mice were fed approximately 1000 infective eggs through a stomach tube. Eggs were suspended in a half saturated solution of sucrose for feeding.

Eggs were collected and embryonated by the method previously described (Wright, 1961). Embryonation was carried out at either room temperature or at 30°C. Infective eggs were stored in a cold room at 8°C. The initial eggs used in the study were brought from the Ontario Research Foundation, Toronto, Canada. This infection was gradually lost for unknown reasons. A new infection was established with eggs collected from a *Peromyscus leucopus* trapped at the Rocky Mountain Biological Laboratories, Colorado.

*Trichuris myosatoria* were collected from nutria (*Myocastor coypu*) kindly supplied by the Texas Game and Wildlife Commission, Sheldon Reservoir, Texas.

Free-living soil nematodes were collected from flower beds on the Rice University campus, while marine nematodes were collected from sediment samples taken at Galveston Island.
Methods for the Light Microscope

The staining methods used for the light microscope are listed in Table I along with reference and fixation. The rationale of these methods is discussed in greater length in standard texts on histochemistry (Gomori, 1952; Lillie, 1954; Pearse, 1960). Only brief comments on the methods will be made here.

Specimens of *Capillaria hepatica* were fixed both *in situ* in small pieces of liver, and after being dissected free of host tissue. Specimens of *Trichuris myocastorius* were fixed following removal from the wall of freshly opened unwashed caeca of nutria, or were fixed *in situ* after gently flushing away caecal contents and excising the portion of the wall to which the worm was attached.

Individual worms were fixed in Helly's, Bouin's, Zenker's acetic, or Rossman's picric acid-alcohol-formalin fixative for one hour. Worms in larger tissue blocks were fixed in the same fixatives for six to eight hours. Some blocks of *C. hepatica* infected liver were fixed in Zenker's acetic fixative for 13 and 24 hours. The latter procedure gave very brittle tissues which were difficult to section. The shorter periods of fixation gave better results. Formalin fixation was carried out in the cold for 24 hours or longer, unless the tissue was to be used for enzyme demonstrations. Worms were fixed *in situ* in Flemming's and Elft-
man's fixatives for a period of three days.

Tissues were embedded in Tissuemat (melting point 60-62°C), and sectioned at 5 microns.

In order to resolve many of the structures being studied, maximum resolution with the light microscope was required. A binocular microscope equipped with acromatic objectives and a centerable substage condenser with a numerical aperture of 1.3 was used with a 500 watt, ribbon filament, light source. Both 10 and 12 power oculars were used. Slides were observed under oil immersion with the substage condenser oiled to the slide.

Carbohydrates.—The periodic acid-Schiff method (PAS method) according to Pearse (1960) was used to visualize carbohydrates. This method depends upon the mild oxidation of the 1,2 glycol linkage of polysaccharides to form aldehyde groups which are active in recoloring the leuco-basic fuchsin Schiff reagent.

Control sections were treated in various ways to determine what classes of polysaccharide compounds were responsible for the staining observed. Thus, sections from which oxidation in periodic acid was omitted, demonstrate the stain contributed by aldehydes present prior to treatment with periodic acid. Glycogen was selectively removed from control sections by incubation at 37°C for 1½ hours in fresh human saliva, or in 0.1% malt diastase (in pH 7.0
phosphate buffer, or in glass-distilled water). Compounds resistant to enzymatic removal were concluded to be lipids if staining was precluded, or if its intensity was significantly decreased by extraction with pyridine for 14 hours at room temperature. Staining resistant to both enzymatic removal and extraction with pyridine was considered to be due to either mucopolysaccharide or mucoproteins.

**Lipid.-** The demonstration of lipid by sudan black B is based upon the greater solubility of this dye in lipids than in propylene glycol, the solvent of the staining solution. Suitable controls may be carried out to characterize the nature of the lipid colored in this manner. Thus, cold acetone, or cold methanol-ether, extracts neutral lipids while pyridine extracts most phospholipids as well as neutral lipids (Pearse, 1960).

Fixation in Elftman's fixative followed by staining with hematoxylin and eosin, and Baker's acid hematin test is considered to be more specific for the detection of phospholipids. In these methods, neutral lipids are largely extracted during dehydration and clearing prior to embedding, while phospholipids are fixed by chromation in the tissues and rendered stainable with hematoxylin dyes; simultaneous fixation and chromation occurs in Elftman's fixative, while controlled chromation follows fixation in calcium formol in Baker's acid hematin test.
**Proteins.**—Two general methods were used to demonstrate proteins, the mercury bromphenol blue method and the acrolein-Schiff method. These methods stain proteins without regard to their amino acid content, although van Duijn (1951) has indicated that the acrolein-Schiff method has some selectivity for sulfhydryl, imino and amino groups, and the imidazole ring.

Both the DDD reaction of Barnett and Seligman and the ferric ferricyanide reaction (Adams, 1956) were carried out to localize proteins having a high content of sulfhydryl groups and disulfide linkages. Prior to staining disulfide bonds were reduced to form sulfhydryl groups by incubation for 30 minutes in alkaline thioglycolate at room temperature. Control sections were incubated in 0.1 M sodium iodoacetate at 37°C for 20 hours in order to block the reactive sulfhydryl groups before staining. Sections of mouse skin were stained along with nematode tissues as a further control.

The Millon reaction for proteins high in tyrosine content was also carried out. Sections were mounted either in glycerin jelly or in the usual synthetic resin and were examined immediately.

**Enzymes.**—Both acid and alkaline phosphatase methods were attempted, using the Gomori methods. No enzymatic activity remained in tissues embedded in 50–52°C paraffin after fixation for four hours in cold calcium formol. Fro-
zen sections of worms in situ cut from fresh frozen tissues, or from tissues fixed in cold calcium formol for four hours, were not suitable since the sections of worms were either crumpled beyond use or were lost entirely from the section during handling. Acid phosphatase was demonstrable in living worms, fresh frozen worms, or worms fixed for four hours in cold calcium formol, providing the enzyme method was performed on these whole tissues prior to embedding in paraffin. For this method, worms were freed of excess host tissue before fixation and incubation. Ten percent polyvinylpyrrolidone was added to the incubation medium to minimize the diffusion of reaction products during incubation. Living and fresh frozen tissues were post-fixed in calcium formol overnight. Alkaline phosphatase activity could not be demonstrated by this method.

Control tissues were incubated in the medium lacking substrate, or were incubated in the complete substrate medium after being placed in boiling distilled water for five minutes.
<table>
<thead>
<tr>
<th>Stain</th>
<th>Reference</th>
<th>Fixative*</th>
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<tbody>
<tr>
<td><strong>General Oversight Techniques</strong></td>
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<td></td>
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<tr>
<td>Tetrachrome</td>
<td></td>
<td>H, Z, B, R, C.</td>
</tr>
<tr>
<td>Masson's Trichrome</td>
<td>Lillie, 1954</td>
<td>H, Z, B.</td>
</tr>
<tr>
<td>Mallory's Phosphotungstic acid hematoxylin</td>
<td>Lillie, 1954</td>
<td>H, BF.</td>
</tr>
<tr>
<td><strong>Cytoplasmic Basophilia</strong></td>
<td></td>
<td></td>
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<tr>
<td>Toluidine Blue O</td>
<td>Pearse, 1960</td>
<td>H, Z, B, BF.</td>
</tr>
<tr>
<td>(Kramer and Windrum method)</td>
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<tr>
<td><strong>Nucleic Acids</strong></td>
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<tr>
<td>Feulgen Reaction</td>
<td>Pearse, 1960</td>
<td>Z, C.</td>
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<tr>
<td><strong>Carbohydrates</strong></td>
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<tr>
<td>Periodic Acid-Schiff</td>
<td>Pearse, 1960</td>
<td>R, C, AAF.</td>
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<td>...counterstained with</td>
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<tr>
<td>1) Allochrome</td>
<td>Lillie, 1954</td>
<td>AAF.</td>
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<tr>
<td>2) Naphthol Yellow S and Hematoxylin</td>
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<td>BF.</td>
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<td>3) Erlich's Hematoxylin</td>
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<td>R.</td>
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<td>4) Erlich's Hemalum</td>
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* Abbreviations of fixatives are as follows: H= Helly's, Z= Zenker's acetic, B= Bouin's, R= Rossman's, C= Carnoy's, BF= Buffered formalin, AAF= Lillie's alcohol-acetic acid-formalin, Fl= Flemming's, E= Elftman's, CaF= Baker's Calcium chloride formalin, ET= Ethanol-trichloracetic acid, A= Acetone.
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<th>Stain</th>
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<th>Fixative*</th>
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<td>Toluidine Blue O (for metachromasia)</td>
<td>Pearse, 1960</td>
<td>H.</td>
</tr>
<tr>
<td>Alcian Blue (for acid mucopolysaccharides)</td>
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<td>H.</td>
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<tr>
<td><strong>Lipids</strong></td>
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<td>CaF.</td>
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<td>Mercury Bromphenol Blue</td>
<td>Mazia et al.,</td>
<td>H, Z, B, BF.</td>
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<td>Acrolein-Schiff</td>
<td>van Duijn, 1961</td>
<td>H, B, BF.</td>
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<td>DDD Reaction</td>
<td>Pearse, 1960</td>
<td>CaF, ET.</td>
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<td>Ferricferricyanide Reaction</td>
<td>Adams, 1956</td>
<td>CaF, ET.</td>
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<td><strong>Enzymes</strong></td>
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<td>Acid Phosphatase</td>
<td>Lillie, 1954</td>
<td>CaF, A, fresh frozen.</td>
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<td>Gomori, 1956</td>
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<tr>
<td>Alkaline Phosphatase</td>
<td>Lillie, 1954</td>
<td>CaF, A, fresh frozen.</td>
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Methods for the Electron Microscope

Specimens of *G. hepatica* were obtained by lightly compressing the infected livers of mice between glass plates; worms thus localized were cut into small pieces with a clean razor blade in a drop of cold fixative and transferred to weighing bottles containing about 1 cc. of fixative.

Four fixatives were used: Caulfield's modification of Palade's veronal acetate-buffered osmium tetroxide fixative (Caulfield, 1957), 1% osmium tetroxide buffered with s-collidine (Bennett and Luft, 1959), Dalton's dichromate-osmium tetroxide fixative (Dalton, 1955), and Luft's 1.2% permanganate fixative in 0.9% NaCl (Luft, 1956).

The tissues were dehydrated rapidly in changes of cold 35%, 70%, and 95% ethanol for five minutes each, and finally in at least two changes of absolute ethanol for 10 to 15 minutes each.

The tissues were then infiltrated with a 3:1 mixture of butyl and methyl methacrylate. The pieces of tissue were transferred to number 00 gelatin capsules containing the same methacrylate mixture with 1% benzoyl peroxide as catalyst. The methacrylate was polymerized at 60°C.

Thin sections were cut with hand-made glass knives on a Porter-Blume microtome. Sections were placed on grids punched from either copper or nickel electroplate screen-
ing, the former having 200 mesh per inch, the latter 100 mesh. They were supported on these grids by celloidin films. The sections of osmium-fixed tissues were stained routinely with lead hydroxide (Dalton and Zeigel, 1960), although some were stained with uranyl acetate (Watson, 1958). Before observation in the microscope, sections were sandwiched with a thin layer of carbon.

Sections were examined with an RCA EMU 3F electron microscope. Electron micrograph negatives were taken at magnifications of 2400 to 17,500 on Kramer class III photographic plates.

Methods for Vital Staining

Both neutral red and methylene blue staining solutions were prepared by diluting a small drop of a concentrated stock stain in distilled water with the desired dilution medium: Tyrode solution, twice concentrated Tyrode solution, half diluted Tyrode solution, saline, or distilled water. Since neutral red is not highly soluble in salt solutions these stains were prepared immediately before use.

Portions of worms of _G. hepatica_ were dissected from the livers of freshly killed mice, and stained either in deep well slides, or in shallow preparations on normal microscope slides. The former preparations were sealed with vaseline, placed in an incubator at 37°C, and examined at irregular intervals; the worms were removed to normal microscope
slides for observation to provide a preparation thin enough
to be viewed with the high dry objective of the compound
microscope. Thin preparations, initially prepared on normal
microscope slides, were examined either at room temperature,
or with the aid of a microscope warming stage at 37°C (body
temperature of the host), using a high dry objective and 12
power oculars giving a final magnification of 675 times.

Methods for Colloidal Gold Uptake

Four rats infected with *G. hepatica* received injections of 0.5 to 0.75 ml. of a 50% dilution of a colloidal
gold suspension (potassium gold chloride in distilled water)
in the caudal vein. Injections were first given when the
rats had been infected with *G. hepatica* for 23 days. Twenty
four hours after the first injection, a second injection
was given; the rats were killed one-half hour after this
last injection. Worms were harvested from the liver for
electron microscopy as described in an earlier section. The
worms were fixed in 1% osmium tetroxide buffered with s-collidine. Methods for embedding and sectioning the worms were
identical to those described previously.

Methods for Incubation in Altered
Physiological Solutions

Specimens of *G. hepatica*, dissected from the livers
of freshly killed mice, were incubated *in vitro* in various
concentrations of mouse serum. It is impossible to obtain
whole worms free of host tissue as the worms are tightly coiled in the liver of the host, and connective tissue is laid down around the worms early in the infection. Portions of worms as long as could be rapidly dissected free of host tissue were used in the experiments. The portions of worms were placed in small watch glasses, each containing 1 ml. of serum at the dilutions required. The watch glasses were covered with coverslips, sealed with vaseline, and kept at 37°C for a period of 15 minutes. The portions of worms were then fixed in Gaulfield's modification of Palade's osmium tetroxide fixative for electron microscopy. The tissues were dehydrated and embedded in methacrylate as described in an earlier section. The following concentrations of mouse serum diluted with glass-distilled water were used: full strength serum, 25:1, 12:1, and 6:1 serum. Sucrose was added to one sample of serum to give it an osmotic pressure twice that of normal serum. When sections were cut from these tissues for the electron microscope, an area near the middle of the portion of worm was chosen for sectioning. This was done in order to avoid confusion of physiological effects with possible artifacts which might occur where internal tissues were exposed to incubating solutions.

Worms were perfused in vivo with Tyrodes solutions of various concentrations by perfusing the liver of mice infected with C. hepatica 24 days earlier. At this age of
infection, very little host connective tissue would have proliferated around the worms and hence, one might be more certain that the perfusing medium would actually reach the worm. The perfusion apparatus was essentially that of Yolles et al. (1947). The perfusion solution was kept in a water bath at 40°C. The mouse was anaesthetized, the body wall opened, and the hypodermic needle of the perfusion system inserted into the hepatic sinus. The angle at which the needle was held determined the area of the liver being perfused. The effectiveness of the perfusion could be followed as the liver was blanched. Perfusion was carried out for five minutes, then the mouse was killed, and the worms fixed in cold 1% osmium tetroxide prepared in the same solution as that used for perfusion. Further processing of the tissue for electron microscopy was identical to methods described previously. The following perfusion solutions were used: full strength Tyrodes solution, 50:1, 25:1, 12:1, and 6:1 dilutions of Tyrodes solution with glass-distilled water, and Tyrodes solution prepared with twice the normal concentration of potassium chloride.
RESULTS

The Hypodermis

**The Lateral and Ventral Chords**

The lateral and ventral hypodermal chords may be described together since their only point of difference is in the extent of the bacillary band contained within them. The dorsal chord may better be considered in relation to the interchordal hypodermis.

Two types of cells may be discerned in the lateral and ventral hypodermal chords. One of these, the gland cell, corresponds to the lateral hypodermal gland cell referred to by Chitwood and Chitwood (1950). The remaining cell type may be termed the non-glandular cell. No sufficiently detailed description of these cells exists in the literature. A review of the results of electron microscopy is, therefore, necessary in order to establish a terminology which will render subsequent descriptions more comprehensible.

The hypodermal chords are a single cell in thickness. The non-glandular cells are in contact with the cuticle at the apical surface and rest basally upon a thin basement membrane. They ensheath the gland cells both apically and basally; thus, the gland cells are not in contact with
FIG. 1 DIAGRAM OF THE GLAND AND NON-GLANDULAR CELLS OF THE LATERAL AND VENTRAL HYPODERMAL CHORDS OF CAPILLARIA HEPATICA

BM = BASEMENT MEMBRANE
BL = BOUNDARY LAYER
CM = CAP MATERIAL
G = GOLGI ZONE
LA = LAMELLAR APPARATUS

c,s = LAYERS OF CUTICLE
PC = PORE CHAMBER
TB = TERMINAL BAR
 сахарозамещение = GLYCOCEN
either the cuticle or the basement membrane. A pore through
the cuticle is located above each gland cell. The apical
cell membrane of the gland cell is recessed from the pore,
and forms a region of highly infolded cell membrane which
is termed the lamellar apparatus. Stretching across the
pore at the level of the base of the cuticle is the boundary
layer. Associated with the outer surface of this layer is
a granular substance, the cap material. The region between
the boundary layer and the lamellar apparatus is the pore
chamber. This terminology is summarized in the diagram,
Figure 1.

Electron micrographs indicate that the fine struc-
ture of the gland cell is remarkably constant throughout
the length of the worm. However, at both the anterior and
posterior extremities of the worm, the cells assume a nearly
spherical shape, are fewer in number and are more widely
spaced. Throughout the mid portion of the worm, including
the regions occupied by the posterior portion of the esopha-
gus, the vagina, and the uterus, the cells are columnar in
shape and more densely aggregated. It is from these regions
that classical descriptions of the cells have been made—
when observed in face view, the cells appear irregularly
hexagonal, while the pores and pore chambers appear as bright
spots surrounded by refractive halos.

There is no discernible difference in the gland cells
between male and female worms, although the cells occur only in the lateral chords of the male, whereas they occur in both the lateral chords and the ventral chord of the female.

Sections of worms ranging in age from 10 to 46 days were examined in order to determine whether the morphology of the gland cell changes with age. A gradual transformation from a nearly spherical to a more columnar shape and an increase in the number of gland cells during larval growth, was observed. The cells are large and spherical and widely spaced in worms 10 to 12 days old (fourth stage larvae). By 16 days (late fourth stage larvae) the cells are more abundant and more closely packed. It was not possible with the resolution of the light microscope to obtain evidence of the differentiation of gland cells from other cells of the larval hypodermis. No change was seen in the morphology of the cells in adult worms ranging in age from 19 to 46 days (worms 21, 23, 26, 27, 31, 37, 40, and 46 days old were examined).

As there is no apparent difference in the morphology of the gland cells of the anterior or posterior regions of the worm (beyond a transition from a spherical to a columnar shape), between male and female worms, or between worms of varying ages, no reference to these factors shall be made in subsequent descriptions. However, most observations were made from worms ranging from 20 to 34 days of age, that is,
shortly after reaching maturity.

Light Microscopy

With the general techniques listed in the first portion of Table I (pages 21 and 22), the gland cell may be distinguished through the light microscope by the fact that the apical pole of the cell is associated with a conspicuous cuticular pore (Figs. 2-4). A zone of striations, the lamellar apparatus, can be distinguished, separated from the pore by a refractile area, the pore chamber. The nucleus is located in the middle of the cell below the lamellar apparatus.

A pronounced acidophilia of the lamellar apparatus of the gland cell is demonstrated by phloxine-methylene blue, hematoxylin and eosin, tetrachrome, and Masson's trichrome stains. Phloxine-methylene blue and toluidine blue O stains demonstrate a faint reticular pattern of basophilia surrounding the nucleus, being somewhat more concentrated near the middle of the cell, but extending into the basal region of the cell as well (Figs. 9-10).

The material of the pore chamber of the gland cell remains unstained in sections stained with hematoxylin and eosin or phloxine-methylene blue. However, it is stained with general connective tissue dyes, the fast green of the tetrachrome method, and the aniline blue of the trichrome method. With these two stains, there may appear to be direct continuity of the pore chamber material with the
similarly stained host tissue fluids surrounding the worm. In most sections, however, the inherent refractive quality of the pore chamber material imparts a distinctive, yellowish hue to the stain in this area.

The results of staining of the gland cell with the tetrachrome method vary greatly; the degree of staining with either the chromotrope-orange G dyes or the fast green dye depending on the intensity of the initial chromotrope-orange G stain and upon the length of mordanting prior to staining in fast green. Thus, although the pore chamber of the gland cell generally stains green, it may retain the chromotrope stain in some sections. In other section, heavily stained with chromotrope-orange G, although the pore chamber stains green, the cap material may occasionally be seen staining heavily with chromotrope. The staining of the lamellar apparatus may vary from entirely green through green at the apical margin only, to entirely stained with chromotrope. The basal region of the gland cell remains largely unstained with this method.

The lamellar apparatus and pore chamber of gland cells stains lightly orange with Mallory's phosphotungstic acid hematoxylin (Fig. 7). The intensity of this orange stain is considerably less than that in the inner layer of the cuticle and in the host collagen.

The non-glandular cells exhibit a cytoplasmic baso-
philbia slightly increased over that of the gland cells, but lack an acidophilic component. The reticular pattern of basophilia is more intense in the apical region of the cells, but extends also into the basal region. The nuclei, located near the middle of the cells, are frequently ovoid, generally smaller than the nuclei of gland cells, and have a more dense chromatin network. Fine granules stained with chromotrope are seen in the apical cytoplasm of non-glandular cells following staining with the tetrachrome method. The basal cytoplasm remains largely unstained.

The histochemical methods used to characterize the hypodermis are listed in Table I (pages 21 and 22).

Nucleic acids.- Desoxyribonucleic acid was demonstrated by means of the Feulgen reaction. Only a very faint staining of the nuclei of worms was obtained after acid hydrolysis for 25 minutes followed by staining in Schiff reagent for 30 minutes, although strong reactions were obtained with much shorter hydrolysis times in host nuclei. DNA could be demonstrated only in the nuclei.

The degree of cytoplasmic basophilia can generally be correlated with the cytoplasmic ribonucleic acid content (Deane and Porter, 1960). It was, therefore, not considered necessary to demonstrate RNA by more critical methods. The fine pattern of cytoplasmic basophilia previously described in the hypodermal cells probably reflects the distribution
of cytoplasmic RNA.

Carbohydrates.—Heavy deposits of glycogen were found in the non-glandular cells (Fig. 11). The localization of glycogen in the non-glandular cells but not in the gland cells was verified by counterstaining the PAS preparations with Erlich's hematoxylin, Erlich's hemalum, Lillie's allochrome, or napthol yellow S and hematoxylin.

No stain attributable to mucopolysaccharides or mucoproteins was detected in the hypodermis. Since the basement membrane of the intestine stains for these compounds, the absence of detectable stain in the hypodermal basement membrane may be due to the thinness of this membrane in comparison to that of the intestine.

No metachromasia was observed in water mounts of sections stained with toluidine blue O.

Lipids.—The lipid content of the hypodermis was determined by sudan black B treatment of frozen sections cut from calcium formol-fixed tissues. Sections were extracted with cold acetone or methanol-ether to remove neutral lipids and with pyridine to remove phospholipids. Coloring, apparently attributable to phospholipid, was obtained in the lamellar apparatus of gland cells on two occasions (Figs. 12-13). This coloring was not affected by previous extraction of sections for two hours in cold acetone,
or for two or 14 hours in cold methanol-ether. The intensity of the color was markedly decreased, however, in sections extracted at room temperature for 14 hours in pyridine. Subsequent repetitions of the method did not give identical results.

Further indication of phospholipid in the lamellar apparatus of gland cells was obtained from control sections stained by the PAS method for glycogen. The lamellar apparatus stained faintly PAS positive both before and after glycogen digestion, but this staining was precluded from sections extracted with pyridine.

Baker's acid hematin test for phospholipids did not stain the lamellar apparatus. Similarly, no evidence of chromation of phospholipids of the lamellar apparatus was found in sections of tissue fixed in Elftman's fixative.

Small droplets of sudanophilic lipid, extractable with cold acetone or methanol-ether, were readily demonstrable with sudan black B in the basal region of both the gland cells and non-glandular cells. Osmiophilic droplets of similar size and distribution were seen in thick sections cut from tissues fixed in osmium tetroxide for the electron microscope and viewed in the phase contrast microscope. These droplets were generally seen in greater number in the non-glandular cells than in gland cells.
Proteins.—The two general protein stains, bromphenol blue and acrolein-Schiff, demonstrated a protein component in the lamellar apparatus of the gland cell, and a lack of protein, at least in concentrations sufficient to be visualized, in the basal region of the cell. Small, stained granules appeared in the mid region of the cell just beneath the lamellar apparatus in sections stained with bromphenol blue. The material within the pore chamber remained unstained with bromphenol blue (Fig. 14), but stained moderately darkly with the acrolein-Schiff method (Fig. 15). The cap material was not clearly distinguished by either method, although a fine, moderately dark line across the pore was seen in many cells with both stains. This line may represent the boundary layer alone, or the boundary layer and some cap material.

A light diffuse staining with the acrolein-Schiff reaction and a more intense staining with the bromphenol blue reaction characterized the apical ends of the non-glandular cells, while their basal regions remained unstained.

In the hope of characterizing the protein material of the pore chamber previously indicated by the acrolein-Schiff technique, methods specific for sulfhydryl groups and disulfide linkages (DDD reaction and ferric ferricyanide method), and for the amino acid tyrosine (Millon reaction)
were carried out. Neither sulfhydryl groups nor disulfide linkages could be detected in the pore chamber of gland cells, although maturing ova gave a weak reaction for sulfhydryl groups and the protein shell of the egg gave a strong reaction for disulfide linkages. Only diffuse staining was obtained in the worm with the Millon reaction for tyrosine, although a slightly more intense reaction, of doubtful significance, was seen in the pore chamber material and lamellar apparatus of gland cells.

Enzymes.- Although both alkaline and acid phosphatase methods were performed on comparable material, only acid phosphatase activity could be demonstrated. Lead deposition, as a result of enzymatic activity, was found associated with the lamellar apparatus, pore chamber, and cap material of gland cells in unfixed tissues incubated three hours, fresh frozen tissue and sections of calcium formol-fixed tissues incubated two and three hours. However, no lead was deposited in the pore chamber when 10% polyvinylpyrolydione was added to the incubation medium and the length of incubation was decreased to 30 minutes or one hour; lead was deposited in the cap material of some cells, especially in tissues incubated for the longer periods (Figs. 16-17). The localization of the reaction product was verified by counterstaining sections with hematoxylin and eosin and by studying the slides with phase microscopy. Control tissues
incubated in medium lacking substrate, or immersed for five minutes in boiling distilled water and incubated with the complete substrate medium gave no trace of lead deposition.

**Polarized Light Microscopy**

Both living worms and unstained sections of fixed worms were studied with polarized light. The cuticle was seen to be birefringent in whole living worms, but not in sections. The material within the pore chamber of the gland cell is not birefringent.

**Electron Microscopy**

The most characteristic feature of the fine structure of the gland cell is the elaboration of the apical cell membrane into the lamellar apparatus. Figure 26 shows that the cell membrane folds back upon itself producing the complex system of lamelliform membranes which constitute the lamellar apparatus. The membranes anastamose and rebranch irregularly near the base of the lamellar apparatus. The form of the membranes as seen in oblique sections is shown in figures 23 and 25. Small vesicles, 60 µm in diameter, of similar density to the interlamellar spaces are located at the base of the lamellae (Figs. 19 and 24). Frequently a linear array of these vesicles extends into the cell from the base of the interlamellar space. In poorly fixed tissues, the entire base of the lamellar apparatus may appear
as a mass of vesicles (Fig. 41). The membranes of the base of the lamellar apparatus are widely dilated in tissues fixed with Dalton’s dichromate-osmium fixative (Fig. 40).

Mitochondria are more numerous near the base of the lamellar apparatus. Some mitochondria lie between the membranes of this region, while some are also found in the basal region of the cell. The mitochondria are elongate with lamelliform cristae and have a matrix considerably more dense than the cytoplasmic ground substance.

The nucleus is located below the lamellar apparatus. The chromatin pattern is finely dispersed. The outer nuclear membrane, presumably continuous with the endoplasmic reticulum, bears numerous ribosomes on its outer surface. The endoplasmic reticulum itself is represented only by scattered profiles of membranes bearing ribosomes. These profiles are frequently seen near the periphery of the cell or near lipid droplets which may occur in the basal region of the cell. Ribosomes, not associated with membranes, are found in groups within the cytoplasmic ground substance.

The golgi system of the gland cell consists of an aggregation of vesicles, smaller in diameter than those associated with the lamellar apparatus, containing a dense material. Some golgi zones include somewhat larger vesicles which may be interpreted as dilated membrane sacs. These aggregates are frequently bounded by membranes of the gran-
ular endoplasmic reticulum (Figs. 19 and 24). Golgi zones are usually seen towards the basal side of the nucleus.

The extracellular material of the pore chamber may be homogeneous in appearance, or may have a filamentous texture. The dense, discrete nature of the pore chamber material is shown most clearly in micrographs of worms incubated in hypotonic media. Here, the membranes of the lamellar apparatus may be distorted and torn away from the pore chamber material. Although the membranes have undergone violent disruption, the pore chamber material is of a normal density, and retains its shape. In cells having dilated membranes of the lamellar apparatus from fixation in Dalton's dichromate-osmium fixative, the density of the dilated spaces is markedly less than the density of the pore chamber material above it. These observations indicate that the material of the pore chamber has the properties of a gel-like plug.

The boundary layer and cap material vary in appearance. The boundary layer appears, in sections, to consist of two irregular dense lines, the inner being approximately 20 μm thick, the outer being more variable (Figs. 19 and 46). These layers are not continuous with the outer layers of the cuticle. In some sections, the boundary layer is indistinguishable from the cap material. The cap material usually appears as a finely dispersed, granular material which is more diffuse farther from the boundary layer. The cap mate-
rial may be torn away from the boundary layer when worms are dissected free of host tissue. Neither the boundary layer nor the cap material is preserved by potassium permanganate fixation (Figs. 18 and 20). The position of the former may be noted as a leached line, while no trace of the latter can be seen. The appearance of the boundary layer in many micrographs suggests that it may be formed by condensation of the material of the pore chamber, and hence, may represent only the outermost aspect of this gel-like plug altered by factors of the external environment. The nature of the cap material remains unknown.

The gland cells are completely ensheathed by the non-glandular cells except for the space over the cuticular pore (Figs. 18-21). Thus, the non-glandular cells, but not the gland cells, associate directly with the cuticle and lie upon the basement membrane which is continuous across the hypodermal chords and the cell bodies of the somatic muscle cells. The line of contact of the apical cell membrane of the non-glandular cell with the cuticle is irregular. There is some interdigitation of basal cell membranes between the gland and non-glandular cells, especially where the gland cells are more spherical in shape.

The most striking feature of the fine structure of the non-glandular cells is the presence of large quantities of glycogen in the basal three-quarters to two-thirds of
the cell (Figs. 19-22). With osmium fixation, the glycogen
assumes the form of irregular aggregates of moderately dense
particles, larger in size than ribosomes (Fig. 24). With
permanganate fixation, glycogen stains as large, dense bodies
(Fig. 21). Lipid droplets are frequently found in the re-
gion of glycogen storage.

The majority of organelles are located in the apical
cytoplasm of the non-glandular cells, a region in which lit-
tle glycogen occurs. Mitochondria, similar to those of the
gland cell are found chiefly in this apical region of the
cell. Endoplasmic reticulum is represented by short profiles
of ribosome-bearing membranes, located principally in the
apical cytoplasm or near the periphery of the cell. Scat-
tered groups of free ribosomes may be found apically, or in
smaller concentrations in the peripheral cytoplasm. Vesicles
varying in size and shape may be located in the cytoplasm
immediately below the cuticle. Some of these vesicles bear
ribosomes on their outer surface, indicating that they are a
part of the endoplasmic reticulum.

The golgi system is represented by aggregates of
small vesicles, similar in size to those of the golgi zones
of the gland cells (Fig. 23). Frequently, a single, larger
vacuole is included in an aggregate. These golgi zones are
often bounded by profiles of the granular endoplasmic reti-
culum. More than one golgi zone may be found in a single
cell. Golgi zones may be situated in the apical cytoplasm, within the region of glycogen storage, or close to the basal cell membrane.

The nucleus is situated near the middle of the cell, within the region of glycogen storage. The chromatin network is finely dispersed. The outer nuclear membrane bears ribosomes on its surface.

Where non-glandular cells are adjacent to each other, the lateral cell membranes appear to be thickened apically by a dark, diffuse material which appears along the inner surface of the cell membranes (Fig. 24). This thickening of the lateral cell membranes is similar to terminal bars of vertebrate epithelia. However, no fibrillar elements are associated with the dense material in the non-glandular cells. The space between the cell membranes is more uniform here than in regions of the cells lacking this development. Terminal bars do not occur between gland cells and non-glandular cells.

Observations on Related Species

Four other species of the genus Capillaria were examined with the light microscope in order to determine whether the structure of the hypodermal gland cells of the bacillary band as revealed in C. heptatica is representative
of these cells in other species of the genus*. The species examined were \textit{C. corvorum}, \textit{C. ovopunctatum}, \textit{C. contorta}, and \textit{C. sp.} from \textit{Sturnis} sp. Although many of the specimens were not optimally fixed for histochemical methods, examination of sections stained with hematoxylin and eosin revealed that the structure of the gland cells in these species is similar to that in \textit{C. hepatica}.

The association between the single bacillary band of \textit{C. contorta}, and the somatic musculature is of interest. The hypodermal chord containing the bacillary band is covered internally by the somatic muscle cells, except in its most lateral region, in the same manner as is the interchordal hypodermis.

Specimens of \textit{Trichuris myocastoris} obtained from the caecum of nutria were sectioned for the light microscope. The sections were stained with hematoxylin and eosin, tetra-chrome, and the PAS method. The gland cells of the bacillary band appear to be similar in structure to the gland cells of the genus \textit{Capillaria}. However, interpretation of the sections is more difficult as the cells, although taller, are more narrow. The distribution of glycogen in the body wall is identical to that in \textit{C. hepatica}. It was also noted that nuclei are located in the interchordal hypodermis of

*I wish to thank Prof. C. P. Read for making available specimens of the following species: \textit{C. corvorum}, \textit{C. ovopunctatum}, and \textit{Capillaria} sp. from \textit{Sturnis} sp. Dr. F. Fisher kindly loaned sections of \textit{C. contorta}.}
Trichinella spiralis

Whole adult specimens of Trichinella spiralis were examined after clearing in glycerin*. No gland cells could be seen.

A limited number of sections of free-living marine and terrestrial, aphanid nematodes were examined with the light microscope in order to compare the structure of the lateral hypodermal gland cells of these groups with the gland cells of the bacillary bands of trichurid nematodes.

The gland cells are apparently different in the three ecological groups. The gland cells of Acanthocheilonema cobbi, a marine nematode of the family Chromadoridae, appear to contain refractile non-staining globules which occupy most of the cell. This material may be squeezed through the cuticular pore if the nematode is compressed. Neither eosinophilic striations, as seen in the gland cells of bacillary bands, nor pigment granules, as described in Anonchus mirabilis by Chitwood and Chitwood (1950), were seen. In the free-living, terrestrial nematode studied, Aporcelaimus sp. of the family Dorylaimidae, only a single cell type could be discerned in the hypodermal chords. The cytoplasm of these cells is considerably basophilic containing large eosinophilic vacuoles or droplets. Although a pore through the cuticle could be identified in some sec-

*I wish to thank Dr. C. J. Weinman for providing mice infected with Trichinella spiralis.
tions, no underlying cell could be clearly associated with it. However, in many poorly fixed, whole specimens of this species, a cell with "plasmolysed" contents was seen below each cuticular pore. Presumably this is the hypodermal gland cell.

Attempts were made to fix specimens of *Trichuris*, *Acanthocheila*, and *Aporcelaimus* for the electron microscope. Adequate fixation was not achieved in any of these species, although sufficient preservation was obtained in some specimens of *Trichuris* to show that the structure of the lamellar apparatus of these gland cells is similar to that in *G. hepatica* (i.e., a highly infolded cell membrane).

Although the hypodermal glands appear similar in structure in *Capillaria* and *Trichuris*, both genera of parasitic nematodes having bacillary bands, these cells do not appear to be closely related to the lateral hypodermal gland cells of free-living groups. The structure of the gland cells appears to be different in free-living marine and terrestrial species.

**The Dorsal Chord and Interchordal Hypodermis**

**Light Microscopy**

The dorsal hypodermal chord is identifiable in cross sections as a narrow strip of basophilic tissue separating the two subdorsal muscle bands (Figs. 2 and 5). This hypo-
dermal chord cannot be clearly identified in all sections. The tissue has a reticular pattern of basophilia somewhat more dense than that of non-glandular cells of the lateral and ventral chords. Nuclei have not been seen in this tissue in serial sections stained with hematoxylin and eosin. Glycogen could not be demonstrated in the dorsal chord.

The interchordal hypodermis is a thin sheet of tissue between the cuticle and the somatic muscle tissue. It may be discerned only as a thin, faintly basophilic line. Glycogen can not be seen here, but lipid droplets are demonstrable with sudan black B. No further details could be visualised with the light microscope.

Electron Microscopy

The dorsal hypodermal chord consists of two longitudinal rows of tall cell processes. These processes contain numerous short profiles of granular endoplasmic reticulum (Figs. 28 and 29) and few or many mitochondria similar in form to the mitochondria of other hypodermal cells (Fig. 30). Lipid droplets are occasionally seen. No sections including a nucleus have been obtained. A thin basement membrane, which is continuous with that of the somatic muscle bands, underlies the bases of these processes. Apically, the cytoplasm merges into the thin sheet of hypodermal tissue forming the interchordal hypodermis.

The tissue of the interchordal hypodermis is approx-
imately 300-400 μm thick. Scattered free ribosomes, short profiles of granular endoplasmic reticulum, occasional mitochondria, and lipid droplets, are recognizable in this tissue (Fig. 35). Nuclei have not been observed in the interchordal tissue. In some micrographs, cell membranes can be seen a short distance beyond the cell bodies of the lateral chord cells, apparently dividing the cytoplasm of these cells from that of the interchordal hypodermis (Fig. 27). These cell membranes are not seen in all micrographs while in some, cell membranes may be seen further within the interchordal area. This tissue, where it makes contact with the cuticle, has the same irregular surface as do the non-glandular cells of the lateral and ventral chords.

The Cuticle

Light Microscopy

The cuticle can be resolved into two layers with light microscopy (Figs. 7-8). The inner of these layers is faintly acidophilic, staining intensely with the fast green of the tetrachrome method, and intensely orange with Mallory's phosphotungstic acid hematoxylin. The general protein stains, bromphenol blue and acrolein-Schiff, stain this layer darkly. The outer layer of the cuticle exhibits the opposite staining characteristics to the inner layer: it is faintly basophilic, stains lightly with fast green,
appears light orange with Mallory's phosphotungstic acid hematoxylin, and stains only lightly with the bromphenol blue and acrolein-Schiff procedures. Unlike the inner layer, it stains faintly with the PAS method, both before and after treatment with saliva, malt diastase, or pyridine. No sulfhydryl groups or disulfide bonds were detected in either layer of the cuticle.

No lipid could be demonstrated in the cuticle with sudan black B. However, a dark line within the cuticle can be seen in sections of tissue fixed in Elftman's and Flemming's fixatives for three days and stained with hematoxylin and eosin. This dark line probably indicates the presence of lipids, principally phospholipids, chromated during fixation. It is difficult to state with certainty the position of this line within the cuticle. It probably corresponds to the second layer of the cuticle as revealed by electron microscopy. If so, it is probable that the staining characteristics of the inner and outer layers of the cuticle as seen in the light microscope can be attributed to the first and third layers of the cuticle as seen in the electron microscope.

Electron Microscopy

The cuticle may be resolved into six layers (Fig. 24). The first or innermost layer, and third layers are of moderate density, appearing finely filamentous. The filamentous
texture of the first layer is oriented in a transverse manner around the worm, while the filaments of the third layer are oriented in a longitudinal direction (Figs. 24 and 36). The inner surface of the first layer is irregular in contour where it is associated with the hypodermal cells. It is approximately 300-600 μ thick over most of the length of the worm. The second layer of the cuticle appears as a dense band approximately 35-50 μ thick (Figs. 18, 24, and 36). Fine vertical striations within this layer may be seen in some micrographs. There is no sharp demarcation between this layer and the first and third layers. Deep irregular grooves in the third cuticular layer form gross circular striations in the cuticle. The depth of these grooves varies with the region of the body, being greatest and most irregular in the mid region of the worm. The outer three layers of the cuticle appear as thin dark lines, each 10 μ thick, following the outer contour of the third layer (Fig. 24).

No difference was seen in the structure of the cuticle overlying the somatic musculature from that overlying the hypodermal chords.

The Somatic Musculature

Light Microscopy

The elongate, spindle shape of the somatic muscle
cell can be seen in whole worms cleared in either lactophenol or glycerin. The cell appears flask-shaped when sectioned through the mid region. The enlarged cell body, containing the nucleus, faces the pseudocoelom, while the narrow contractile portion of the cell lies against the interchordal hypodermis (Figs. 5-6).

An irregular region of acidophilia along the sides of the contractile region, or entirely filling the contractile region, was revealed by staining with phloxine-methylene blue, and with hematoxylin and eosin. The cell body remained unstained, except for the basophilic nucleus, located near the base of the contractile region, and an extremely fine, reticular pattern of cytoplasmic basophilia surrounding the nucleus and spreading to the periphery of the cell.

The acidophilic cytoplasm of the contractile region of the cell stained light orange with Mallory's phosphotungstic acid hematoxylin. Careful observation revealed a series of six to 10 longitudinal ridges stained dark blue lying against the inner surface of the cell membrane in this region. The ridges were more clearly seen in some sections in which the orange stain was lacking (Fig. 8). These ridges occur against the cell membrane facing the hypodermis as well as against cell membranes adjacent to other muscle cells.

Glycogen occurs in both the cell body and in the
contractile portion of the cell. In the cell body, the glycogen occupies the area unstained by such stains as hematoxylin and eosin (Fig. 11).

Scattered lipid droplets, extractable with acetone or methanol-ether, are demonstrable within the cell body by treatment with sudan black B. Longitudinal ridges, colored intensely, appear in the contractile region with this method. This coloration may be due to phospholipid since it occurred even after extraction with cold acetone. These ridges are similar in distribution but smaller than those seen in sections stained with Mallory's phosphotungstic acid hematoxylin.

The areas of cytoplasmic basophilia, the nucleus, and the cell membrane stained with the general protein methods, bromphenol blue, and acrolein-Schiff. As well, the acidophilic component of the contractile portion of the cell stained faintly and the longitudinal ridges on the inside of the cell membrane stained intensely.

The longitudinal ridges in the contractile region of the cell which stain with Mallory's phosphotungstic acid hematoxylin, sudan black B, bromphenol blue, and acrolein-Schiff, are probably all identical.

Electron Microscopy

The contractile portions of adjacent muscle cells are separated by a considerable space, varying in width,
which contains a material of moderate density (Figs. 33 and 35-37). It can be seen, where three muscle cells meet (Fig. 36) or where muscle and hypodermal tissues are adjacent, that this intercellular material lines the outer surface of the entire muscle cell. No interface between two adjacent layers of this intercellular material can be distinguished. The thickness of this material decreases considerably over the cell body.

The cell membrane of the lateral surface of the contractile portion of the cell forms irregular vertical grooves which loosely interdigitate with similar grooves of neighboring cells (Figs. 34 and 38). In the contractile region, irregularly spaced areas of dense material form longitudinal ridges along the inner surface of the cell membrane (Figs. 33-37). Frequently these ridges appear to be paired between adjacent cells. Similar ridges may also occur along the cell membrane facing the hypodermal tissue.

Within the contractile region of the cell, the myofilaments are centrally distributed. They are usually separated from the cell membrane by a zone of peripheral cytoplasm (Figs. 32-33).

The cell body of the muscle cell is characterized by an abundance of glycogen (Figs. 31-32). The nucleus is located near the juncture of the cell body and contractile region. Mitochondria, scattered profiles of the granular
endoplasmic reticulum, and free ribosomes are found in the cytoplasm around the nucleus and in the peripheral cytoplasm (Fig. 33). Golgi zones, consisting of aggregates of small vesicles and a few flattened membrane-bound sacs, may be found near the nucleus or in the peripheral cytoplasm adjacent to the pseudocoelom.

Vital Staining

Staining in Isotonic Neutral Red Solutions

A sequence of staining reactions was seen when the progress of neutral red staining of *G. hepatica* was followed using a microscope equipped with a warming stage. Within the first two minutes after the slide was prepared, the ova were deeply and uniformly stained throughout the worm. By five or six minutes, the intestine and uterus showed a diffuse stain, and in a few regions of the worm, a faint pink wash of stain was visible in the hypodermis. Concomitant with the staining of the hypodermis, the diffuse stain of the uterus and intestine became less obvious while small, lightly stained vacuoles appeared. By the time the diffuse staining of the hypodermis reached maximum intensity, the vacuoles of the intestine and uterus were distinct, while the diffuse stain of these organs was barely distinguishable. Although the hypodermis was obviously stained, the
lamellar apparatus and pore chamber of the gland cells were
unstained and appeared light yellow in contrast to the dark
pink of the basal region of the cells. Vacuoles of stain
were seen in the basal portion of hypodermal cells of some
regions of the worms within 10 minutes. The intensity of
the staining of these vacuoles progressively increased while
the diffuse staining decreased. Distinctly stained vacuoles
were seen in the hypodermis of all regions of the worm with-
in 15 minutes of preparing the slide. By 50 minutes after
preparing the slide, the uniformly large, deeply stained
vacuoles had migrated, coming to lie immediately below the
cuticle. At this time it was seen that the vacuoles occurred
only within the non-glandular cells, while no stain was seen
in the lamellar apparatus or pore chamber of the gland cells.
The distribution of the vacuoles was confirmed by examining
the preparation under oil immersion. The vacuoles were
approximately one to two microns in diameter. The stain
remained in these vacuoles in living worms at 37°C for four
to six hours.

The first stain seen in the worms was in the intes-
tine and reproductive tract, rather than in the hypodermis.
This fact suggested that visible stain entered the hypodermis
from within the worm rather than across the cuticle. In
order to test this, worms were incubated in Tyrodes neutral
red at 37°C until the intestine and reproductive tracts were
stained, but were removed before the dye entered the hypodermis. They were washed in Tyrodes, and incubated at 37°C in fresh Tyrodes lacking neutral red. Within one hour, there was only light staining of the intestine and reproductive tract, no diffuse stain in the hypodermis, but distinctly stained vacuoles in the apical region of the non-glandular cells.

Neutral red is clearly concentrated into vacuoles by the non-glandular cells of the hypodermis. The dye is apparently acquired by these cells from the internal region of the worm, rather than from the external medium. It is unknown, however, whether neutral red enters the gland cell at some period.

Neutral red staining was repeated several times in both Tyrodes solution and saline. Although the pattern of staining was identical in all instances, the time required varied. The process was slowest at room temperature and some regions of the worm never did acquire stain. Various rates of staining were seen along the body of the same worm at 37°C, but at this temperature all regions did stain in time. This regional variation along the body was most strikingly seen in the hypodermis, but was also seen in the staining of the intestine and reproductive tracts.
Staining in Osmotically Varied Neutral Red Solutions

Having determined the sequence of staining reactions in isotonic Tyrodes solution, experiments were carried out to see whether the pattern or rate of staining could be altered by changing the osmotic values of the external medium. Worms were stained in Tyrodes diluted 1:1 with distilled water, in twice concentrated Tyrodes solution, and in distilled water.

A pattern of staining identical to that described from isotonic staining solutions was seen in both diluted and concentrated Tyrodes solutions. Vacuoles seemed to appear more rapidly in the diluted Tyrodes. Estimates of the rate of staining in different slides are difficult to compare since the concentration of stain was not precisely controlled. Also, as discussed above, some regions of the body lag behind others in staining. In some experiments, the initial Tyrodes neutral red solution was replaced during observation by diluted Tyrodes neutral red, by drawing a drop of the diluted staining solution under the coverglass. This appeared to speed the rate of staining. The converse did not appear to affect the rate.

Worms placed in a distilled water solution soon died, but in some instances, a staining of the lamellar apparatus and pore chamber of the gland cell was noted. It is doubtful that this staining can be attributed to normal physio-
logical processes.

Staining with Methylene Blue

Vital staining of *G. hepatica* with methylene blue gave final results seemingly similar to neutral red staining. However, the staining proceeded by an obviously different mechanism. Worms stained rapidly in concentrated methylene blue solutions in Tyrodes, saline, or distilled water. The intestine and reproductive tract were lightly stained, while large vacuoles of stain similar to the neutral red vacuoles, as well as a series of smaller, darkly stained droplets, appeared in the hypodermis immediately below the cuticle. The large vacuoles were seen only in the non-glandular cells of the hypodermal chords, while the small droplets were seen in the interchordal hypodermis as well. The small droplets were probably lipid droplets demonstrated earlier with sudan black B and osmium tetroxide. These droplets were visible, but not stained in neutral red preparations.

Staining was not so uniformly achieved in dilute staining solutions. In these solutions, some stain was seen to rapidly penetrate broken ends of the worms, staining all organs of the worm readily; however, over most regions of the worm no staining occurred within two hours, or at the most, a faint staining of the hypodermis only occurred.

It appears that methylene blue, in low concentrations,
does not penetrate the cuticle of the living nematode rapidly. It is doubtful that the intense staining obtained in concentrated solutions can be attributed to physiological processes of the worm.

The Uptake of Colloidal Gold

Large vacuoles containing a dense diffuse material were seen with electron microscopy in tissues of worms taken from a host which had received injections of colloidal gold (Figs. 48-50). Similar vacuoles were never seen in worms from uninjected hosts. It was assumed that the contents of the vacuoles was colloidal gold. Dense material similar to that in the vacuoles was seen in the intestinal lumen. The cells of the intestine contained many vacuoles of varying sizes. Fewer vacuoles were seen in non-glandular cells of the hypodermal chords. The vacuoles did not appear in the gland cells. A few small vacuoles were seen in the proliferating zone of the ovary. No vacuoles were seen in the muscle cells, or in the interchordal hypodermis.

The Effects of Incubation in Altered Physiological Solutions

In order to determine whether the hypodermal gland cells might function in osmotic or ionic regulation, worms were experimentally exposed to physiological solutions of
varying osmotic and ionic concentrations, and the effects on the fine structure of the cells examined. However, it is difficult to evaluate the results of electron microscopy on worms incubated in solutions of varying tonicities. This is largely because of our lack of precise knowledge of factors involved in the fixation of tissues for the electron microscope. Even isotonic solutions cause damage to the fine structure of cells (Pease, 1960).

In both groups of experiments carried out (in vitro incubation in serum, and in vivo perfusion of infected livers with Tyrode solutions), control tissues, which were treated with presumably isotonic solutions, were considerably altered. Most notably, the lamellar apparatus tended to fragment into a mass of vesicles (Fig. 41). In general, the fixation after experimental treatment was poor. Dilutions of experimental media of 6:1 or 12:1 with distilled water resulted in extreme leaching and distortion of cell contours (Figs. 45-47). Thus, the membranes of the basal region of the lamellar apparatus were irregularly separated and broken into vesicles, the apical cell membrane of non-glandular cells was separated in places from the cuticle, mitochondria were extremely swollen, and the ground substance of the cytoplasm was greatly leached. These effects were far beyond a physiological level, as all tissues of the worm showed marked leaching and disruption of organelles.
Less marked alteration of the tissues was noted in worms exposed to dilutions of 25:1 Tyrodes or serum (Fig. 44). No changes were noticeable in either the gland or non-gland cells beyond the vesiculation of the basal region of the lamellar apparatus. The pore chamber material appeared to retain its dense nature.

Some change was apparent in the lamellar apparatus of gland cells from worms perfused in vivo with Tyrodes solution diluted 50:1 with distilled water (Fig. 43). Here, the basal regions of the lamellae were dilated while the remainder of the cell and surrounding non-glandular cells were not noticeably altered. The lamellar apparatus of these cells appeared identical to that in worms fixed in Dalton's osmium-dichromate fixative (Fig. 40). The dilation of the membranes caused by this fixative was not prevented when the fixative was osmotically adjusted by the addition of 1.5% sucrose. It does not seem likely that the dilation of the membranes of the lamellar apparatus seen in tissues fixed with Dalton's fixative, or in tissues exposed to dilutions of Tyrodes of 50:1, can be attributed to an osmotic factor.

Tissues of worms incubated in serum to which sucrose was added to render the medium hypertonic, showed no gross changes, except that the tissue was extremely dense and granular.
Tissues of worms from livers perfused in vivo with Tyrodes solution containing extra potassium chloride appeared similar to control tissues (Fig. 42). As well as containing a higher potassium ion content, this perfusing solution was slightly hypertonic to the normal medium of the worms.
DISCUSSION

Hypodermal Gland Cells

Gabe and Arvy (1961) have discussed at length criteria by which one may determine whether or not a cell is glandular. Glandular activity as defined by these authors is a tripartite process composed of (1) injection, the transfer of materials from the surrounding environment into the cell; (2) synthesis, the formation of the secretion product within the cell; and (3) extrusion, the elimination of the secretion product from the cell. Glandular activity is distinguished from the more general process of secretion by the further qualification that the synthetic phase must involve considerable specificity for the production of a secretion product highly characteristic of the particular cell type. They present four classes of criteria for the determination of glandular activity: morphological, histophysiological, physiological, and biochemical data.

Two of these classes of criteria, morphological and histophysiological data, may be considered in order to ascertain whether the hypodermal "gland cells" of bacillary bands are indeed glandular. The last two criteria; physiological data, collected by means of ligation of ducts, stimulation of nerves, and extirpation of glands; and biochemical data, the chemical analysis, artificial synthesis, and substitu-
tion of secretion products, cannot be applied to the present study.

Both electron microscopy and histochemistry have been used to characterize the morphology of the gland cell of *Capillaria hepatica*. No secretion product can be identified by either method. The material of the pore chamber appears to contain protein, but this cannot be related to an extracorporeal accumulation of any similar material. No precursor material can be clearly identified within the cell, as giving rise to the material of the pore chamber. The arrays of small vesicles associated with the base of the lamellar apparatus might be interpreted either as containing material destined for extrusion from the cell, or for incorporation into the cell. The significance of these vesicles shall be discussed more fully later. The presence of a highly developed granular endoplasmic reticulum is a constant feature of cells actively synthesizing proteinaceous secretion products (Palade and Siekevitz, 1956; Fawcett, 1959). The endoplasmic reticulum is represented in the hypodermal gland cell by only short scattered profiles of granular membranes. A complex golgi apparatus associated with a formed secretion product is also characteristic of many gland cells (Palay, 1958). Further, the polarity of secretion of the cell is frequently reflected in the position of the golgi, the golgi being situated towards the side of the cell from
which the secretion product is extruded (Gabe and Arvy, 1961). However, in the hypodermal gland cell of *C. hepatica*, the golgi system is very small, and is usually located towards the basal side of the nucleus, indicating little polarity. The association of the gland cell of *C. hepatica* with the conspicuous pore through the cuticle overlying the cell, remains the only morphological evidence which might suggest that the cell is glandular.

Histophysiological criteria may include the observation of an innate cycle of secretion in the unaltered animal, or cellular changes induced through either physiological alteration or artificial alteration of the organism. No evidence has been found for a rhythmic alteration of any of the organelles of the hypodermal gland cells. Physiological alteration of the entire nematode *in vivo* is difficult to achieve as it must require a more complete knowledge of the host-parasite relationship than is available at present. The attempts to artificially alter the environment of the nematode through the *in vivo* perfusion of infected livers with altered physiological salt solutions, or the *in vitro* incubation of nematodes in diluted mouse serum, offer no evidence for glandular activity in the gland cells.

The significance of the arrays of vesicles associated with the membranes of the lamellar apparatus and of the juxtanuclear aggregates of smaller vesicles of the
golgi system are important in considering the possible function of the lamellar apparatus of these cells. The vesicles of the lamellar apparatus contain a material of similar density to the material of the interlamellar folds. The fact that they frequently occur in linear arrays off the tips of the infoldings suggests a similarity to micropinocytotic vesicles formed at the base of microvilli in cells of the vertebrate nephron (Novikoff, 1961), liver (Trump, 1961), and intestine (Hampton, 1960). Bennett (1956) suggested a mechanism of membrane flow to account for the incorporation of materials into cells by the formation of vesicles. Trump (1961) has shown the uptake of colloidal materials into the micropinocytotic vesicles of cells of the proximal convoluted tubule of the rat kidney. The resolution obtained to date in micrographs of gland cells from worms which had taken up colloidal gold is not sufficient to determine whether small amounts of this material are present in the vesicles associated with the lamellar apparatus.

Goldfischer et al. (1961) have suggested the possibility that small vesicles may function to transport materials either into or out of cells (that is, endocytosis or exocytosis) through a process of membrane flow involving the membranes of the golgi system and endoplasmic reticulum. The phenomenon of exocytosis could be active in the gland
cell of *C. hepatica*, involving the vesicles associated with the lamellar apparatus and those of the golgi system. Of interest in this respect is the extreme lability of the membranes of the lamellar apparatus as seen in the readiness with which the basal regions of these membranes fragment to form vesicles, either in poorly fixed untreated tissues, or in tissues exposed to various physiological solutions. In some micrographs of these tissues, the entire supranuclear region is filled with a mass of vesicles, giving a superficial appearance of an extensive golgi system. Possibly the golgi system may act as a region of concentration (Novikoff, 1961) giving rise to small vesicles which increase in size as they migrate toward the lamellar apparatus. Fusion of these vesicles with the membranes of the lamellar apparatus would release their contents into the extracellular medium. Addition of this membrane material to the basal region of the lamellar apparatus must require, simultaneously, a dissolution of the membranes at some point, since there does not appear to be an increase in the extent of the lamellar apparatus either in rhythmic patterns within the worm, or with increasing age of the worm. Flow of the membranes from the basal to the apical region would facilitate the movement of materials into the pore chamber. Dissolution of the membrane might take place from the apical region of the lamellae. The lamellae in
this area are more stable, however, as witnessed by the fact that they are unaffected when the basal regions become vesiculated. This may be evidence against the flowing of the membranes of the lamellar apparatus. These speculations are, at best, tentative as experimental analyses capable of testing them have not yet been devised.

The localization of acid phosphatase activity in the gland cell may be related to transport phenomena associated with the lamellar apparatus. However, two interpretations may be drawn from the localization of the enzyme reaction product visualized with the light microscope: either the localization may be false due to non-specific adsorption of reaction products onto membranes, or it may represent the actual enzyme localization indicating something of the physiological capabilities of the lamellar apparatus. In unfixed tissues incubated for long periods (1 to 3 hours) in osmotically unadjusted solutions, the reaction product, lead sulphide, deposited in the pore chamber; however, such deposition did not occur in tissues incubated for shorter periods (30 minutes to 1 hour) in osmotically supplemented solutions. Lead sulphide was deposited in the boundary layer and/or cap material of some cells in the latter tissue. Considering the nature of these structures, it is likely that the deposition seen here represents non-specific adsorption rather than enzymatic activity. A
similar interpretation could be placed upon the deposition of lead sulphide in the membranes of the lamellar apparatus, since one would expect to find alkaline phosphatase rather than acid phosphatase associated with these membranes. The observations of Essner et al. (1958), Molbert et al. (1960), Novikoff (1960), Clark (1961) indicate that alkaline phosphatase enzymes are localized in cell membranes, while those of Novikoff (1960), Essner and Novikoff (1961), and Holt and Hicks (1961) indicate that acid phosphatase enzymes are associated with the hydrolytic enzyme-containing bodies, lysosomes. Acid phosphatase is also frequently associated with the golgi regions of cells (Novikoff, 1961). It was impossible to determine whether acid phosphatase activity occurred within the golgi apparatus of the hypodermal gland cell as this organelle is small, and any reaction product present here could not be clearly distinguished from that of the lamellar apparatus. If, as previously suggested, the golgi system of gland cells does form vesicles which migrate and fuse with the lamellar apparatus, one might expect the vesicles to carry with them some acid phosphatase activity. This would be true especially if the enzyme molecules formed an integral part of the vesicle membrane. The identity of the acid phosphatase molecule with molecules of structural proteins is implied in the chromatographic separation by Barka (1961) of both PAS positive materials
and acid phosphatase enzymes from liver lysosomes. [See also the isolation of both phospholipids and acid phosphatase activity in golgi fractions of homogenates of epididymis by Kuff and Dalton (1959).] This speculative explanation for the localization of acid phosphatase is most attractive as it may indicate that the cell transports materials across the membranes of the lamellar apparatus by the mechanism of exocytosis.

The elaborate infoldings of the cell membranes which form the lamellar apparatus of hypodermal gland cells resemble the infoldings of the basal membranes of cells which function in water and ion transport. Extensive infoldings of cell membranes between which mitochondria are oriented are characteristic of the cells of the distal convoluted tubule of the vertebrate nephron, secretory duct cells and serous cells of the submaxillary salivary gland (Pease, 1956), and cells of the hind gut and Malpighian tubules of insects (Noirot et Noirot-Timothée, 1960; Smith and Littau, 1960). Mitochondria are not so uniformly associated with the infoldings of the basal cell membranes of the cells of the ependymal epithelium of the choroid plexus, epithelial cells of the ciliary body of the eye (Pease, 1956), or the cells of the labyrinth of the green gland of Gambarus sp. (Anderson and Beams, 1956). The cell membranes of cells presumably functioning in ion secretion are similarly modi-
fied; the infoldings of the cell membranes are continuous from base to apex in the chloride cells of the seagull and petrel (Doyle, 1960), and the elaboration takes the form of anastamosing and rebranching tubules within the chloride cells of the guppy (Straus and Doyle, 1961). [See Holliday (1962) for the current status of chloride secretion by the chloride cells of fish gills.] The similarity of the membranes of the lamellar apparatus of hypodermal gland cells to the elaboration of the membranes in the above cell types provides strong circumstantial evidence that the hypodermal gland cells may function in either ionic or osmotic regulation. Although the experimental alteration of the osmotic and ionic content of the external medium of the worms did not reveal any physiologically significant changes in the morphology of the gland cells, this may reflect only the lack of refinement in experimental approach. Similar inability to alter the tubules of the chloride cells of guppies by keeping the fish in either fresh or salt water was reported by Straus and Doyle (1961).

Indirect evidence which may indicate that the gland cells function in osmoregulation or ion regulation can be drawn from the uptake of neutral red and colloidal gold into vacuoles within non-glandular cells, but not in gland cells. These materials may have been taken up by the gland cells and eliminated at such a rate that a concentration
sufficient to be visualized never accumulated.

Evidence that nematodes carry out osmoregulation has been given by Panikkar and Sproston (1941), Stephenson (1942), and Weinstein (1952). [See Weinstein (1960) for a more detailed account of early studies.] Their claims have not been supported, however, by measurements of the osmotic pressure of the body fluids because of the small size of the nematodes. The results of Hobson, Stephenson, and Eden (1952) indicate that *Ascaris lumbricoides* may both osmotically and ionically regulate. The possibility that nematodes carry out ionic regulation has not been extensively investigated, although it is thought likely by von Brand (1961). Some support for the suggestion that the hypodermal gland cells of trichuroid nematodes perform one or both of these functions may be found in the fact that the species having bacillary bands lack the typical nematode excretory system (considered by some to be osmoregulatory in function). However, *Trichinella spiralis*, a trichuroid which also lacks an excretory system, does not have bacillary bands, or even isolated gland cells.

In summary, the function of the hypodermal gland cells of the bacillary bands cannot be stated with certainty, although the morphology of the lamellar apparatus is consistent with the suggestion that the cells function in either osmotic or ionic regulation.
Some comments must be made on the system of terminology used to describe the gland cells. Prior to 1961, no descriptions of the gland cells were sufficiently detailed to involve a specialized terminology. However, Wright (1961) gave an introductory description of the gland cells of the bacillary band of *G. hepatica* in which the terms "neck region" and "region of striations" were used to describe the pore chamber and lamellar apparatus, respectively. In view of the electron microscopy presented here, the term "neck region" for an extracellular area is clearly misleading, while the term lamellar apparatus describes the structure of the "region of striations" more accurately. The terms pore chamber, boundary layer, and cap material, proposed here, may be replaced in the future as the nature of these materials is more thoroughly understood. At present, separate terms for these regions seem justified, although, as suggested in the description of electron microscopy, the boundary layer may be no more than the outer condensed margin of the pore chamber material; however, the differential staining of the pore chamber material, the boundary layer and cap material complex with the tetrachrome method may indicate a greater difference in composition. There appears to be no similarity in electron micrographs between the structure of the material of the pore chamber and that constituting the cap material.
Non-Glandular Cells

The non-glandular cells of the hypodermis form a complete cellular layer around the body of *G. hepatica*. The multicellular structure of the lateral and ventral hypodermal chords can be clearly seen in electron micrographs, but the structure of the interchordal hypodermis and dorsal hypodermal chord is more obscure. Cell membranes are seen in many electron micrographs subdividing the interchordal hypodermis at various points across the interchordal region; however, nuclei have not been seen in this tissue either with the light or electron microscope. In some instances, no cell membranes can be seen in this area; the cytoplasm of the dorsal chord tissue and of the outermost non-glandular cells of the multicellular chords appear to be continuous with the interchordal cytoplasm. Nuclei have not been seen in the tissue of the dorsal chord. [Chitwood and Chitwood (1950) have also noted the absence of nuclei from the dorsal chord in the mid region of the body of some larval aphaeidian nematodes.] These observations suggest that the interchordal hypodermis may be formed by flat irregular processes of the non-glandular cells of the lateral multicellular chords which extend across the interchordal regions and are reflected internally at the dorsal line to form the dorsal chord. Organelles are more sparsely distributed in the processes of the
interchordal region than in non-glandular tissue of hypoderma chords.

Both light and electron microscopy reveal that the non-glandular cells of the multicellular chords are one of the chief sites of glycogen storage in the nematode body. This confirms earlier histochemical studies (von Kemnitz, 1912; Lee, 1960). The fact that large quantities of glycogen are concentrated in the basal three-quarters of the cells probably indicates a polarization of the metabolic activities of the cell. Although a few mitochondria may be found in the lateral and basal cytoplasm, there are many more mitochondria in the apical cytoplasm. Similarly, profiles of the endoplasmic reticulum and free ribosomes occur in greater concentrations in the apical cytoplasm. From the distribution of mitochondria, one would expect the apical region of the cytoplasm to have a high oxidative capacity and to be rich in chemical energy. Studies of the vertebrate hepatic cell show that during times of more rapid glycogen synthesis there is a relative increase in the amount of agranular endoplasmic reticulum which associates specifically with the glycogen (Fawcett, 1955; Porter and Bruni, 1960). Karrer (1960a,b) has attempted, without success, to implicate the golgi apparatus in glycogen synthesis or storage. No biochemical experiments have been done to give direct evidence for the functioning of any organelle
in the synthesis of glycogen. As there appears to be no specific association between any organelle of the non-glandular hypodermal cells and glycogen, the origin of the glycogen in the body wall cannot be deduced.

The presence of glycogen stores does not appear to impede the passage of colloidal materials and vital dyes into the cells, or the transport of these materials through the cytoplasm of the cells. It is possible that the Golgi system may be involved in this process. Golgi zones may be found in the cytoplasm of either the basal or apical regions of the cells. Occasionally a single, larger vesicle is associated with the small vesicles of the Golgi zone. These large vesicles may be the origin of colloidal gold and neutral red vacuoles. Further studies following the uptake of colloidal gold by the worm will be fruitful in answering these questions.

The cell membrane of the non-glandular cells is intimately associated with the cuticle. However, there is no evidence of pore canals or protrusions of hypodermal cells passing into the cuticle. The question of whether the cuticle is a secretion product of the hypodermal cells, or is formed by a condensation of the hypodermal cell protoplasm (Chitwood and Chitwood, 1950) cannot be studied in adult worms. There is no evidence of a specific association of organelles with the cuticle which might indicate a continual
replacement or renewal of the cuticle; however, as discussed above, the apical cytoplasm of the non-glandular hypodermal cells is probably an energy rich, highly oxidative region. Whether this metabolic polarization is solely a result of glycogen deposition, is associated with the maintenance of the cuticle, or is important in the transport of small molecules through the cuticle cannot be adjudged.

The marked differences in organelle distribution between the tissue of the interchordal hypodermis and the non-glandular cells of the hypodermal chords probably reflect the physiological activities of these regions. As the gland cells of the chords do not associate with the cuticle, it is only the non-glandular tissue that need be considered when appraising the physiological activities that may take place across the cuticle (possibly gas and water exchange). It may be suggested that in C. hepatica such activities are largely restricted to the regions of the hypodermal chords. In species having more narrow chords, one might expect a thicker interchordal hypodermis containing a greater concentration of organelles and, therefore, capable of greater physiological activity. It is of interest to note that in Trichuris myocastoris, a species having much narrower hypodermal chords, nuclei could be seen with the light microscope in the interchordal hypodermis.
The Cuticle

It is difficult to relate the six layers of the cuticle of *G. hepatica* as revealed by electron microscopy to layers described by other workers from light microscopy of other nematodes. The outer three layers of the cuticle of *G. hepatica* are osmiophilic, but are not as heavily stained with osmium as one would expect if lipid were the major component, as suggested by Bird and Deutsch (1957) for the outer layer of the cuticle of *Ascaris lumbricoides*. The first and third layers of the cuticle of *G. hepatica* are chemically similar, since they stain with the same dyes (fast green, aniline blue, Mallory's phosphotungstic acid hematoxylin), but are not identical, the first layer exhibiting a greater intensity of stain. The staining reactions of the first layer indicate that it is composed of a collagen-like protein, while the third layer may contain a protein more closely related to matracin, the elastin-like protein characteristic of *Ascaris* cuticle. Fiber layers are absent from the cuticle of *G. hepatica* and appear to be absent from the other species of the genus *Capillaria* which were studied, and from *Trichuris myocastoris*. As the fiber layers of the cuticle of *Ascaris* and related species contain a collagen-like protein, ascarocollagen, it is possible that the first layer of the cuticle of *G. hepatica* may be homologous to the fiber layers of these nematodes,
but that in *G. hepatica* and related species, condensation of this collagen-like protein to form fibers does not occur. The second layer of the cuticle of *G. hepatica* does not appear as a discrete layer, but rather, it resembles a condensation of material between the first and third layers. However, the results of fixation and chromation of the cuticle with Elftman's and Flemming's fixatives indicate that this second layer may contain phospholipid.

The Somatic Musculature

The results of light and electron microscopy, from this study, cast doubts upon the identity of both the myofibers and structural fibrils identified in muscle cells of other species. The longitudinal contractile myofibers of nematode muscles are classically described as long, ribbon-like structures. The fiber is usually diagrammed with its width oriented at right angles to the cell membrane. Such discrete fibers cannot be seen with light microscopy in *G. hepatica*. Some stains (Mallory's phosphotungstic acid hematoxylin, sudan black B, acrolein-Schiff, bromphenol blue) demonstrate longitudinal ridges of irregular contour lying against the cell membrane in the contractile region. The stains indicate that the ridges contain both lipid (probably phospholipid) and protein. In electron micrographs, dense thickenings are seen along the inner surface
of the cell membrane of this region, which are probably the ridges described from light microscopy. The ridges may function as supporting structures, possibly as semi-rigid rods, which might tend to return the muscle cell to its extended length after contraction. In electron micrographs, these ridges are clearly distinguishable from the much finer myofilaments, which occur centrally within the cytoplasm of the contractile region.

The electron micrographs of Hinz (1959) illustrate a much more complex system of supportive fibrils in the muscle cells of *Parascaris equorum*. In this species, infoldings of the cell membrane occur along the contractile region, giving rise to a system of tortuous tubules projecting into the cell at right angles to the outer cell membrane. Each system of tubules appears to surround a longitudinal, dense fiber, which is approximately circular in cross section. This complex of infolded cell membrane, tubules, and fiber are interpreted by Hinz as a supportive fibril. Myofilaments are located between these fibrils and within the central cytoplasm of the contractile region of the cell. Hinz suggests that the classical myofibers of *Parascaris* muscle are groups of myofilaments separated by supportive fibrils which are identical with the fibrils first described by Apathy (1894).

In view of the distribution of myofilaments in
*Parascaris* and *G. hepatica*, and of the staining reactions of the longitudinal ridges of the contractile region of the muscle cell of *G. hepatica*, it seems more likely that the myofiber of earlier studies is, in fact, a visualization of the supportive elements associated with the cell membrane, rather than groups of myofilaments.

The muscle cells of *G. hepatica* are coated externally by a layer of moderately dense, diffuse material which is thicker between contractile regions of cells than over the cell bodies. It is possible that this material is responsible for the adherence of muscle cells to each other, and to the hypodermis, which must occur for efficient muscle contraction. The irregular, radially oriented grooves formed on the sides of the contractile region of the muscle cell of *G. hepatica* may function similarly by loosely interlocking with those of neighboring muscle cells.
SUMMARY

The present study which has combined both light microscope histochemistry and electron microscopy, confirms many observations of light microscopists on the body wall of nematodes, questions some interpretations, and allows some speculation on the physiological capabilities of the body wall through knowledge of cell structure and organelle distribution in the tissues.

The hypodermis of *Capillaria hepatica* forms a complete cellular layer around the body. The hypodermis is separable into the following regions: three multicellular hypodermal chords which are lateral and ventral in position, thin interchordal regions, and a dorsal hypodermal chord.

Two cell types occur within the multicellular hypodermal chords: the "gland" cell (that is, the lateral hypodermal gland cell referred to by Chitwood and Chitwood, 1950), and the non-glandular cell. No evidence has been found indicating that the "gland" cells are, indeed, glandular. However, until further details on the functioning of these cells can be obtained, no purpose would be served by proposing a new name. The structure of the gland cells suggests that they may function in water or ion balance. The non-glandular cells are rich in glycogen, the predominant

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storage product of the body wall, and exhibit a distribution of organelles which probably indicates a polarization of the metabolic activities.

The interchordal hypodermis is an extremely thin layer, probably formed by flat processes of the non-glandular cells of the multicellular hypodermal chords. No glycogen is found here, and organelles are sparsely distributed.

The structure of the dorsal hypodermal chord is difficult to discern. It may be formed by the internal reflection at the dorsal line of terminal enlargements of the processes constituting the interchordal hypodermis.

Externally, the hypodermal tissue is in intimate contact with the cuticle. The line of contact between hypodermal tissue and cuticle is irregular, but no specific protusions from the hypodermis penetrate the cuticle.

Internally, the hypodermis is exposed to the pseudocoelom in the regions of the hypodermal chords, and is adjacent to the cells of the somatic musculature in the interchordal regions. No specialized processes serve to attach the interchordal hypodermis to the muscle cells. It is suggested that the extracellular material surrounding muscle cells functions in cell adhesion. A thin basement membrane lines the inner surface of the hypodermal chords and the somatic musculature.

The cuticle consists of six layers. In contrast to
the structure previously reported for many nematodes, the cuticle of *Capillaria hepatica* lacks fiber layers.

The structure of the muscle cell is described in detail and the identity of the myofibers of classical descriptions is questioned. Light and electron microscope observations indicate that these myofibers may be a visualization of a system of apparently supportive elements, while the actual myofilaments, of much smaller dimensions, are located more centrally within the contractile region of the cell.
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Note: All illustrations are of the species *Capillaria hepatica*.

Figure 1. Summary diagram of the structure of the gland and non-glandular cells of the multicellular chords of *Capillaria hepatica*. (page 29)

Figure 2. Cross section through the entire body of an adult female worm. The two dorsal bands of somatic musculature of the body wall are seen at the top of the section to either side of the dorsal hypodermal chord (arrow), while the ventral musculature is seen below, to either side of the multicellular ventral hypodermal chord. The broad multicellular, lateral hypodermal chords occur between the areas of dorsal and ventral musculature. The uterus, containing three eggs, occupies most of the central area of this cross section. The intestine is situated to the lower right of the uterus.

Bouin's fixative, tetrachrome stain, X 1,000.
Figure 3. Cross section of a lateral, multicellular hypodermal chord. Several gland cells may be seen below pores in the cuticle (arrows). The pore chamber material is stained darkly with chromotrope in this section and cannot be clearly separated from the lamellar apparatus which appears beneath it as an area of striations also stained with chromotrope.

   Bouin's fixative, tetrachrome stain, X 1,200.

Figure 4. Longitudinal section through the body wall, showing gland cells in the lateral hypodermal chord. The uterus, containing eggs is seen across the bottom of this photomicrograph.

   Bouin's fixative, tetrachrome stain, X 1,300.
Figure 5. Cross section through the dorsal somatic musculature. The narrow, contractile region of the cell lying against the interchordal hypodermis is darkly stained with both chromotrope and fast green, while the cell body, facing the pseudocoelom is not stained. The dorsal hypodermal chord (arrow) stains more darkly than do the cell bodies of the muscle cells.

Bouin’s fixative, tetrachrome stain, X 1,500.

Figure 6. Cross section through the ventral musculature and ventral hypodermal chord. A gland cell within the ventral chord, but below the level of focus, can be seen at the arrow.

Bouin’s fixative, tetrachrome stain, X 1,500.
Figure 7. Cross section showing the two layers of the cuticle as seen in the light microscope. A lateral hypodermal chord is seen to the left, while the dorsal musculature is seen above in this cross section.

Helly's fixative, Mallory's phosphotungstic acid hematoxylin, X 1,300.

Figure 8. Darkly stained ridges can be seen along the inner surface of the cell membrane in the contractile region of the muscle cell. The section is cut slightly obliquely to the direction of the ridges. Two layers can be seen in the cuticle overlying the somatic musculature.

Helly's fixative, Mallory's phosphotungstic acid hematoxylin, X 1,500.
Figure 9. Cross section stained with Toluidine Blue O to demonstrate cytoplasmic basophilia; photographed using a red filter. Note the faint basophilia of the hypodermal cells, especially in comparison to the basophilia of the uterus and intestine.

Helly's fixative, Toluidine Blue O, X 1,000.

Figure 10. Higher magnification of tissue similar to that shown in figure 9. The basophilia of the hypodermal cells can be seen. The non-stained areas in the apical poles of the cells are pore chambers and lamellar apparatuses of the gland cells.

Helly's fixative, Toluidine Blue O, X 1,300.
Figure 11. Glycogen can be seen in the non-glandular cells of the hypodermal chords, in the cell bodies of the muscle cells, and in the cells of the intestine. The dorsal musculature is located towards the upper right, the ventral musculature towards the lower left.

Rossman's picric acid-alcohol-formalin fixative, PAS reaction, X 1,300.
Figure 12. The presence of phospholipid in the lamellar apparatus of gland cells, and droplets of neutral lipid in both gland and non-glandular cells of the hypodermis is indicated in this frozen section treated with sudan black B.

Calcium formol fixative, sudan black B, X 900.

Figure 13. Tissue similar to that shown in figure 12. Note the absence of lipid from the pore chamber. Nuclei appear as light spheres. The large black granules are contaminating sudan black B deposits.

Calcium formol fixative, sudan black B, X 1,000.
Figure 14. Note the staining with mercury bromphenol blue of the lamellar apparatus of gland cells, and the lack of stain in the pore chamber (arrow). The basal region of both the gland and non-glandular cells of the hypodermis and cell bodies of muscle cells are not stained.

Helly's fixative, mercury bromphenol blue method, X 1,000.
Figure 15. Note the staining of both the lamellar apparatus of the gland cell and the material within the pore chamber (arrow) in this section stained with the acrolein-Schiff method for proteins.

Helly's fixative, acrolein-Schiff method, X 900.
Figure 16. Lead sulphide, the reaction product of the acid phosphatase method, can be seen in the lamellar apparatus of gland cells in the lateral chords and ventral chord.

Calcium formol fixative, acid phosphatase method, X 1,000.

Figure 17. A preparation similar to that shown in figure 16, counterstained with hematoxylin and eosin. Enzyme reaction product can be seen in both the lamellar apparatus and, in some cells, in the cap material; no reaction product occurs in the pore chamber.

Calcium formol fixative, acid phosphatase method, counterstained with hematoxylin and eosin, X 1,100.
Figure 18. The cellular construction of the lateral hypodermal chord may be seen in this low power electron micrograph. Three gland cells can be seen in which the plane of section passes through the cuticular pore above the cell. As well, four other gland cells can be seen. Non-glandular cells surrounding these gland cells contain small dense granules of glycogen. The larger dense bodies with clear centers which occur in both the gland and non-glandular cells are incompletely fixed lipid droplets. Many mitochondria can be seen in the non-glandular cells immediately below the cuticle. Note that neither the boundary layer, nor cap material associated with the gland cell is preserved by this fixative.

Potassium permanganate fixative, X 7,800.
Figure 19. The structure of the gland cell is seen in this section of a lateral hypodermal chord. The cuticle, of which the three inner layers are distinguishable, is seen in the upper left corner, while the pseudocoelom is seen as a moderately dense zone across the lower right corner of the micrograph. The hypodermis is separated from the pseudocoelom by a basement membrane (large arrow). Within the gland cell, small vesicles can be seen associated with the base of the lamellar apparatus. Portions of membranes bearing ribosomes and free ribosomes can be seen throughout the cell. Ribosomes occur also on the outer nuclear membrane. Mitochondria are distributed throughout the cell, while three lipid droplets can be seen in the basal region of the cell. A golgi zone, consisting of an aggregate of small vesicles bounded by endoplasmic reticulum can be seen in the portion of a gland cell in the upper right corner of the micrograph (small arrow). The filamentous texture of the material within the pore chamber can be seen as well as the boundary layer and diffuse, granular cap material.

Glycogen appears as aggregates of moderately dense particles, lighter, and larger than ribosomes. Note the occurrence of glycogen in the non-glandular cells, but not in the gland cells.

Osmium tetroxide fixative, X 10,000.
Figure 20. The relationship between the gland and non-glandular cells of the hypodermis is clearly shown in this micrograph. Glycogen appears as irregular, dense granules. The position of the boundary layer may be noted as a faintly leached line (arrow). Debris of host tissue is seen at the right above.

Potassium permanganate fixative, X 9,400.
Figure 21. The membranes of the lamellar apparatus with mitochondria oriented between them can be seen in this micrograph. Glycogen can be seen in the non-glandular cells.

Potassium permanganate fixative, X 21,200.
Figure 22. The structure of the non-glandular hypodermal cell can be seen in this micrograph. Note the distribution of glycogen in the non-glandular cell, and the concentration of mitochondria in the apical cytoplasm just below the cuticle. Golgi zones can be seen in both the non-glandular cells and in the gland cell on the left of the micrograph.

Osmium tetroxide fixative, X 19,000.
Figure 23. The pore chamber and lamellar apparatus of gland cells, the apical cytoplasm of the non-glandular cells, and the cuticle are seen in this oblique section through a multicellular hypodermal chord. Mitochondria, free ribosomes, and profiles of granular endoplasmic reticulum can be seen. Glycogen can be seen in the non-glandular cells. Oblique sections through terminal bars between non-glandular cells can also be seen (small arrow). A golgi zone can be seen in a non-glandular cell (large arrow).

Osmium tetroxide fixative, X 15,000.
Figure 24. Oblique section through the apical region of a gland cell (lower right), surrounding non-glandular cells, and the cuticle. Profiles of the granular endoplasmic reticulum, free ribosomes, and mitochondria can be seen in the gland cell. The vesicles associated with the basal region of the lamellar apparatus are also shown. A golgi zone is situated above the lipid droplet. Within the non-glandular cells, glycogen, a few profiles of granular endoplasmic reticulum, and mitochondria can be seen. A single large vesicle is associated with the vesicles of the golgi zone in one non-glandular cell. The structure of the terminal bar between adjacent non-glandular cells is also shown (arrows). The outer three dense layers of the cuticle can be distinguished.

Osmium tetroxide fixative, X 27,000.
Figure 25. The form of the membranes of the lamellar apparatus as seen in oblique section, can be seen in this micrograph. Note the abundance of mitochondria in the apical cytoplasm of the non-glandular cells.

Potassium permanganate fixative, X 20,000.

Figure 26. The infolding of the cell membrane to form the membranes of the lamellar apparatus, and the anastomosis and rebranching of these membranes can be seen in this micrograph. Note also the presence of mitochondria between the membranes of the lamellar apparatus.

Potassium permanganate fixative, X 25,800.
Figure 27. The continuity of the cytoplasm of the interchordal hypodermis with that of the outermost cell of the lateral hypodermal chord can be seen in this micrograph. Cell membranes dividing the interchordal cytoplasm can be seen at the arrow. The relationship of both the cuticle and the musculature to the interchordal hypodermis is also shown.

Potassium permanganate fixative, X 19,500.
Figure 28. The two processes forming the dorsal hypodermal chord are seen in this micrograph (small arrows). The basement membrane (large arrow) separates these processes from the pseudocoelom. Muscle cells occur to either side of the dorsal chord.

Osmium tetroxide fixative, X 9,000.

Figure 29. Detail of the dorsal chord shown in figure 28, showing the continuation of the processes of the chord with the interchordal hypodermis. Note the abundance of granular endoplasmic reticulum in the dorsal chord tissue.

Osmium tetroxide fixative, X 18,100.
Figure 30. The cell processes forming the dorsal hypodermal chord are seen in cross section in this micrograph. The chord at this point is low and is partially overlain by the cell bodies of adjacent muscle cells. Note the continuity of the cytoplasm of the chord tissue with that of the interchordal hypodermis. Many mitochondria are present in the cytoplasm of the dorsal chord processes.

Potassium permanganate fixative, X 18,000.
Figure 31. The juncture of the somatic musculature and lateral hypodermal chord is seen in this micrograph. Several gland cells can be seen in oblique section. The myofilaments within the contractile region of the muscle cells are not preserved by this fixative. Glycogen can be seen in the cell bodies of the muscle cells. The majority of the mitochondria of the muscle cell occur between the nucleus and the contractile region.

Potassium permanganate fixative, X 16,800.
Figure 32. Several muscle cells are seen in cross section in this micrograph. The interchordal hypodermis can be seen between the cuticle and the muscle cells. The pseudocoelom (arrows) is located between the thin basement membrane of the body wall and the closely appressed, multilaminar basement membrane of the intestine. A cell of a multicellular hypodermal chord is situated at the top of the micrograph.

Osmium tetroxide fixative, X 14,600.
Figure 33. Cross section through the contractile regions and part of the cell bodies of several muscle cells. Myofilaments can be seen within the central cytoplasm of the contractile region, while dense thickenings can be seen along the cell membranes in this region. Intercellular material can be seen between the contractile regions of the cells. A golgi zone is situated beside the nucleus of one cell.

Osmium tetroxide fixative, X 23,400.
Figure 34. Lateral hypodermal chord, interchordal tissue, muscle, and cuticle are seen in this oblique section. Note the distribution of myofilaments in the muscle cells, the presence of dense thickenings along the inner surface of the muscle cell membranes, and the irregular contours of the cell membrane along the sides of the contractile region of the muscle cells. Pseudocoelom is seen between the musculature and hypodermal chord in the lower left hand corner of the micrograph.

Osmium tetroxide fixative, X 8,600.
Figure 35. The myofilaments and dense thickenings of the cell membrane of muscle cells are seen in this oblique section of the contractile region of four muscle cells. Intercellular material can be seen between the cells. The interchordal hypodermis contains mitochondria (arrow) and lipid droplets.

Osmium tetroxide fixative, X 31,400.
Figure 36. Cross section through the contractile region of muscle cells showing the central distribution of myofilaments, dense thickenings of the cell membrane, and intercellular material. Note the space between layers of intercellular material at the arrows.

Osmium tetroxide fixative, X 25,800.

Figure 37. Higher magnification of tissue similar to that shown in figure 36, showing the dense thickenings along the cell membranes adjacent to interchordal hypodermis as well as along the sides of the contractile region.

Osmium tetroxide fixative, X 40,600.
Figure 38. Dense thickenings on the inner surface of the cell membrane, myofilaments, and mitochondria are seen in this oblique section through the contractile regions of muscle cells. Note the presence of intercellular material and grooves along the sides of the cell formed by the convolutions of the cell membrane.

Osmium tetroxide fixative, X 29,000.

Figure 39. Two golgi zones are shown in the basal region of the cell bodies of muscle cells. Mitochondria and free ribosomes can also be seen. The pseudocoelom is situated in the right hand corner of the micrograph.

Osmium tetroxide fixative, X 25,600.
Figure 40. The dilation of the membranes of the basal region of the lamellar apparatus caused by fixation in Dalton's osmium-dichromate fixative, can be seen in this micrograph. Note the lesser density of the material between the dilated membranes compared to the density of the material within the pore chamber.

Dalton's osmium-dichromate fixative, X 22,600.
Figure 41. The vesiculation of the basal regions of the membranes of the lamellar apparatus of gland cells can be seen in this micrograph from control worms taken from a liver perfused in vivo with undiluted Tyrodes solution.

Osmium tetroxide in Tyrodes buffer, X 17,900.

Figure 42. Vesiculation of the basal region of the lamellar apparatus of the gland cell, similar to that produced in control tissues, is seen in this tissue from a worm perfused in vivo with Tyrodes solution supplemented with extra potassium chloride.

Osmium tetroxide in Tyrodes KCl buffer, X 29,200.
Figure 43. The dilation of the basal region of the membranes of the lamellar apparatus of the gland cell is seen in this micrograph of tissue from a worm perfused in vivo with Tyrodes solution diluted 50:1 with distilled water. Note the similarity to the dilation of these membranes caused by fixation in Dalton's osmium-dichromate fixative.

Osmium tetroxide in 50:1
diluted Tyrodes buffer, X 17,900.

Figure 44. This gland cell, from a worm perfused in vivo with Tyrodes solution diluted 25:1 with distilled water, does not appear to have been greatly altered.

Osmium tetroxide in 25:1
diluted Tyrodes buffer, X 17,300.
Figure 45. Extreme leaching of the cytoplasm, swelling of mitochondria, and disruption of cell membranes can be seen in this micrograph from a worm incubated in vitro in serum diluted 12:1 with distilled water. Note the separation of the apical cell membrane of the non-glandular cells from the cuticle.

Osmium tetroxide fixative, X 11,000.

Figure 46. The leaching and distortion of cell membranes is clearly seen in this micrograph of tissue comparable to that shown in figure 45. The structure of the boundary layer of the pore chamber is emphasized.

Osmium tetroxide fixative, X 21,900.
Figure 47. The extreme leaching of the cytoplasm and distortion of cell membranes resulting from incubating worms in serum diluted 6:1 with distilled water can be seen in this micrograph. Note the vesiculation, as well as the irregular dilation, of the membranes of the lamellar apparatus.

Osmium tetroxide fixative, X 17,900.

Figure 48. Two colloidal gold vacuoles (arrows) can be seen in non-glandular cells of the hypodermis in this micrograph of worms from a host previously injected with colloidal gold.

Osmium tetroxide-collidine fixative, X 19,600.
Figure 49. Many small vacuoles containing colloidal gold can be seen in the non-glandular hypodermal cells in this micrograph. Two larger vacuoles may be seen in the portion of intestine in the lower left corner of this micrograph.

Osmium tetroxide-collidine fixative, X 22,300.

Figure 50. Many complex vacuoles containing colloidal gold may be seen in the intestinal cells in this micrograph. Colloidal gold is also located within the lumen of the intestine (arrow), in a small vacuole in the hypodermal tissue in the upper left corner, and within the epithelial cell lining the ovary in the lower right corner of the micrograph.

Osmium tetroxide-collidine fixative, X 10,700.