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STUDIES ON THE MORPHOLOGY,
FUNCTION, AND PHYLOGENETIC IMPLICATIONS
OF THE ACANTHOCEPHALAN ABSORPTIVE SURFACE

by

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INTRODUCTION

"Problems concerned with the origin and the relationships of the Acanthocephala have received but little attention at the hands of investigators. The usual avenues for directing inquiries into phylogeny yield but scanty convincing evidence of direct relationship between the Acanthocephala and other animal groups. The reasons for this condition are found in the specialization which has accompanied perfect adaptation and complete organic adjustment to the parasitic existence. Many other parasitic organisms, through either their anatomy or their ontogeny reveal indisputable evidences of relationship with free-living forms which occupy a natural position in the scheme of classification. ...coordination of conspicuously specialized with unmodified forms becomes possible down through not only the class and the order but frequently to the category of family or even of genus. Such possibilities are wholly lacking for the Acanthocephala, for the entire group stands apart in a condition of isolation that has baffled most investigators who have given thought to the problem. Agreement is even lacking as to the phylum of the animal kingdom to which this group shows closest relationship."

H. J. Van Cleave (1941)

Some 29 years have passed since Van Cleave's review of the relationships of the Acanthocephala and as of this date no indisputable evidence linking these organisms to another animal group has been presented. To place the acanthocephalans in a taxonomically tenantable position, both Van Cleave (1948) and Hyman (1951) raised this group to the rank of an independent phylum.

Hyman pointed out that only two phyla, the Platyhelminthes and the Aschelminthes, should be considered as possibly related to the Acanthocephala. (Hyman regarded the three classes Turbellaria, Trematoda and Cestoda to comprise the Platyhelminthes and the six classes Rotifera, Gastrotricha, Kinorhyncha, Priapulida, Nematoda, and Nematomorpha, a loose assemblage, to comprise the Aschelminthes.) She reviewed the various evidences point-by-point and arrived at the conclusion that "the
general structure is rather on the aschelminthic side, whereas the embryology presents more points of resemblance with the platyhelminths."

Although evidence has been presented to link the acanthocephalans to virtually all of the classes of the above two phyla (note Hyman, 1951), most of the arguments have favored either the Cestoda or the Priapulida. Van Cleave (1924, 1941, 1948) became an advocate of the contention by the Russian Kholodkovsky (1897) stating that the Acanthocephala and Cestoda shared common origins and, at the least, should be classified in adjacent taxa. Van Cleave felt that the evidences of relationship were of a degree denoting long separation. Chitwood (1940) and Petrochenko (1952) have also forwarded arguments for affinities of these two groups.

More attention has been focused on the possible relationship of the Acanthocephala and Priapulida. This idea apparently originated with Schepotieff (1908) and Rauther (1909) and laid the groundwork for the grouping of the Acanthocephala and Priapulida with the Rotifera, Gastrotricha, Kinorhyncha, Nematoda, and Nematomorpha by Meyer (1932-33) into a phylum which he termed Aschelminthes. Meyer (1928, 1932-33) was of the opinion that the acanthocephalans, priapulids and kinorhynchs were closely related. Lang (1953, 1963) has perpetuated and elaborated on Meyer's ideas and has suggested dividing the aschelminths into two groups, one group comprising the Rotifera, Gastrotricha, Nematoda and Nematomorpha, the other group comprising the Acanthocephala, Priapulida and Kinorhyncha.

Golvan (1958) in a monographic work has re-evaluated the problem of acanthocephalan phylogenetic relationships. He examined the possibility of affinities with the Turbellaria, Trematoda, Cestoda, Rhynchocoela, Rotifera, Kinorhyncha, Gastrotricha, Priapulida, Nematoda, Nematomorpha, Annelida, Tardigrada and Arthropoda. As a result of this study, Golvan
supported the position of Meyer, considering the acanthocephalans to be an aschelminth group most closely related to the priapulids. He also supported Van Cleave's contention that the acanthocephalans should constitute a phylum as well as suggesting that the other aschelminth groups be elevated to phylum level and all be grouped in the super-phylum Aschelminthes. (Hyman (1959) has also suggested that each of these groups might constitute a phylum.) Of interest, Colvan described and diagrammed a hypothetical Protacanthocephala, a primitive ancestor with features more clearly illustrating the proposed similarities of the Acanthocephala and Priapulida.

Meyer (1932-33) also briefly considered similarities between the rotifers and acanthocephalans. The possibility of this relationship was again advocated by von Haffner (1950) but abruptly dismissed by Hyman (1959). More recently, Remane (1963) has opened this door and suggested that a phyletic affinity between these groups is very probable.

Upon what bases have these investigators founded their opinions? Hyman (1951) lists systematically a number of acanthocephalan characters which have been used including the general structural plan (pseudocoelomate), the division of the body into presoma and trunk, an armed retractable proboscis, superficial segmentation, the presence of a "cuticle", a synctial nucleated epidermis containing spaces (lacunae) and appearing striated, body wall musculature of circular and longitudinal layers, origin of the pseudocoel, division of the pseudocoel by partitions and "mesenteries", the appearance of the reproductive system, the close relationship of the nephridial and reproductive systems, eutely, embryology and various other features. Extensive charts comparing the characters of the acanthocephalans with those of other invertebrate groups
are found in Chitwood (1940) and Golvan (1958).

It is not the purpose of this writer to re-evaluate the arguments concerning each of these characters simply because this approach has brought us little closer to an answer than was evident in Van Cleave's introductory paragraph of 29 years ago. As Nicholas and Hynes (1963) have pointed out, elements of each character might be used to support arguments for affinities with either the Platyhelminthes or Aschelminthes. New approaches to the problem are required. One means is a re-investigation of certain features of the acanthocephalans using new tools and techniques. Such a means is provided by the electron microscope.

This instrument has proven invaluable in recent years in resolving many taxonomic problems in the Prokaryota and, perhaps to a greater extent, in the Protozoa. A careful examination of selected features of the acanthocephalans, platyhelminths and aschelminths with the electron microscope should allow a re-evaluation of the taxonomic worth and significance of many morphological features. Such a literature has begun to grow; however, these studies are generally concerned only with specific structures in specific organisms examined and rarely have been applied to the problem of phylogenetic affinities.

In the course of the present investigation, one feature of the acanthocephalans--the structure of the trunk wall--has been studied in detail with the electron microscope and the results have been used to consider the problem of phylogenetic relationships. The rat acanthocephalan, Moniliformis dubius, was utilized since this helminth was already being maintained in the laboratory where the research was to be carried out. Such a standardized laboratory infection provided a ready consistent source of material and facilitated the reproduceability of experimental work.
Recently, accounts of the ultrastructure of the surface of several acanthocephalans including *M. dubius* have been published (refer to the literature review). These papers have stimulated the inception of the present work by varying in interpretation, lacking in detail, and creating more questions than providing answers about the structure of the body wall.

This investigation is divided into three parts:

1) The detailed morphology of the trunk wall of *M. dubius* was studied with the electron microscope. Particular attention was paid to the absorptive surface of this helminth and those structural features associated with the extracytoplasmic and intracytoplasmic aspects of the absorptive surface.

2) Certain experimental procedures as detailed in the text were carried out. These experiments contributed information about specific structural and functional properties of the absorptive surface and trunk wall of *M. dubius*.

3) The morphology of the acanthocephalan body wall was compared to that of the equivalent priapulid, cestode and rotifer structures. The priapulid body wall has not been previously investigated; therefore, specimens of *Priapulus bicaudatus* were examined and the fine structure of the priapulid surface was described. Micrographs of the cestode and rotifer integuments were included to facilitate comparisons.
LITERATURE REVIEW

The reader is referred to the extensive review of acanthocephalan biology by Nicholas (1967) as well as to the reviews of body wall structure and function by Lee (1966) and Bird and Bird (1969).

Early observations on body wall structure were made by workers including Baltzé, Hamann, Kaiser, Kilian, Leukart, Saefftigen, Travassos, and Van Cleave. Anton Meyer (1932-33) reviewed these findings while adding the results of his own research. The following summarizes a translation of his interpretations.

Meyer considered the integument to correspond to a single layered epithelium. Features common to the integument of all acanthocephalans are: 1) the syncytial construction 2) the fibrillar structure in many layers 3) a cytoplasmic vascular system constantly delineated but without true walls (lacunar system) and 4) either primary, ameboid giant nuclei in small numbers or amitotically originating nuclear fragments in great numbers. The integument consists of the following layers (parts): 1) a very thin cuticle (Cuticula) 2) a thin cuticular striated zone (Streifenzone) 3) a diversely thick and complex felt fiber layer (Filzfaserschicht) and 4) a diversely thick and simple radial fibril layer (Radianfibrillenschicht). The subcuticular Filzfaserschicht and Radianfibrillenschicht together were termed the hypodermis. Meyer recognized that these "layers" were not sharp and represented cytoplasmic zones.

The cuticle measured up to 1 micron thick in some species but
considerably thinner in others. Two elements were detected in the Streifenzone—small radial pore canals and radially spread elements of the cuticle that could not be clearly separated from the cuticle parallel to the surface. The Filzfaserschicht was believed to be a composite of several layers containing a continuation of the radial fibrils of the deeper zone and circular, longitudinal and oblique tangential fibers. This layer is thin in some species but may constitute up to two-thirds of the hypodermal thickness of other species. The Radiarfibrillenschicht is the basal layer of the integument and contains radial fibrils, the nuclei and the vessels. Meyer cited extensive evidence for contractility of these fibrils and considered this to be an almost proven phenomenon.

There are two types of integument: a) with large ameboid shaped nuclei present in such small numbers that large cytoplasmic areas are free of nuclei (for example, Moniliformis) or b) with numerous small nuclei arising through amitotic fragmentation which are generally equally distributed throughout the integumental cytoplasm. Nuclear morphology and distribution in the acanthocephalans are extremely diverse with many examples being cited by Meyer. He noted that the large primary nuclei are abnormal, lacking much of the detail common to the nuclei of most animal cells.

The vascular system should be regarded as vessels (not lacunae) since they are constant. They are an intracytoplasmic structure of the integument limited to the Radiarfibrillenschicht and quite diverse in form and distribution. The vessels are often bounded by the fibrils of this region with their basal boundary continuous with the inner aspect of the integument. Meyer reported these vessels to contain resorbed nutritive juices.
Meyer also observed that the integument contains Sudan-positive fat droplets of all sizes as well as less easily identified materials.

Since the publication of Meyer's monograph the major contribution by light microscopists to knowledge about the structure and composition of the integument has come from histochemical studies. Information contributed by chemists during this same period are linked to the cytological findings.

Recently, Crompton (1963) and Crompton and Lee (1963) have reported an additional integumental layer external to the cuticle in *Polymorphous minutus* and *Macracanthorhynchus hirudinaceus*. This epicuticle is thin, less than one micron thick, and was detected by alcian blue staining. Nicholas and Mercer (1965) found a similar layer on the surface of *Moniliformis dubius* which was PAS-positive and insensitive to salivary digestion. Wright and Lumsden (1968) further experimented with the epicuticle of *M. dubius*. This layer was stained by the PAS and periodate-silver methenamine reactions, stained metachromatically at pH 2.8 with toluidine blue, and bound alcian blue, Thorotrast, and colloidal iron at pH 2.4 to 2.1. These investigators attributed these staining properties to the presence of neutral polysaccharides and/or glycoproteins in the epicuticle.

The cuticle has been investigated histochemically by Mueller (1929), Monne (1959) and Crompton (1963). Mueller reported sulfur in the cuticle of *Macracanthorhynchus hirudinaceus*. Monne found a fibrin-like protein in the cuticle of *Polymorphus botulus* and *P. boschadis* and suggested stabilization of this cuticle by polyphenolquinone tanning. Using a number of histochemical techniques combined with treatment of whole
worms by various chemical reagents, Crompton (1963) concluded that the cuticle of Polymorphus minutus consists of a lipoprotein containing -SH groups and -SS- linkages and that this lipoprotein is a main structural component of all parts of the body wall. He also questioned the validity of the suggestion of Monne regarding polyphenolquinone tanning.

Little histochemical information has been presented on the structural elements of the other layers of the body wall. Crompton (1963) found the fibers or pores of the Streifenzone to have almost the same staining properties as the cuticle. The fibers of the Filzfaserschicht when stained in a like manner appeared to contain less sulfur. Nicholas (unpublished) has reported successful isolation of these felt-like fibers from M. dubius.

Glycogen has been detected histochemically in the body wall of Macracanthorhynchus hirudinaceus (von Brand, 1939, 1940), Leptorhynchoideas thecatus and Echinorhynchus coregoni (von Brand, 1940), E. coregoni, E. gadi, Pomphorhynchus bulbocollis, Neoechinorhynchus cylindratus and N. emydis (Bullock, 1949b) and Polymorphus minutus (Crompton, 1965). Von Brand and Bullock reported heavy glycogen concentrations in the subcuticular region and in the lacunar canals. Von Brand (1939, 1966) stated that the lacunar canals play a major role in the transport of glycogen about in the body wall. Crompton (1965) commented on the lability of body wall glycogen and suggested that glycogen in the lacunar channels could be an artifact of fixation. He occasionally found deposits in the lacunar canals and was the first to detect glycogen in the Filzfaserschicht. Bullock commented on the apparent variation of detectable body wall glycogen in correspondence to the nutritional state of the worms.

The histochemical detection of glycogen in acanthocephalans has been
substantiated by chemical means. Von Brand (1939, 1940) was the first to isolate this glycogen and noted that the integument of male _M. hirudinaceus_ contained 80% of the total body glycogen. Ward (1952), Read and Rothman (1958) and Dunagan (1964) confirmed Bullock's observation that the levels of stored polysaccharide drop during starvation of acanthocephalans. Read and Rothman also reported a diurnal fluctuation of the glycogen levels in _M. dubius_ in the rat host. Male specimens contain more glycogen per unit weight than female worms according to von Brand (1940) and Graff and Allen (1963), but Rothman and Read (1958) and Laurie (1959) obtained results which conflict with this.

A fraction of isolated _M. hirudinaceus_ polysaccharide was found to be resistant to digestion by filtered saliva (von Brand, 1939) and upon further analysis appeared to be galactogen (von Brand and Saurwein, 1942). A polysaccharide resistant to saliva digestion was detected in the body wall of _P. minutus_ by histochemical means (Crompton, 1965).

The presence and distribution of lipid droplets in the body wall has been studied by von Brand (1939), Bullock (1949b) and Crompton (1963). Von Brand found fat localized in the subcuticular regions of the body wall and noted a lower concentration in the head or presoma and, in the female, in the most posterior regions. Somewhat different results were reported by Bullock who found more lipid in the presoma. Bullock observed large concentrations of fat droplets around the lacunar canals.

Beames and Fisher (1964) have studied the neutral lipids and phospho-lipids of _M. dubius_ and _M. hirudinaceus_ by chemical means. _M. dubius_ contained about four times more lipid than _M. hirudinaceus_ and, in both species, males contained almost twice the total lipid present in females. The higher fat content was predominately glycerides. C\textsubscript{10} through C\textsubscript{20}
fatty acids were detected with C_{18} acids accounting for over 70% of the total acids. Eighty per cent of the total fatty acids were unsaturated. Ethanolamine, serine, choline and inositol phospholipids were found as well as plasmalogens. Cholesterol was identified as 50-80% of the total unsaponifiable lipid present.

A number of enzymes have been localized by histochemical techniques in the integument. Lipase and alkaline phosphatase were detected in the body wall of several acanthocephalans by Bullock (1949a). Bullock (1958) investigated the localization of alkaline phosphatase in 23 species and found activity concentrated in the Streifenzone and inner regions of the Radiarfibrillenschicht; however, certain species including all eight Neochromeinorhynchidae examined had no detectable activity in the integument. The distribution of alkaline phosphatase, non-specific esterase and leucine aminopeptidase in P. minutus was studied by Crompton (1963) and Crompton and Lee (1963). Alkaline phosphatase activity was highest in the Streifenzone, particularly in the region of the body spines, and was also present in the hypodermal layers. The lacunar channels contained the maximum non-specific esterase activity. All body wall layers other than the cuticle and epicuticle showed non-specific esterase activity in the region of the spines while in the other parts of the body wall activity predominated in the Filzfaserschicht. Leucine aminopeptidase activity was found in the Streifenzone and Radiarfibrillenschicht. More recently, Rothman (1968) has localized alkaline and acid phosphatase in the "pore canals" of the Streifenzone at the fine structural level.

Ten oxidoreductase enzymes including alcohol dehydrogenase, glutamate dehydrogenase, glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and succinate dehydrogenase
were localized in particles predominately found in the inner aspects of the Streifenzone and the Radiarfibrillenschicht of *P. minutus* by Crompton (1965). He concluded that the particles were mitochondria when their distribution was compared with earlier morphological findings at the ultrastructural level (Crompton and Lee, 1965). Similar results were obtained by the localization of succinic dehydrogenase and NADH dehydrogenase in the integument of *M. dubius* (Bryant and Nicholas, 1966). Crompton (1965) pointed out that mitochondrial distribution closely parallel glyco- gen distribution.

Considering the unusual nature of the body wall nuclei of acanthocephalans, it is surprising that they have received so little attention. Van Cleave (1914) noted that, at least in the early stages of development, each species has a constant number of nuclei in fixed positions. He concluded that these organisms are eutelic or possess a constant cell number in relation to body size. No mitotic nuclear division occurred after an early point in development. Van Cleave (1928, 1932, 1951) studied the large ameboid primary nuclei of some species and the amitotically occurring nuclear fragments of other species. He discussed eutely as a biological phenomenon (1932). Only Crompton (1963) has investigated the histochemistry of the integumental nuclei. He found two main types of nuclear inclusions—one staining with methyl green (type A) and one staining with pyronin Y (type B). The judicious use of the Feulgen technique for DNA and ribonuclease digestion showed that type A contains RNA and DNA and type B contains only RNA. These inclusions had been termed chromatic bodies or nucleoli by Graybill (1902). Crompton found some of the inclusions to have unstable central regions which he suggested might be vacuoles.
The integument of M. dubius rests on a connective tissue layer which is PAS-positive, diastase stable and stains like collagen with Mallory's triple and Van Giesen's stains (Nicholas, 1967).

The first published reports on the ultrastructure of the adult acanthocephalan body wall were brief notes on M. hirudinaceus (Rothman and Rosario, 1961), M. dubius (Nicholas and Hynes, 1963) and P. minutus (Crompton and Lee, 1963). Since the appearance of these abstracts, more extended descriptions of the integument of M. dubius (Nicholas and Mercer, 1965), P. minutus (Crompton and Lee, 1965), Pomphorhynchus laevis (Stranack et al., 1966) and Acanthocephalus ranae (Hammon, 1967) have appeared. These species represent two of the three orders comprising the Acanthocephala (Hyman, 1951). A comparison of the texts and plates of these four papers show that although the terminology and interpretations of the authors differ greatly, the essential features of the body walls of all four species are quite similar. Because of these differences, each of these four papers will be summarized using the authors' terminology.

Nicholas and Mercer (1965) described the epicuticle (layer I) as a finely fibrous extracellular fringe and suggested renaming it the cuticle. Layer I is separated from the other integumental layers of M. dubius by an 80 A trilamellar membrane. A second more electron-dense "membrane" was reported subjacent to the plasma membrane. The layers (II to V) below this sub-plasma membrane were regarded as cytoplasmic. Layer II contains a dense meshwork interspersed with less dense cytoplasmic pores running perpendicular to the surface and tapering as they approach the body surface. The meshwork constitutes a branching septa which was interpreted as being the striations visible in the light microscope. Membrane bound vesicles often fill the pores of the meshwork. The plasma membrane
and sub-plasma membrane appear to dip down into the pores and these authors suggested that the vesicles could be formed by invagination and pinching off of the plasma membrane. Prominent fibers extend up to this meshwork and were found to be composed of finer elements termed tegumentary fibers. A cytoplasmic layer (layer III) lies between the meshwork of layer II and layer IV. This cytoplasmic layer contains the previously mentioned vesicles, tegumentary fibers, and mitochondria. Mitochondria were found throughout layers III, IV, and V and were somewhat atypical in appearance having only limited numbers of cristae. Lipid droplets, Golgi clusters and other membranous profiles are common in these layers. Particles 150-400 Å were found throughout the cytoplasm with most of these particles being identified as glycogen. The presence of RNA in the integument was detected with fluorescence microscopy but none of the particles could be identified as ribosomes with certainty. Ribonuclease digestion did not eliminate the smaller population of these particles. Nuclear features resemble those of higher animals. Nicholas and Hynes observed a double-contoured membrane at the base of the tegument. This membrane has numerous large vesicles on its cytoplasmic surface which were interpreted as pinching off from or coalescing with the membrane. The tegumentary fibers appear to insert on this membrane at sites of membrane thickenings. Underlying the body wall is a basement membrane and an extracellular space containing fine fibrils showing a periodicity. These fibrils were later shown to be digested by Clostridium histolyticum collagenase at pH 7.6 (Nicholas, 1967). Nicholas and Hynes (1965) found the structure of the integuments of the males, females and immature worms to be quite similar.

Appearing soon after the previous report was a paper on the fine structure of the body wall of P. minutus by Crompton and Lee (1965).
Crompton (1963) had earlier hypothesized that the striped layer "may be considered to have a function of facilitating the absorption of nutrients into the body wall by means of a series of components whose ends are separated and supported by the thin, inert cuticle. These components of the striped layer may be fibres, pores or strands of cytoplasm." Crompton and Lee described both the trunk or metasomal body wall and the presomal body wall. The terminology of Crompton (1963) as derived from Meyer was used.

Five layers are present in the metasomal body wall. The epicuticle is an irregular disposition on top of the cuticle. The outer layer of the cuticle is a trilamellar membrane which is penetrated by many pores opening into the canals of the striped layer. Underlying the membrane is a thin homogeneous region constituting the remainder of the cuticle. The striped layer was interpreted as being the homogenous material surrounding the canals. The canals each extend from a pore in the cuticle to the felt layer and were considered to be the features after which the layer was named. The pores are narrower than the canals and are lined with the membrane of the cuticle. An electron-dense material was found in the canals, and in discussion, the possibility that these canals extend to the lacunar canals was offered. The felt layer contains many fibrous strands arranged in all planes which appear to be hollow or less electron-dense in the center. Lacunar canals were described in this layer and in the radial layer as thin-walled and filled with a homogeneous substance. Vesicles, smooth membrane profiles and glycogen particles were found in the felt and radial layers. No rough endoplasmic reticulum nor free ribosomes were identified by methyl green/pyronin Y staining indicated at the light microscope level the presence of cytoplasmic RNA. The radial
layer contains fewer fibrous strands but more and larger lacunar canals than the felt layer. Mitochondria were detected in the felt and radial layers and reported to be concentrated around the lacunar channels. Many lipid droplets were found in the radial layer of the posterior portions of the trunk wall and the nuclei were found but not described. The plasma membrane of the inner surface of the body wall is greatly infolded with the ends of the folds forming small vesicles containing a material suggested to be lipid. The body wall rests on a basement membrane.

Trunk spines are formed from the body wall, with a cuticle and a thin striped layer having fewer canals, covering a core of compacted fibers which is an extension of the felt layer.

The body wall of the presoma is similar to that of the metasoma with a fibrous partition separating these two regions. The striped layer contains fewer canals and each is filled with a dense material said to be similar to lipid. The radial layer is thinner and tapers gradually until it is absent from the proboscis wall. The membrane of the inner surface is similarly infolded but the dense droplets are absent.

Stranack et al. (1966) found no epicuticle on the surface of P. laevis. The cuticle consists of a trilamellar membrane, a layer of moderately dense material and a narrow band of more dense material pierced by pores of about 150 A diameter. The membrane dips down into the pores and is continuous with tubular channels which are reported to connect to the endoplasmic reticulum deep in the body wall. A homogeneous honeycomb matrix forms a framework around these channels. The felt layer contains an irregular tubular array of smooth membranes termed endoplasmic reticulum with these tubular profiles becoming more scattered in the deeper cytoplasm. The fibrils of the felt layer are arranged in three
layers and are surrounded by profiles of endoplasmic reticulum in the radial layer. Mitochondria are seen in the felt layer but are more numerous in the radial layer. A variety of vesicular and membranous structures as well as the nuclei are evident in the radial layer. Each nucleus is delineated by a nuclear envelope with a 150 A space between the membranes. 400 A nuclear pores are present but not clearly defined. The nuclear ground substance contains nucleoli, smaller electron-dense aggregations occurring in both nucleus and nucleoli, and scattered granules. The lacunar canals are quite large, contain a finely granular matrix and are traversed by fibers and endoplasmic reticulum. The membrane of the inner surface of the body wall is extensively infolded into tubular channels permeating the cytoplasm. The connective tissue layer internal to the body wall contains 100 A fibrils.

The features of the body wall of *A. ranae* essentially agree with those of the three species previously discussed. Hammond (1968) detected a thin homogeneous epicuticle but suggested that this was not an integral structural part of the body wall. The trilamellar plasma membrane continues through pores in the cuticle as the bounding membrane of canals which penetrate into the helminth. The homogeneous layer beneath the plasma membrane was termed the cuticle and is bounded internally by a sub-plasma "membrane." A matrix of dense material lies below this and is penetrated by pores through which the canals pass. There is a space of electron-transparent material between this matrix and the sub-plasma membrane. The matrix generally runs only 1 micron in depth but the canals are much deeper. The region of the matrix is recognized as the Streifenzone of Meyer but the lower region or canal layer is interpreted as the area which appears striated with the light microscope. The canals contain a material
which is continuous with and similar in appearance to the material comprising the epicuticle. The features of the Filzfaserschicht and Radiarfibrillenschicht are similar to those previously described. The lacunar canals, some of which appear to be limited by membranes, contain a granular material as well as dense bodies similar to lipid droplets. Nuclei were observed but not described. A thick electron-dense layer of what might be lipid covers the surface of the presoma and fills the canals. The pores in the cuticle of the presoma are more widely spaced than those of the metasoma. The inner portion of the matrix of the Streifenzone is broken into a series of lamellae. Unique to the presoma is a system of fine fibers running parallel to the body surface between and below these lamellae. The bases of the canals appear to connect with a series of lacunae containing similar lipid-like material. No distinct Filzfaserschicht nor Radiarfibrillenschicht exist. An inner layer of the presomal wall contains a system of conspicuous fibers.

Wright and Lumsden (1968) described the ultrastructure of the epicuticle of *M. dubius*. This layer is composed of filaments of about 25 Å diameter extending more or less perpendicularly from the free surface of both the metasomal and presomal integuments to form a branching mat of about 0.5 micron thickness. A similar epicuticle of considerably greater thickness is present on the cystacanth surface. The filaments comprising the epicuticle appear firmly anchored in the outer electron-opaque leaflet of the surface membrane.

The surfaces of three acanthocephalans, *Acanthocephalus ranae*, *Echinorhynchus truttae* and *Pomphorhynchus laevis* have been examined with the scanning electron microscope (Hammond, 1968b). A thick coating, perhaps the epicuticle, obscured the surface details of *E. truttae* and
P. laevis. Numerous surface pores were evenly distributed over all aspects of the surface of A. ranae. The thin epicuticle did not obscure details. No difference in distribution was detected nor were the pores arranged in any particular pattern. A discrete mass of material apparently similar to that seen in thin sections (Hammond, 1967) was associated with each proboscis pore. This material was removed by storage of the worms in 70% ethanol. A number of pits seen on the proboscis surface were assumed to be sensory structures.

Since the acanthocephalans lack a gut, it is tacitly assumed that all nutrients and excretory products pass across the outer surface of the body wall; however, little is known about the particular membrane processes involved. Indirect evidence has shown that this membrane is permeable to sugars, (certain monosaccharides and the disaccharide, maltose), organic acids and ethanol (Laurie, 1957, 1959; Read and Rothman, 1958; Graff, 1964; Crompton and Ward, 1967a,b).

Recently, Crompton and Lockwood (1968) have explored the mechanism of glucose uptake by in vitro incubations of P. minutas under anaerobic conditions. These authors state that their results are compatible with the assumption that a carrier system is involved in the transport of glucose. The carrier system is half-saturated at an external concentration of about 0.25 mg/ml and approaches saturation when the external concentration reaches 2.0 mg/ml. P. minutas was shown to be capable of absorbing glucose against a concentration gradient.

The permeation of amino acids in M. dubius and M. hirudinaceus in vitro was studied by Rothman and Fisher (1964). Methionine was accumulated by both sexes of both worms against a concentration gradient and the rate of uptake was non-linear with respect to concentration. The uptake of
methionine was inhibited by other amino acids and these inhibitions were reciprocal. The combined effect of four neutral amino acids on the uptake of methionine could be predicted by the equation formulated by Read et al. (1963).

Edmonds (1965) demonstrated the appearance of L-leucine-C\textsubscript{14} in the tissue of M. dubius in vivo when the rat host received the amino acid orally or intraperitoneally. The amount of label recovered from worm tissue in intraperitoneal experiments was low. He also showed that L-leucine-C\textsubscript{14} uptake by M. dubius in vitro is inhibited by DL-valine, DL-serine and DL-methionine.

A similar series of in vivo experiments by Edmonds (1965) demonstrated the appearance in worm tissue of the label from sodium-P\textsuperscript{32} which had been administered orally or intraperitoneally to the rat host. Again, more label was present in the worms after oral administration to the rat.

Bullock (1949a) first suggested the possibility of lipid absorption by acanthocephalans particularly by the wall of the presoma. Pflugfelder (1949) demonstrated that a lipid-soluble dye, Scharlach R. appeared in the body wall of Acanthocephalus ranae after the starved frog host was fed a mixture of pork fat and the dye. It has been assumed that many of the pigments found in acanthocephalans are taken up by the worms and originate from the arthropods which the vertebrate hosts ingest. Such a pigment has been identified as a lipid-soluble carotene by Van Cleave and Rausch (1950).

Crompton and Lee (1965) raised the question of fat absorption after observing the predominance of a lipid-like material in the pore canals of the presoma of P. minutus. These authors argued that fat is absorbed by the presoma, possibly through the lemnisci, into the body cavity and
resorbed by the inner surface of the trunk wall into the trunk wall cytoplasm. Hammond (1967) argued in favor of fat excretion after observing a similar phenomenon on the surface of the presoma of *A. ranae*. Hammond (1968b) tried to substantiate this argument with his scanning microscope observations. Specific accumulations of lipid-like material at each pore of the presoma might indicate excretion. This problem was more carefully considered by Hammond (1968a). Using autoradiographic techniques with the light and electron microscopes, the label from glyceryl tri [oleate-9,10-3H] was found to accumulate in the trunk wall, particularly in the Radiarfibrillenschicht of *A. ranae*. At 60 minutes, no label was evident in the presoma or lemnisci. Much of the label was associated with lipid droplets. Corresponding experiments with the lipid soluble dyes, Scharlach R, Sudan Black and Lipid Crimson suggest that lipids are taken up by the trunk wall and stored in the presoma and lemnisci.

Edmonds and Dixon (1968) reported the uptake of mixture of carbon particles and thorium dioxide in vitro and carbon particles in vivo; however, an examination of the published figures does not confirm the reported pinocytotic activity. The tracer molecules are located in the "pore canals" and, for all purposes, are still on the external side of the surface membrane. The presence of the electron-opaque particles in the lumen of the canal do seem to establish the continuity of the lumen of this canal with the external environment.
MORPHOLOGY OF THE ABSORPTIVE SURFACE

MATERIALS AND METHODS

General

The terms applying to the various larval stages of *M. dubius* (egg, acanthor, acanthella, cystacanth) are used by this author as currently accepted by Chandler and Read (1961), Nicholas (1967) and King and Robinson (1967). The term **juvenile** will refer to sexually immature worms from the definitive host whereas **adult** refers to sexually mature worms.

Morphological and experimental studies primarily utilized juvenile worms obtained from the rat host between 14 and 21 days after infection. To simplify terminology these worms are referred to as **x days old** meaning that they were obtained *x* days after infection of the definitive host (a minimum of 24 hours being 1 day, a minimum of 48 hours being 2 days, etc.). During the two to three week post-infection time period, *M. dubius* maintains its highest rate of growth (Fisher, unpublished). This period of rapid development was selected as being most suitable for a study of body wall morphology and function. In addition, the worms were of an optimal size for handling and experimentation.

The morphology of the absorptive surface of *M. dubius* was examined at various periods up to ten weeks after infection. The two to three week period after infection was found to be representative of all juvenile and adult worms other than those obtained during the
first 24 hours post-infection. To examine developmental changes in the morphology of the absorptive surface during this period, cystacanths as well as juveniles obtained from the rat host 1, 2, 6, 12, and 24 hours post-infection were prepared for electron microscopy.

**Biological**

The acanthocephalan, *Moniliformis dubius* Meyer, 1933, was maintained in the laboratory utilizing the Sprague-Dawley laboratory rat (Holtzman Co., Madison, Wisconsin) as the definitive host and the American cockroach, *Periplaneta americana*, as the intermediate host.

Eggs were obtained by cutting gravid female worms into small pieces in a beaker of tap water and shaking. This material was strained through several layers of cheese cloth to remove the remnants of the adult worms and washed several times by allowing the eggs to settle, decanting and adding fresh tap water. Eggs were concentrated on filter paper using a Buchner funnel, mixed with an equal portion of apple scrapings and fed to the roaches as a moist mass on a minimum amount of filter paper. The roaches were maintained in black-painted glass tanks and provided with water and powdered Purina Laboratory Chow or Holtzman rat food *ad libitum*.

Infected larvae (cystacanths) were obtained from the hemocoel of the roach ten or more weeks after infection. Each roach was decapitated, the rear end clipped off and the hemocoel flushed with tap water. Male 80-90 gram Holtzman rats were infected orally with 25 cystacanths each. Before being infected, these rats were allowed a minimum of three days conditioning after arrival in the animal quarters. Rats were maintained in all wire mesh 12"x15"x20" cages in groups of 10-15 and provided with water and Purina Laboratory Chow *ad libitum*. Gravid worms could be obtained
approximately 42 days after infection of the rat.

**Electron Microscopy**

Rats were killed by cervical dislocation and the small intestine quickly removed. *M. dubius* was fixed either *in situ* by injection of fixative into the intestine or after removal from the host intestine. Some worms were washed briefly in the tris-maleate buffered Krebs-Ringer saline (KRTM) of Read *et al.* (1963). Worms were dropped into several drops of fixative on a piece of bite wax (Mizzy Inc., Clifton Forge, Va.) and given a few minutes to relax and "firm up." The anterior and posterior one-fifth of each worm was cut off and discarded. The remaining tissue was cut into thin transverse slices with a razor blade and transferred to fresh fixative in a vial. Cystacanths were obtained as described previously, the "cyst" removed and the worms fixed as above.

This writer encountered some difficulty in finding a fixation technique which would provide consistent results and most fully facilitate interpretations. Often it was felt that the solution to this problem lay hidden in the conoction of Shakespeare's three witches in *Macbeth*—

"Fillet of a fenny snake,
In the cauldron boil and bake;
Eye of newt, and toe of frog,
Wool of bat, and tongue of dog,
Adder's fork, and blindworm's sting,
Lizard's leg, and howler's wing;
For a charm of pow'rfull trouble
Like a hell-broth boil and bubble."

Of the many fixations attempted, the following variation of the original glutaraldehyde-osmium technique of Sabatini *et al.*, (1963) proved to be most satisfactory.

The tissue was fixed at room temperature in 6% glutaraldehyde containing 0.5 mM CaCl$_2$ buffered to pH 7.3 with 0.1 M monobasic-dibasic sodium
phosphate buffer. The glutaraldehyde reagent (50% w/w Biological Grade, Fisher Scientific Co.) was first shaken with excess barium carbonate to remove contaminating glutaric acid and then centrifuged. The purified supernatant was used.

At the end of three hours, the fixative was poured from the vials and the tissue was then washed with three 20 minute changes of 0.1 M phosphate buffer, pH 7.3, containing 0.5 mM CaCl₂ and 0.15 M (5%) sucrose. The tissue was post-fixed 90 minutes at room temperature in phosphate buffered 1% osmium tetroxide containing 0.5 mM CaCl₂ and 0.03 M (1%) sucrose.

After fixation, the tissue was rapidly washed with two changes of tap water. A portion of the tap water was decanted and 95% ethanol was added drop-by-drop with gentle agitation. Decanting and adding 95% ethanol was continued until the tissue was dehydrated to 95% ethanol (Philpott, 1965). Similarly, 100% ethanol was added drop-by-drop until dehydration appeared complete—the entire procedure taking about 20 minutes. The absolute ethanol was replaced by four 20 minute changes of fresh absolute ethanol. Again using the drop-by-drop method, the ethanol was quickly replaced with propylene oxide followed by a mixture of equal parts of propylene oxide and Epon (Luft, 1961) (47 ml. Epon 812, Ring Chemical Co., Houston; 33 ml. nadic methyl anhydride; 20 ml. dodecenylsuccinic anhydride; 1.6 ml. n,n-dimethylbenzylamine). The vials of tissue were then left uncapped on the top of a warm oven from six hours to overnight. At the end of this period, the tissue was exposed to two one hour changes of fresh plastic at room temperature. During the last 15 minutes of each of these two changes, the vials were placed in a vacuum oven at 60°C and gently pumped down. After these changes the tissue was placed in fresh warm (60°C) plastic in silicone rubber flat-embedding molds and polymerized at
60-70°C for a minimum of two to three days.

Thin sections displaying silver to grey inference colors were cut on diamond knives in a Sorvall "Porter-Blum" MT-2 Ultra-Microtome, collected on copper grids and double stained five minutes in 2% aqueous uranyl acetate pH 4.8, (Watson, 1958) and five minutes in lead citrate (Reynolds, 1963). Sections were examined and photographed in an RCA EMU-3F or a Phillips EM-200 electron microscope.

Epoxy sections 0.5 to 1.0 micron in thickness were mounted on glass slides and examined and photographed unstained using phase optics. A Leitz Ortholux microscope with Periplan GF 10x oculars, Pv 20/0.45 (PHANC) and Pv Fl Oil 70/1.15 (PHELB) objectives, a Heine phase contrast condenser and a Nikon Microflex AFMC with 1.3 x relay were utilized.

Glycogen

Cytoplasmic glycogen in the tissues of M. dubius appears as a particle of rather uniform size and composition. No evidence for two major groupings of molecular size (alpha and beta particles) as is common in such cells as rat hepatocytes (Drochmans, 1962; Barber et al., 1966) or tapeworm cells (Lumsden, 1965; Reissig and Colucci, 1968) was obtained. To further facilitate comparisons, M. dubius glycogen was prepared by a variation of the cold water isolation technique of Bueding and Orrell (1964) as modified by Colucci et al. (1966). Sedimentation runs of purified glycogen were carried out in a Beckman Spinco Model E Analytical Ultracentrifuge using the AN-D 2020 rotor. Runs were conducted at 9000 rpm, at 20°C, with the glycogen dissolved in glass-distilled water at concentrations of 0.35%, 0.525% and 0.7%. Photographs were taken using Schlieren optics. Glycogen from the rat tapeworm Hymenolepis diminuta was similarly prepared as a
control. Glycogen from this tapeworm previously has been studied by Orrell et al. (1964) with similar techniques.

Isolated glycogen was examined with the electron microscope after negative staining with 2% phosphotungstic acid-NaOH, pH 6.6 as detailed in Kay (1965). Copper grids were coated with a Parlodion film followed by carbon coating in a Kinney vacuum evaporator. Glycogen was dissolved in the sodium phosphotungstate and samples dried down on the coated grids. Glycogen was also prepared by powdering over a drop of sodium phosphotungstate pieces of worm which had been frozen in liquid nitrogen. The surface film was picked up on coated grids and allowed to dry.
OBSERVATIONS AND RESULTS

General

Specimens of Moniliformis dubius were generally located in the anterior second and third fifths of the rat small intestine. The worms were attached to the gut mucosa by their thorny anterior end and had to be pulled free with fingers or a pair of forceps. Worms fixed in situ were recovered unattached and could be flushed from the gut lumen a short period of time after injection of the fixative. Freshly removed worms are flaccid to the touch, highly motile, writhing about, white in color and moniliform in appearance. Young worms appear somewhat rounded but older specimens are distinctly flattened. When these worms are maintained in KRTM at room temperature, they become firmer and more round in shape after a period of time; however, specimens incubated at 37°C in KRTM or various experimental media based on this buffered saline retain their flaccid and flattened appearance.

Worms were separated on the basis of sex either in KRTM when being pre-incubated for an experiment or in the fixative before being cut up. More female worms were recovered from the rat host than males. This was readily apparent when segregating a specific number of male worms for an experiment.

This study of body wall morphology and function was limited to a portion of the metasomal or trunk body wall. The anterior one-fifth of Moniliformis consisting of the presoma or proboscis-associated body wall and the posterior one-fifth containing the gonads were discarded. It was hoped that any regional variations in structure and function might be avoided and the study system might be standardized. This was desirable with respect to the reported differences between the acanthocephalan presomal
and metasomal body walls.

The body wall occupies the greatest portion of the cross-sectional area of the helminth in young juveniles. As the worms age, the body wall grows in thickness, but the proportion of this tissue in the cross-sectional area of the worm diminishes greatly.

Light Microscopy

The metasomal body wall of a 16 day old male (Fig. 1) is about 0.11 mm in thickness, roughly one-third the diameter of the worm. The trunk has the appearance of a piece of thick-walled tubing, the smoothness of the outer surface occasionally being broken by shallow longitudinal furrows. The trunk wall cytoplasm is syncytial with no compartmentalization by lateral cell membranes and no connecting intercellular channels between the milieu exterieur and the pseudocoel or body cavity (n.b. the genital pores being special exceptions). The body wall cytoplasm is bounded by an outer and an inner membrane. With phase optics no true trunk wall layers may be detected. Large nuclei are located in proximity to the inner surface. The most prominent cytoplasmic features are numerous lipid droplets which range in size up to 8 microns or more in diameter. Often these lipid droplets appear to be fused into much larger accumulations. Only occasional patches of cytoplasm and a zone some 16 microns wide below the outer surface of the body wall are relatively free of these lipid droplets. Internal to the trunk wall is an irregular connective tissue layer, circular and longitudinal layers of muscle fibers and the pseudocoel and its contents.

As seen at a higher magnification (Fig. 2) numerous striations of about 4-6 microns in length located perpendicular to the outer surface of the trunk wall characterize the Streifenzone or striped "layer". This
feature appears to be more a modification of surface architecture than an indication of a structural layer. Careful examination of light micrographs even suggested that these features might be pits.

**Electron Microscopy**

**General**

Any new terminology introduced in this thesis is intended to be of a descriptive nature rather than link specific names to specific structures. Over the years, studies of invertebrate form and function have produced a specific set of nomenclature for each major phylogenetic grouping. Often one term was applied to a structure which either appeared to be similar in various taxa or was thought to be an analogous structure in taxa which were believed to be closely related. With the advent of the electron microscope and its facility of improved resolution, such use of terminology and implications of affinities in structure and origin often have been shown to be highly misleading. An example of such a misused term is the word **cuticle**.

The electron microscope has revealed a clearly defineable set of architectural features which are common to eucaryotic cells. These are the terms used in this study to describe the features of the absorptive surface of *Moniliformis dubius*. Generally the terminology used follows Fawcett (1966). The terminology of Meyer (1932-33) is used to designate cytoplasmic regions of the body wall.

**Streifenzone** (Figs. 3 to 22)

The general features of the Streifenzone are depicted in Figs. 3 and 4. This term refers to a region limited to the outer 4 to 6 microns of the body wall. For the purposes of this study the Streifenzone is considered to be the absorptive surface of the acanthocephalan and is given
primary attention. In regard to the worm physiology, it is assumed that this is the regulating interface between parasite and host.

The acanthocephalan surface has many structural analogies to the lumenal surface of the rat intestinal epithelial cell (see Cardell et al., 1967). There is a thick extracellular carbohydrate-rich surface coat, a characteristic surface membrane, features of surface area elaboration and cytoplasmic structural elements to support these elaborations.

**Surface Crypts**

The area of the absorptive surface of *M. dubius* is greatly increased by numerous pits or surface crypts. These crypts extend 2 to 4 microns below the surface of the worm and constitute the striped element of the Streifenzone. The crypts are very irregular in shape being roughly conical. The broadest portion of the crypts is usually found near their basal ends and measures about 0.40 microns wide. The crypts narrow at their apex to a slender neck measuring 120-240 A in diameter. A given crypt may be connected to the surface by one, two, or possibly more necks (Figs. 5 and 6). Morphologically, there appears to be continuity between the lumen of each crypt and the milieu exterieur; however, the lumen of the neck region does contain a loose meshwork of fine filaments (Figs. 10,11).

The surface crypts do not interconnect although they are frequently found in close apposition to one another. Occasionally the trilamellar membranes of two crypts touch and the cytoplasmic leaflets of the membranes appear to fuse (Inset, Fig. 15).

Small cytoplasm containing vesicles and myelinated structures are of common occurrence in the crypts. These are believed to be small protrusions from the sides of the crypts which appear isolated as a result of the plane of section. A finely flocculent material of an unknown nature fills the
crypts.

Calculations were made to estimate the degree to which the surface crypts amplify the area of the surface of *Moniliformis dubius*. A series of micrographs from a 21 day old male worm were utilized. The length of 27 surface crypts averaged 2.92 microns and the widths of 28 crypts averaged 0.38 microns. An examination of micrographs taken from sections cut en face to the surface of the worm produced an estimate of 15 crypt openings per square micron of surface.

Surface area was calculated by considering each surface crypt to be a cone, with the crypt height corresponding to the height of the cone and with the width of the crypts being used as the diameter of the base of the cone. This type of estimation contains two major errors which would tend to offset each other. First, the crypts, being of irregular shape, would actually have more surface area than cones of equal dimensions (height and width). Second, the number of crypt openings on the surface of the worm do not necessarily indicate an equal number of crypts as some crypts have two or possibly more necks.

On this basis, the surface area of a given surface crypt was calculated to be 2.881 square microns with 43.22 square microns of crypt area per square micron of surface. By adding that square micron of surface to the area of surface infoldings in the square, the surface area of this 21 day old male *M. dubius* was estimated to be amplified 44 times.

**Terminal Web**

Below the surface membrane is a matrix of about 2 to 4 microns in thickness composed of tightly compacted fine filaments. This matrix is considered to be analogous to the terminal web of many epithelial cell types. The terminal web is permeated by large pores through which the
surface crypts extend and may act as a cytoplasmic skeleton providing a framework for crypt structure as well as strengthening the external aspects of the worm as a whole. The filaments of the matrix are clearly seen in longitudinal profile only in sections tangential to the worm surface and appear as chains of tiny beads or stacked discs. The filaments are arranged in interlocking arrays circling around each surface crypt. The outer aspect of the terminal web is condensed into a more dense 100 A sheet lying below the surface membrane (Fig. 7). Continuous with this dense sheet are other small condensations of the terminal web forming an annulation encircling the neck region of each surface crypt (Figs. 7, 10, 11).

A 260 A thick cytoplasmic layer free of filaments lies between the surface membrane and the dense apical portion of the terminal web. In a like manner, the membrane of each surface crypt is separated from the web by a cytoplasmic sheath 650 A or more in thickness; however, the condensed filaments of each annulation form a cincture in contact with the membrane of the neck of the crypt (Fig. 10, 11, 13). The terminal web extends into the body wall cytoplasm only to the approximate level of the bases of the surface crypts (Figs. 15 to 17).

**Surface Coat**

External to the surface membrane of *Moniliformis dubius* is a surface-associated coating of carbohydrate-containing material. This feature is considered to be analogous to the extracellular coating of the absorptive surface of vertebrate intestinal epithelial cells termed surface coat by Ito (1965). The surface coat measures between 0.26 and 0.32 microns thick and is composed of tiny mucous-like filaments and clumps. A comparison of Figures 8 and 13 suggests that this pubigerous material is composed of
a number of net-like layers or small patches of net-like material laid
one-upon-another parallel to the surface membrane. A layer of this material
is closely applied to the other leaflet of the trilamellar surface membrane
(Fig. 9). The surface coat may extend for a short distance into the necks
of the surface crypts (Fig. 10) but no morphologically recognizable ele-
ments of this material have been found in the crypt lumens nor is there a
coating evident on the lumenal surface of the crypt membranes.

Membranes

The surface membrane of _M. dubius_ is typically tri-lamellar in ap-
pearance with no indication of asymmetry as is common to the surface
membranes of many absorptive surfaces. The membrane is thick (115 A) with
the electron-lucent middle leaflet constituting some 55 A of this. The
material was not studied under conditions of sufficient resolution to
make observations of membrane substructure. The tri-lamellar membrane of
the surface crypt (Fig. 12) is much thinner—only about 95 A thick. This
transition in thickness occurs about at the point of maximum constriction of
the crypt neck (Figs. 10 and 11). The crypt membrane has some indications
of structural asymmetry with the lumenal leaflet being more distinct than
the cytoplasmic leaflet. The electron-lucent middle layer is about 30 A
thick.

Cytoplasmic Inclusions and Organelles

The cytoplasm proximal to the basal portions of the surface crypts
and the cytoplasmic zone of several microns thickness below the crypts are
rich in inclusions and organelles. The following descriptions will also
apply to the constituents of the deeper cytoplasm of the body wall.

Filaments

Bundles of fine long filaments running in a direction radial to the
central axis of the body are common in the cytoplasm of *M. dubius*. These filament bundles are found coursing from the deeper cytoplasm into the cytoplasmic sheaths lying between the surface crypts and the terminal web (Figs. 4, 16, 17). In some micrographs, these cytoplasmic filaments extend along the surface crypts to the annulations and possibly insert on these or other portions of the terminal web. Filament bundles are discussed further in regard to the Radiarfibrillenschicht and basal region of the body wall.

Microtubules

Cytoplasmic microtubules of 240 A diameter and 1.5 microns or more in length are commonly found in the Streifenzone (Figs. 4, 8, 14, 16) and deeper cytoplasm. Interestingly, these structures frequently occur in the cytoplasmic sheath surrounding the surface crypts oriented in the direction of the longitudinal axis of the crypts (Figs. 4, 14).

Glycogen

Glycogen particles measuring 200 to 260 A in diameter are found in large numbers throughout the body wall cytoplasm of *M. dubius*. These particles are of uniform size and conformation appearing as single rounded granules with indication of substructure.

Cold-water isolated glycogen forms an opalescent solution when dissolved in water or appears as a fine white powder when dry. Sedimentation patterns of cold-water isolated *Hymenolepis diminuta* glycogen (Text Fig. 1) are indistinguishable from those obtained by Orrell et al. (1964). Two peaks were obtained representing the distribution of this tapeworm glycogen into two molecular weight ranges.

Sedimentation patterns of cold-water isolated *Moniliformis dubius* glycogen (Text Fig. 2) showed only one peak illustrating the more uniform
construction of this molecule \( s_w,20 = 160 \ 10 \). The single peak of the pattern does not give us any information about the degree of polydispersity of this glycogen sample. No variation of sedimentation patterns between glycogen from male worms, female worms or a mixture of glycogen from worms of both sexes was noted. The samples of _M. dubius_ glycogen sedimented at a much slower rate than those of _H. diminuta_. Negative stained glycogen (Text Fig. 3) is similar to glycogen studied in thin sections.

**Lipid Droplets**

Generally occurring in the deeper cytoplasm of the body wall as previously discussed (Fig. 1), scattered lipid droplets of less than 0.5 micron diameter are found in the inner aspects of the Streifenzone (Figs. 16, 18). Lipid deposits in the body wall are rounded, varying from less than 0.5 to 8 or more microns in diameter, with an uneven surface and no enclosing trilamellar membrane. These droplets are smooth or finely granular in texture and of only moderate electron density having only limited affinity for osmium tetroxide. The fringes of the droplets may be slightly more granular and more electron-opaque. Lipid droplets are usually found in pockets of glycogen particles with many of the particles embedded in the fringes of the lipid droplets. Mitochondria are also often intimately associated with the surface of lipid droplets. In the deeper cytoplasm lipid deposits frequently are found in close apposition appearing to be coalescing. The lipid droplets are easily extracted during preparation of the helminth tissue for electron microscopy. Experimentation indicated that lipid extraction occurred during the phosphate buffer washes between the aldehyde and osmium fixations. Limiting the extent of these washes and agitation of the tissue during the washes greatly improved the retention of the lipid droplets.
Text Figure 1  Sedimentation patterns of cold-water isolated Hymenolepis diminuta glycogen 8, 10, 12, 14, and 16 minutes after reaching 9000 rpm (20°C in H₂O; Analyzer angle 50°). The double peak is a result of the glycogen being distributed predominately into two molecular weight ranges. The leading (right-hand) peak corresponds to the morphologically identifiable alpha particle and the following peak to the beta particle.

Text Figure 2  Sedimentation patterns of cold-water isolated Moniliformis dubius glycogen 18, 22, 26, 30, and 34 minutes after reaching 9000 rpm (20°C in H₂O; Analyzer angle 72.5°). The single peak would indicate that the molecular weights of M. dubius glycogen are grouped into one range; however, the single peak is no indication of the degree of polydispersity of this glycogen sample ($s_{w,20} = 160±10$).

Text Figure 3  A negative print of a micrograph of negative stained M. dubius glycogen. This sample was prepared by powdering pieces of a worm which had been frozen in liquid nitrogen and allowing the powder to settle on the surface of a drop of 2% sodium phoshotungstate, pH 6.6. The sample was picked up on a coated (Parlodion and carbon) copper grid. The glycogen particles appear to be of a somewhat uniform size. Some indication of substructure may be seen (arrow). x approx. 150,000.
Vesicles

This term is used in a specific sense to apply to small ovoid structures measuring approximately 0.2 x 0.1 microns common to all parts of the body wall cytoplasm (Fig. 19). Each of these structures is bound by an 95 A thick trilamellar membrane displaying asymmetry—the inner leaflet being more clearly defined than the outer leaflet. A fine filamentous loosely packed material fills each of these vesicles. These structures are further considered in the section on Thorotраст experiments.

Mitochondria

Mitochondria of varying shape, size and appearance occur throughout the body wall cytoplasm. In profile, their shape and size vary from small spherical or oblong structures measuring 0.24 microns at the widest point to highly irregular branching structures up to 1 micron or more in length. These organelles are delineated by a thin outer limiting membrane. Separated from this outer membrane by a space of irregular width is an inner limiting membrane. Projecting into the interior of the mitochondrion are occasional plicate folds of the inner membrane commonly termed crista mitochondriales or cristae; however, the cristae are not always evident in a given plane of section giving these mitochondria a somewhat atypical appearance. Filling the interior of each mitochondrion is a characteristic dense highly compacted granular matrix.

Identification of mitochondria was often based on the presence of the two membranes surrounding the characteristic matrix. Large clusters of mitochondria occur in the cytoplasm of the Radianfibrillenschicht.

Golgi Complexes

These organelles are of common occurrence in the trunk wall of Moniliformis dubius being most frequently found in the inner aspects of the
Streifenzone and the inner region of the body wall (Figs. 18, 31, 37). The features of the Golgi complexes are not clearly delineated. In profile, the organelle consists of a concentric stack of cisternae of varying lengths. Each cisterna consists of a space enclosed by two parallel membranes which join at each terminus. The inner cisternae appear empty whereas the cisternae on the outer fringes of the concentric arrangement contain a material of moderate electron density. A number of small 400 A vesicles containing a similar density may be associated with these outer cisternae as well as occasional small apparently empty vesicles which appear to be coated with a layer of fine filaments on their cytoplasmic surface. No membrane-bound "secretory granules" are found associated with these Golgi complexes.

**Lysosomes**

A number of cytoplasmic vesicular structures are considered to be lysosomes on the basis of positive cytochemical localization of acid phosphatase activity as discussed in a later section. These structures share a number of common features and may be representative of variations of one particular organelle. These lysosomes are delineated by an asymmetric 95 A trilamellar membrane with the inner leaflet being distinctly more dense than the cytoplasmic leaflet. The inner matrix of the lysosomes is characteristically granular and highly compacted. The several types are designated numerically. Type 1 lysosomes (Figs. 18, 19) are round in shape and measure from 1.0 to 1.5 microns in diameter. These lysosomes occur most frequently in proximity to the surface crypt membranes. Type 2 lysosomes appear as long profiles up to 0.7 microns long, 1.1 microns wide and pinched in at the center (Fig. 21). Type 3 lysosomes are large lysosomes, rounded and up to 0.7 microns in diameter, and containing
one large vesicle filled with cytoplasm and perhaps glycogen particles (Fig. 18). It is possible that type 2 lysosomes are discoid shaped with a flattened center and appear as type 3 lysosomes when sectioned en face. Type 4 lysosomes are multivesicular bodies (Fig. 22). These rounded structures of about 0.3 microns in diameter may contain in profile in their matrix up to six or more vesicles ranging in diameter from 450 to 950 A. Each small internal vesicle is bounded by an 95 A trilamellar membrane and contains little electron-opaque material. These lysosomes occur most frequently in the inner extent of the Streifenzone but a few may be found scattered throughout the body wall. A type 5 lysosome is found only in the Radialfibrillenschicht and is described in that section.

Other Cytoplasmic Detail

Occasional profiles of endoplasmic reticulum are encountered in the lower aspects of the Streifenzone (note Fig. 16); however, positive identification is difficult. Rough endoplasmic reticulum is described from the perinuclear cytoplasm in the Radialfibrillenschicht section.

The major architectural features of the Streifenzone are recapitulated diagrammatically in Text Figure 4.

The Absorptive Surface of the Cystacanth (Fig. 23)

The cystacanth body wall shares the major features of that of the adult but is more compact in structure and shows less cytoplasmic detail. The surface coat is composed of compacted short fine filaments and tiny beads and is quite thick (up to 1.4 micron in thickness). Surface crypts are present but are developed poorly being fewer in number and extending only about 0.13 microns into the surface of the cystacanth. An occasional crypt might be as deep as 0.8 micron. The elements of the terminal web closely resemble those of the adult but are less distinct. Few inclusions
Text Figure 4. A diagrammatic summary of the absorptive surface (Streifenzone) of Moniliformis dubius. The reader is referred to the text for supporting details.

An, Annulation
f, Filaments
G, Golgi complex
gly, Glycogen
L, Lipid
M, Mitochondrion
mt, Microtubule
TW, Terminal web
v, Vesicle

Lysosome types
1, Round
2, Flattened
3, Univesicular
4, Multivesicular
and organelles are evident in the outer portions of the body wall other
than numerous glycogen particles and the well organized fibrils of the
Filzfaserschicht.

Developmental Changes in the Absorptive Surface

Twenty-four hours after infection of the rat host the features of the
absorptive surface of _M. dubius_ are indistinguishable from those of older
specimens. Samples of worms 1, 2, 6, and 12 hours post-infection were
fixed and examined to determine the time and sequence of changes. It is
interesting that even 1 hour after infection all of the juvenile worms
were attached by their thorny proboscis to the mucosa of the rat small
intestine. These rats were experimentally infected in the early afternoon
when their stomach contents should provide little hinderance to rapid
passage of the acanthocephalan larvae. Juveniles recovered 1 hour post
infection were flattened, elongated and more flaccid than the rounded,
somewhat compact cystacanths.

Numerous crypts of about 1.5 micron in depth are in evidence in the
surface of juveniles 1 hour after infection of the rat (Fig. 24). These
crypts are different from those of the adult but represent a radical change
from the cystacanth structure. The surface coat still resembles that of
the cystacanth with occasional areas of evident disruption. The fibers of
the Filzfaserschicht are no longer tightly packed as in the cystacanth. Six
hour old worms have a surface morphology (Fig. 25) which even more resembles
that of the adult. The features of surface area elaboration and the cyto-
plasmic skeletal features are clearly defined. Surface coat morphology
has assumed the appearance of that of older worms. Only the clear de-
lineation of the cytoplasmic Streifenzone remains to be completed.

_Filzfaserschicht_ (Figs. 26 to 30)
Immediately below the Streifenzone is a cytoplasmic region containing numerous closely packed, randomly arranged fibrils. This zone was termed the Filzfaserschicht by Meyer (1932-33). The major feature of this zone is fibrils of irregular cross-sectional profile 0.06 to 0.12 micron in diameter and indeterminable length. These fibrils are to be composed of a number of very thin highly compacted filaments. Also found in the Filzfaserschicht are numerous glycogen particles, vesicles, occasional lipid droplets up to about 2 microns in diameter, mitochondria and occasional lysosomes.

The fibrils of the cystacanth Filzfaserschicht are tightly packed and organized roughly into an outer circular layer, a middle longitudinal layer and an inner circular layer (Fig. 30). Radially oriented fibers course through the middle circular layer. Glycogen particles frequently occur between the tightly packed fibers.

Radiarfibrillenschicht (Figs. 31 to 37)

General

The Radiarfibrillenschicht constitutes about four-fifths of the thickness of the body wall ranging from the inner region of the Filzfaserschicht to the membrane delineating the inner aspect of the trunk wall. The most notable features of this region are the large bundles of filaments coursing radially through the cytoplasm, numerous large lipid droplets and nuclei of the coenocyte. No evidence providing any firm conclusions about the structure and nature of the acanthocephalan lacunar system was obtained.

The filament bundles consist of large numbers of parallel tightly packed 30 A filaments coursing through the body wall cytoplasm in a manner analogous to the spokes of a wheel connecting the hub and the rim. These bundles are as thick as 0.7 micron.
The Radiarfibrillenschicht contains large concentrations of glycogen particles, vesicles, lipid droplets up to 8 microns or more in diameter and larger aggregations of lipid droplets, numerous mitochondria, Golgi complexes and a variety of lysosomes. Common to this region of the body wall are large cytoplasmic pockets containing closely packed glycogen particles or large numbers of mitochondria. Found in the perinuclear cytoplasm are distinct profiles of endoplasmic reticulum with ribosomes studding the cytoplasmic surface of the membranes (Fig. 34). These are the only identifiable ribosomes in the cytoplasm of the body wall of M. dubius.

The lysosomes which occur most frequently in the Radiarfibrillenschicht are the multivesicular body (type 4 lysosome) and a lysosome termed the type 5 lysosome which is distributed only in this zone (Fig. 31). These rounded structures of about 0.45 micron diameter are delineated by a tri-lamellar membrane. The internal aspect of this membrane is lined by a 360 A thick layer of fine filaments and the lysosome contains a finely flocculent or filamentous material.

Nuclei (Figs. 33 to 36)

Large nuclei measuring up to 50 microns or more across their profile are found in the basal region of the coenocytic body wall of Moniliformis dubius. These nuclei are irregular in shape and delineated by a highly convoluted nuclear envelope (Fig. 33). At a low magnification, these nuclei contain little recognizable heterochromatin; however, as many as 5 or 6 nucleoli may be seen in one section.

The nuclear envelope is thin, consisting of two parallel 75 A tri-lamellar membranes separated by a space of less than 160 A. For this reason sections generally catch the nuclear envelope out of profile. The
perinuclear cistern is traversed by pores of some 690 A diameter which presumable allow a means of communication between the nuclear and cytoplasmic ground substances. A small concentration of electron-dense material often fills these pores and extends into the ground substance for a distance of several hundred A on either side of the pores. Occasional patches of this fine material are found on the inner aspect of the nuclear envelope.

The nuclear ground substance is finely flocculent and contains scattered dense granules up to about 450 A diameter and only a few scattered patches of heterochromatin. The peripheral heterochromatin common to nuclei of most cells is not apparent.

The somewhat compact nucleoli are oblong or rounded in shape and range up to 3 microns or more in diameter. They consist of a central zone compacted and dense, surrounded by a peripheral region containing numerous 150 A granules embedded in a fine matrix. The inner homogeneous zone is termed the pars amorpha, while the periphery represents a compacted nucleolenema. The granules resembles cytoplasmic ribosomes and are considered to be the nucleolar site of ribonucleoprotein.

Of particular interest are pockets up to 1.2 microns in diameter occurring in the pars amorpha and containing numerous electron-opaque rounded or oblong granules measuring up to 1000 A long or 550 A in diameter (Fig. 36). Of common occurrence, up to several of these pockets may be found in one nucleolus yet they are not found in every nucleolus or nucleus.

Inner Surface of the Body Wall (Fig. 37)

The membrane of the inner surface of the trunk wall of _M. dubius_ is
highly involuted with numerous thin finger-like surface area elaborations of about 450 A diameter penetrating up to 1.2 microns into the body wall cytoplasm. These thin channels are delineated by an 85 A trilamellar membrane displaying no apparent asymmetry and in their lumen contain a sparse amount of fine filamentous or condensed material. The filaments of the bundles characterizing this Radiarfibrillenschicht insert into small patches of dense material closely applied to the cytoplasmic aspect of this inner membrane. This is highly reminiscent of the relationship of tonofilaments to the cytoplasmic aspects of a desmosome. Common to the cytoplasm of this basal region are glycogen particles, vesicles, Golgi complexes and type 5 lysosomes. Internal to the basal membrane of the body wall is a finely amorphous basal lamina about 500 A thick. The underlying connective tissue matrix contains a loose mat of thin 150 A fibrils of indeterminable length. These fibrils are hollow in cross-section and appear beaded along their length.
FUNCTIONAL ASPECTS OF THE ABSORPTIVE SURFACE

MATERIALS AND METHODS

Lanthanum

Despite substantial morphological evidence for the continuity of the surface crypt lumen with the milieu extérieur, an unequivocal demonstration of this feature was regarded as desirable. The recent introduction by Revel and Karnovsky (1967) of the technique of occluding extracellular space in tissues with an electron-opaque lanthanum complex provided the means. This technique also allowed an investigation of whether each surface crypt were a separate structure or simply a portion of a complex interconnecting system.

The lanthanum complex was prepared by dissolving four gm of lanthanum nitrate in 95 ml glass-distilled water, titrating this solution with 0.01 N NaOH to pH 7.8 and correcting the total volume to 100 ml.

Nineteen day old worms of both sexes were fixed at room temperature in 6% glutaraldehyde containing 0.5 mM CaCl₂ and 1% lanthanum buffered to pH 7.3 with 0.1 M s-collidine-HCl. At the end of four hours the tissue was washed with three 20 minute changes of 0.1 M collidine buffer, pH 7.3, containing 0.5 mM CaCl₂, 1% lanthanum and 10% sucrose and post-fixed 90 minutes in 1% collidine-buffered osmium tetroxide with 0.5 mM CaCl₂ and 1% lanthanum. All other details of processing tissue for electron microscopy previously have been discussed. Thin sections were studied and photographed with and without heavy metal staining.

Magnesium Sulfate
An experiment was run to determine whether the cytoplasmic filaments comprising the annulations around the neck of the surface crypts were of a contractile nature. It was reasoned that a common narcotizing agent, magnesium sulfate, might relax these filaments and cause the neck to open up. The osmolarity of the saline component of KRTM (Read et al., 1963) was determined to be 240 mOsm using an osmometer (Advanced Instruments, Newton Highlands, Mass.). A magnesium sulfate solution of equivalent osmotic strength (0.22 M) was substituted for this saline to make a tris-maleate buffered magnesium sulfate. Thirty-three day old male worms were incubated in this buffered saline for periods up to three hours in length and prepared for electron microscopy.

Thorotrast

Revel (1964) introduced a method for staining methacrylate thin sections with colloidal thorium dioxide as a means of detecting "acid mucopolysaccharides" with the electron microscope. This technique was later used on tissue en bloc to detect the presence of the carbohydrate-rich cell coat at the surface of rat cells (Rambourg and Leblond, 1967). Thorium dioxide was used in this study to investigate the nature and extent of the carbohydrate-containing coat associated with the surface of M. dubius. Thorotrast - En Bloc

38 day old male worms were fixed ice cold in 3% glutaraldehyde containing 0.5 mM CaCl₂ and 3% sucrose buffered to pH 7.3 with 0.1 M phosphate buffer. After three hours, the worms were washed in three 20 minute changes of 0.1 M phosphate buffer with 0.5 mM CaCl₂ and 5% sucrose and post-fixed in 1% phosphate buffered osmium tetroxide containing 0.5 mM CaCl₂ and 1% sucrose. The tissue was allowed to come to room temperature and washed three times in tap water followed by three five minute washes in 3% acetic
acid. The tissue was then soaked in a solution of 3% acetic acid and 4% Thorotrast (a solution of 24% to 26% stabilized colloidal thorium dioxide by volume, Fellows Tetagar Div., Fellows Medical Manufacturing Co., Inc., Detroit, Michigan) overnight. This was followed by three five minute washes in 3% acetic acid and dehydration and embedding for electron microscopy as previously discussed. Thin sections were examined and photographed both stained and unstained with heavy metal salts.

**Thorotrast - on Thin Sections**

Colloidal thorium dioxide did not penetrate the surface crypts of tissue stained en bloc even though the particles appeared to be of a sufficiently small size. It was possible that the particular features of the neck of the surface crypt might be excluding the thorium micelles from entry. To ascertain whether polyanionic compounds were present in the surface crypts, the staining of thin sections became necessary. A modification of Revel's technique was employed. Twenty-one day old male and female worms which had been prepared for morphological study and embedded in Epon were utilized. Thin sections were cut on a diamond knife, collected on gold grids (Ernest F. Fullam, Inc., Schenectady, N. Y.) and stained as follows:

1. 30% acetic acid (v/v): 15 minutes
2. 1% Thorotrast in 30% acetic acid: 20-30 minutes
3. 30% acetic acid: 5 minutes
4. 30% acetic acid: 5 minutes
5. Distilled water: briefly washed

These sections were examined and photographed with no heavy metal salt staining.

**Ruthenium Red**

A technique for staining extracellular materials for electron microscopy with ruthenium red has been developed by Luft (1965). This
reaction was carried out to gain more information about the surface coat of
M. dubius. Unpurified ruthenium red (K & K Laboratories, Inc., Plainview,
N. Y.) was used.

Forty day old male worms were fixed one hour ice cold in a solution
of equal parts of the following:

a. 7.5% glutaraldehyde
b. 0.2 M cacodylate buffer, pH 7.3
c. ruthenium red stock solution in distilled water at 1500-3000 ppm

The tissue was rinsed in three 15 minute changes and one 25 minute change
of 0.15 M cacodylate buffer. Post-fixation was for three hours at room
temperature in a mixture of equal parts of:

a. 6% OsO₄ in distilled water
b. 0.2 M cacodylate buffer, pH 7.3
c. ruthenium red stock

The tissue was rinsed in buffer for one 35 minute change and three 15
minute changes, washed in tap water, dehydrated by drop-by-drop ethanol
additions and embedded in Epon. Sections were examined both unstained
and stained with uranyl and lead salts.

**Acid Phosphatase**

The morphology of the surface crypts and a tentative report of acid
phosphatase activity in these structures by Rothman (1967) were suggestive
of the possibility of extracellular digestion. A careful study of acid
phosphatase activity in M. dubius was carried out to explore this poss-
bility as well as to determine sites of lysosomal activity. Techniques
utilized were essentially those of Seed et al. (1967).

Fourteen and eighteen day old male worms were fixed 60 minutes ice
cold in 4% glutaraldehyde buffered to pH 7.3 with 0.1 M sodium cacodylate
buffer. Following fixation, the tissue was washed briefly in ice cold
cacodylate buffer containing 5% sucrose. Fifty micron sections were cut
on a Sorvall TC-2 "Smith and Farquhar" Tissue Sectioner (Ivan Sorvall, Inc., Norwalk, Conn.) according to Smith and Farquhar (1963). The cacodylate buffer was replaced by two ice cold washes of 0.1 M tris-acetate buffer, pH 5.0, containing 5% sucrose. The sections were incubated with shaking at 37°C for 30 minutes in 8.3 mM substrate, 25 mM tris-acetate buffer, pH 5.0, and 2.5 mM lead nitrate. Sodium glycerophosphate (Fisher Scientific Co.) and p-nitrophenylphosphate disodium (Calbiochem) were used as substrates. Controls were run by omitting the substrate from the incubation medium. Following incubation, the sections were washed in three changes of tris-acetate buffer containing 5% sucrose, post-fixed 60 minutes ice cold in 1% osmium tetroxide buffered to pH 7.3 with 0.1 M sodium cacodylate, dehydrated in ethanol and embedded in Epon. Thin sections were examined and photographed unstained and stained with heavy metal salts.

The Uptake of Large Molecules

General

The morphology of the surface crypts, crypt acid phosphatase activity and lysosomal activity in the region of the crypts suggested the digestion and/or uptake of large molecules by this absorptive surface. Studies were carried out with tracer molecules to detect and to achieve an understanding of these activities. The tracer molecules employed were ferritin (5x crystallized--Mann Research Laboratories, New York), colloidal thorium dioxide (Thorotrast), horseradish peroxidase (Type II, Sigma Chemical Co., St. Louis) and Chlorella protein C14 (New England Nuclear, Boston).

In Vitro Incubation Technique

Fourteen day old male M. dubius were removed from the rat intestine
and pre-incubated 30-45 minutes in KRTM (Read et al., 1963). The incubation mixture consisted of KRTM containing 12.5 mM sodium bicarbonate and the tracer molecule. Controls were run on all incubations by omitting the tracer molecule from one sample. The incubation mixture was equilibrated with a 95% N₂-5% CO₂ mixture prior to introducing the worms and all incubations were conducted with constant gassing. The incubation vessels (25 ml Erlenmeyer flasks) contained three worms in 5 ml (rarely 10 ml) of medium. A two hole #00 rubber stopper with Pasteur pipets inserted in the holes provided the gas inlet and outlet. Worms were incubated for 60 minutes at 37°C in a shaking water bath.

**Ferritin**

*M. dubius* was incubated in ferritin at concentrations of 1, 5 and 50 mg/ml in total volumes of 5 ml or occasionally 10 ml. A sample of worms which had been incubated in 50 mg/ml ferritin was processed to localize acid phosphatase activity as previously described.

**Thorotrast**

Worms were incubated in 5 ml of medium containing Thorotrast at concentrations of 2%, 4% and 20% (i.e. approximately 5, 10, and 50 mg/ml colloidal thorium dioxide).

**Horseradish Peroxidase**

Worms were incubated in 5 ml of medium containing peroxidase at concentrations of 5 and 10 mg/ml.

**Electron Microscopy**

Worms incubated in ferritin and Thorotrast were processed for electron microscopy as previously described for morphological study.

The cytochemical method of Graham and Karnovsky (1966a, b) for visualization of horseradish peroxidase for the electron microscope was used.
After incubation, the tissue was fixed in glutaraldehyde as above. Following fixation, the tissue was washed 20 minutes in 0.1 M phosphate buffer containing 0.5 mM CaCl₂ and 5% sucrose and then incubated at room temperature for 15 minutes in a medium consisting of 5 mg 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) in 10 ml 0.05 M tris-HCl buffer, pH 7.6, with 0.1 ml of 1% H₂O₂ (freshly prepared from 30% H₂O₂) added just before use. This was followed by four brief washes in distilled water, post-fixation 90 minutes at room temperature in 1% osmium tetroxide buffered to 7.3 with 0.1 M cacodylate buffer containing 0.5 mM CaCl₂, dehydration in ethanol and embedding in Epon.

Thin sections of specimens from the ferritin, thorium dioxide and peroxidase incubations were examined and photographed both unstained and stained with uranyl and lead salts.

**Clorella Protein-C¹⁴**

It was reasoned that the detection of hydrolysis products of a labeled protein sample would provide evidence of digestion in the surface crypts or associated lysosomes as well as a means for estimating the rate of uptake. **Clorella** protein-C¹⁴ (50 µc/mg.) was one of the few available labeled proteins.

Incubations were conducted as described above. Two basic series of experiments were run---1) to determine the effect of a carrier protein (ferritin), 2) to determine the effect of the medium. The samples of the first series consisted of:

- a) medium with Clorella protein-C¹⁴
- b) medium with Clorella protein-C¹⁴ and 1 mg/ml ferritin
- c) medium with Clorella protein-C¹⁴ and 5 mg/ml ferritin

The medium was always KRTM-bicarbonate equilibrated with 95% N₂-5% CO₂ unless otherwise noted and the amount of label was 1 or 2.5 µc./flask.
depending on a particular run. The samples in the second series consisted of:

a) KRTM with Chlorella protein-C\textsuperscript{14} omitting the worms as a control
b) KRTM with Chlorella protein-C\textsuperscript{14}
c) KRTM-bicarbonate equilibrated with 95% N\textsubscript{2}-5% CO\textsubscript{2} and Chlorella protein-C\textsuperscript{14}
d) KRTM-bicarbonate equilibrated with 95% N\textsubscript{2}-5% CO\textsubscript{2}, Chlorella protein-C\textsuperscript{14} and 1 mg./ml carrier (ferritin)

Samples from each incubation (including a pre-incubation medium sample, a post-incubation medium sample and a tissue sample) were fractionated into trichloracetic acid (TCA) insoluble material, TCA soluble material, ethanol insoluble material and chloroform soluble material.

The material was processed as follows:
1) The original sample was homogenized in 5 volumes of 10% ice cold TCA and centrifuged.
2) The pellet was washed several times in 5 volumes of diethyl ether to remove residual TCA, then dissolved in a small volume (0.5 to 1.0 ml) of 0.01 N NaOH.
3) The TCA supernatant was washed several times with 5 volumes of diethyl ether to remove the TCA, 1.2 volumes of 95% ethanol was added to precipitate the glycogen, and the sample was centrifuged. The glycogen was dissolved in a small volume of water, reprecipitated and redissolved several times to insure its purity.
4) The supernatant was extracted with an equal volume of chloroform to produce final aqueous and chloroform soluble fractions.

A sample of each fraction was dried on a planchet (the TCA insoluble sample being first neutralized) and counted in a Nuclear-Chicago gas-flow planchet counter.

Initial experiments showed the Chlorella protein-C\textsuperscript{14} to be a very
heterogeneous preparation containing TCA insoluble, TCA soluble and chloroform soluble material. After this finding, the Chlorella protein-C\textsuperscript{14} was initially "purified" by treating with ice cold 10% TCA several times. The denatured TCA insoluble Chlorella protein-C\textsuperscript{14} was actually used for these studies.
OBSERVATIONS AND RESULTS

Lanthanum (Figs. 38, 39)

The results of fixing *Moniliformis dubius* tissue in solutions containing lanthanum salts unequivocally demonstrated the morphological continuity of the surface crypt lumens with the *milieu extérieur*. The lumen of each surface crypt was fully occluded by the electron-opaque complex of lanthanum salts. No evidence was obtained that the lanthanum crossed the surface membrane or crypt membrane and penetrated the trunk wall cytoplasm. In most cases, no lanthanum was evidenced in the neck region of the crypts and is assumed to have been washed out by preparative procedures; however, an occasional neck was filled with and clearly outlined by the electron-opaque lanthanum (Inset, Fig. 39). In these instances the neck lumen at the level of the annulation appears to contain some fine material which prevents total occlusion by the lanthanum.

The surface coat was clearly stained by the lanthanum (Fig. 38). When sections of *M. dubius* tissue which had been prepared, for example, for morphological study, are examined without uranyl acetate-lead citrate staining, the surface coat cannot be detected. The outer fringes of the surface coat accumulated dense clusters of lanthanum salts perhaps suggestive of a higher affinity for lanthanum or a generalized greater binding capacity for certain compounds by the elements of this region.

Magnesium Sulfate

Incubation of *M. dubius* in a tris-maleate buffered magnesium sulfate solution with shaking at 37°C for periods up to 3 hours in length produced neither any noticeable changes in the morphology of the Streifenzone nor any unusual variation of the neck diameters of the surface crypts.
Thorotrast - EnBloc (Figs. 40, 41)

When specimens of Moniliformis dubius were soaked en bloc overnight in a Thorotrast solution in 3% acetic acid (at approximately pH 2.1) numerous thorium dioxide particles were bound to the material of the surface coat. The results were complex and somewhat unexpected. The surface coat acted as a sieve allowing no thorium particle of a diameter greater than 85 A to penetrate deeper than its outer fringes. Thorium particles of a diameter greater than about 85 A (some particles range up to 300 A or more in diameter) were bound to the outer fringes of the surface coat forming an irregular layer of some 0.08 micron thickness. Particles about 85 A or smaller are observed bound directly to the filaments of all of the deeper elements of the surface coat. A single fine filament of the surface coat will have a single row of small thorium particles bound along its length. The filaments of the deeper elements of the surface coat appear to bind a larger number of thorium particles per unit length and to be more closely packed than the filaments closer to the outer fringes. Numerous thorium particles are also bound to the amorphous layer of the surface coat which is closely applied to the surface membrane.

Although there appears to be no obstacle to the penetration of the surface crypts by Thorotrast, only rarely was a scattered, usually single, particle found in the crypts. The normal limit of penetration of thorium particles into the crypts corresponded with the limit of inward penetration of morphologically recognizable elements of the surface coat—the level of the annulations. Two possibilities are evident. First, there may be no polyanionic material in the surface crypts to bind the Thorotrast and the acetic acid rinses wash virtually all of the particles out of the
crypts. Second, entrance to the crypts is blocked or regulated by some material (for example, very fine filaments as suggested morphologically and by the lanthanum experiments). This material would act as a sieve allowing entrance only of compounds of a certain maximum size or a certain chemical nature. The first possibility seems unlike because of the conformation of the surface crypts. It seems unlikely that virtually all of the thorium particles would be washed out. The selective sieve appears highly likely and would explain the penetration of lanthanum salts and the exclusion of virtually all thorium dioxide particles. The major problem would appear to be establishment of the limits within which a particle could freely permeate the neck of the surface crypts.

Thorotrast - On Thin Sections (Figs. 42, 43)

Application of acidic (approximately pH 1.9) solutions of Thorotrast to epoxy thin sections proved to be a useful technique. Thorium particles were bound to the face of the sections over the site of the surface coat, a material which was previously demonstrated to have the property of binding thorium. Thorium particles were also bound in less dense concentrations over a number of the available surface crypts and their cytoplasmic sheaths. Other surface crypts had few or no thorium particles bound over them and only a very low background of thorium binding was in evidence over the terminal web and the Epon external to the surface coat. The presence of a limited amount of polyanionic material in a population of the surface crypts is, thus, demonstrated and some weight is given to the hypothesis that thorium particles are generally excluded from the crypts during en bloc staining.

Unexpectedly, Thorotrast was bound to the epoxy sections in a density equivalent to the surface coat binding over the profiles of the structures
which have previously been termed vesicles. A re-examination of the morphology of these vesicles (Figs. 44 and 45) suggests that their contents are highly reminiscent of the material of which the surface coat is composed.

Ruthenium Red (Figs. 46 to 48)

Examinations of unstained thick epoxy sections of the worms which had been fixed in ruthenium-containing solutions revealed a general reddish-brown coloration throughout the tissue. When viewed with the electron microscope, this tissue did not have the anticipated distinct staining of the surface coat by the ruthenium red-osmium tetroxide complex as is commonly seen in other tissues (Luft, 1965; Kelly, 1968). Only scattered clumps of electron-opaque material were observed in the surface coat. Closer study of unstained sections did reveal a distinctive staining of the membrane delineating the surface crypts. This staining becomes indistinct as the crypt membrane approaches the neck region and no staining of the surface membrane nor of any of the cytoplasmic membranes of M. dubius was in evidence. At high magnifications, this staining can be seen to be associated with the luminal and cytoplasmic leaflets of the trilamellar membrane. The appearance of the trilamellar structure of this membrane was greatly enhanced in uranyl acetate-lead citrate stained sections of ruthenium stained tissue when compared to sections of material which had been prepared for conventional morphological study.

Acid Phosphatase (Figs. 49-55)

The electron-opaque lead phosphate product indicative of localized enzyme activity was found in the body wall as result of the acid phosphatase incubations. With disodium p-nitrophenyl phosphate as substrate, enzyme activity was localized in the crypts of the absorptive
surface of M. dubius. Using the standard substrate for lysosomal acid phosphatase localization, sodium β-glycerophosphate, enzyme activity was in evidence both in the surface crypts and in numerous vesicular structures assumed to be lysosomes found throughout the body wall. Helminth tissues incubated in control media containing no substrate were free of any electron-opaque precipitate.

The electron-opaque lead phosphate occasionally filled the surface crypts particularly in the neck region but more often was distributed around the periphery perhaps suggestive that the enzyme activity is associated with the crypt membrane. No particular region of the crypts contained a predominating portion of the reaction product; however, activity diminished in the neck region and was not evidenced on the surface of the worm. The reaction product appeared as small granules and clusters of granules generally forming an irregular coating up to several hundred angstroms thick on the lumenal aspect of the crypt membrane.

The identification of type 1 lysosomes was presumed on the basis of their small rounded appearance and their frequent occurrence in proximity to the surface crypts. The other lysosomal types were identified by their tell-tale shapes and characteristic appearances. These lysosomes contained a loose aggregation of lead phosphate in their matrix. Type 4 lysosomes (multivesicular bodies) contained electron-opaque reaction product in the granular matrix but the small internal vesicles were free of lead phosphate. The precipitate in type 5 lysosomes was limited to their periphery perhaps associated with the characteristic thick layer of filaments found closely applied to the inner aspect of their delineating membrane. The lead-phosphate free center of these type 5 lysosomes contained a fine web-like net of material.
No acid phosphatase activity was detected in the characteristic structures which previously have been termed vesicles and have been shown to contain a thorium-binding substance. An apparent scattered cytoplasmic phosphatase activity was found in the inner region of the body wall.

**The Uptake of Large Molecules**

**Ferritin** (Figs. 56 to 65)

Ferritin molecules in association with a mucous-like material were found in patches several microns wide on the outer aspects of the surface coat of *M. dubius*. Occasional ferritin molecules appeared to be bound to the filaments comprising the surface coat. No direct evidence for the passage of ferritin into the surface crypts or the uptake of ferritin was obtained.

As a consequence of the ferritin incubations, numerous electron-opaque concentrations of material ranging up to about 0.1 micron diameter appeared in the surface crypts. Such phenomena occur only at very low frequencies in controls or materials prepared for morphological study.

The density of these accumulations precludes any identification of ferritin molecules which they might contain.

Corresponding to the appearance of the dense concentrations in the surface crypts, similar densities are found in the matrix of the various lysosomal types of the Streifenzone. Such dense accumulations are not present in the matrix of the lysosomes observed in controls nor in specimens prepared for morphological study. These dense concentrations are found only in lysosomes located in the Streifenzone or outer reaches of the Filzfaserschicht. Again, the density of these concentrations prevent any positive identification of ferritin molecules. The density in multi-vesicular bodies (type 4 lysosomes) is located in the matrix among the
small internal vesicles—the particular site in these structures (and in the other lysosomal types) of the acid phosphatase activity.

Worms which had been incubated in ferritin and subsequently incubated for acid phosphatase activity had a substantially increased number of lysosomes in the Streifenzone when compared to tissue which had simply been processed for this enzyme activity.

Thorotrast (Figs. 66 to 73)

To detail the results of the incubations in colloidal thorium dioxide is to recapitulate the findings of the ferritin experiments. The results are virtually identical with only a few exceptions. Patches of thorium and an associated mucous-like material were found in the outer fringes of the surface coat. Occasional small thorium particles were observed in the surface coat bound to the thin filaments comprising this material. Dense accumulations appeared in the surface crypts and in the matrix of lysosomes from the Streifenzone and outer aspects of the Filzfaserschicht after incubation of specimens in Thorotrast. This again is in contrast to controls and specimens prepared for morphological study, in which the appearance of these dense concentrations is of low frequency in the surface crypts and has not been detected in lysosomes. The density of these accumulations again precludes positive identification of the tracer molecule; however, examination of unstained thin sections revealed a very few small (apparent) thorium particles in these densities both in the surface crypts and in the lysosomes. An occasional thorium particle was rarely found in the lumen of the surface crypts not associated with the densities.

Horseradish Peroxidase (Figs. 74 to 79)

The results using horseradish peroxidase as the tracer molecule are
quite different from those of the previous two sets of experiments. The presence of the electron-opaque product of the reaction between the peroxidase molecule and the diaminobenzidine reagent is considered to be indicative of the location of the protein itself. Only traces of the peroxidase are found in the surface coat usually appearing as small clumps or tiny lace-like patches; however, the surface crypts are literally filled with the dense flocculent reaction product. Some surface crypts are entirely occluded by a homogeneous dense material. The tracer molecule is apparently accumulated in the surface crypts. In the vicinity of the crypts and throughout the cytoplasm of the Streifenzone and outer fringes of the Filzfaserschicht, numerous lysosomes of various types are filled with the electron-opaque reaction product indicating the presence of horseradish peroxidase in these organelles. The tissues of worms incubated in control media containing no peroxidase and subsequently incubated in the diaminobenzidine reagent are free of electron-opaque deposits. These studies suggest strongly that horseradish peroxidase is in sequence accumulated in the surface crypts of the absorptive surface of *M. dubius* and then taken up, being found in the lysosomal structures of the adjacent cytoplasm. Not all of the lysosomes in the Streifenzone contain peroxidase. Occasionally two lysosomes of the same type will be observed proximal to each other with only one containing the dense product indicative of the presence of peroxidase.

**Chlorella Protein-C\(^{14}\)**

First, let it be stated that the *Chlorella* protein-C\(^{14}\) is definitely the wrong tool to use in such an investigation. The sample from New England Nuclear was found to contain TCA insoluble, TCA soluble and chloroform soluble fractions. These are presumed to represent respectively
large proteins, small proteins and polypeptides, and lipoproteins. Even though the chloroform soluble fraction (less than 0.1% of the total radioactivity) was eliminated with little difficulty, successive TCA fractionations never reduced the TCA insoluble fraction (from its initial level of greater than 34% of the total radioactivity) to below approximately 12% of the recoverable radioactivity (this is with regard to fractions of only the Chlorella protein sample).

By comparing the radioactivity of the various fractions recovered during these preliminary experiments some qualitative conclusions might be drawn which suggest that these experiments are worth pursuing. First, when several worms were incubated with 50 micrograms of labeled protein for one hour, about 1/6 of this label is recovered from the worms but only about 1/40 of this fraction as assumed hydrolysis products. This indicated that the worms are adept at accumulating the protein from the medium but hydrolysis proceeds at a much lower rate. Second, carrier protein in the form of ferritin increases both of these rates. Third, the medium affects the recovery of TCA soluble material from the worm. The recovered TCA soluble label attributed to hydrolysis by the worms increases from KTRM-bicarbonate equilibrated with 95% N₂-5% CO₂ to the latter medium containing 5 mg/ml carrier ferritin in the ratio of 2:4:11. These findings will, of course, have to be confirmed by repeatable quantitative results using a proper tracer but do suggest that the problem merits investigation.

In the light of the other experiments on the surface of M. dubius, it is suggested that a "proper" label fulfill several criteria: 1) The protein should be small in size (refer to the discussion) and the sample fairly uniform in composition 2) It should be detectable
morphologically or by a cytochemical technique to allow the uptake and digestive processes to be followed. Such requirements could be satisfied by sending a sample of Sigma Type II horseradish peroxidase to New England Nuclear to be tritiated by one of several means.
COMPARATIVE ASPECTS OF THE ABSORPTIVE SURFACE

MATERIALS AND METHODS

Priapulida

Specimens of *Priapulus bicaudatus* were collected from the intertidal zone on the coast of Maine and transported to the Marine Biological Laboratory, Woods Hole. The samples were fixed several hours ice cold in either 6% glutaraldehyde or a mixture of 2% glutaraldehyde and 1% acrolein buffered to pH 7.4 with 0.12 M monobasic sodium phosphate-NaOH (Millonig, 1961) containing 0.5 mM CaCl₂ and 3% sucrose. The tissue was washed free of fixative with several changes of phosphate buffer containing 0.5 mM CaCl₂ and 5% sucrose and flown in this buffer wash in vials packed in ice to Houston. The tissue was post-fixed ice cold 90 minutes in 1% osmium tetroxide buffered to pH 7.4 with 0.12 M sodium phosphate-NaOH containing 0.5 mM CaCl₂ and 1% sucrose, allowed to come room temperature, dehydrated in ethanol and embedded in Epon.

Cestoda

Trypanorhynch cestodes tentatively identified as *Prochristianella penaei* Kruse, 1959, were removed from the spiral valve of several female specimens of the sting ray, *Dasyatis sabina*, which had been caught in Galveston Bay, Texas. These worms were fixed ice cold in 6% glutaraldehyde buffered to pH 7.4 with 0.12 M sodium phosphate-NaOH containing 0.5 mM CaCl₂ and 3% sucrose, washed in phosphate buffer with 0.5 mM CaCl₂ and 5% sucrose, and post-fixed in 1% phosphate-buffered OsO₄ containing 0.5 mM CaCl₂ and 1% sucrose. The tissue was dehydrated in ethanol and embedded in Epon.
Rotifera

Micrographs of the body wall of *Asplanchna brightwelli* and the stomach and body wall of *Philodina acuticorius odiosa* which had been prepared according to the methods of Koehler (1964, 1966) were obtained through the courtesy of Dr. James K. Koehler, Department of Biological Structure, School of Medicine, University of Washington, Seattle.
OBSERVATIONS AND RESULTS

Priapulida (Figs. 80-93)

General

The surface of Priapulida bicaudatus is described briefly as this organism is not the main topic of this thesis. The description applies to the generalized body surface as depicted in the accompanying micrographs. Localized variations of this general structure are found when examining the surface of the numerous warty tubercules on the organism and the surface of the prominent caudal glands. The body surface consists of a thick extracellular cuticle which lies over a simple cuboidal epithelium of comparative thickness. The epithelial cell layer rests on a basal lamina, a connective tissue layer and underlying muscle fibers. Occasional nerve cells intrude between the epithelial cells and the muscle layer. Close junctions are formed between the membrane of the nerve cell and the basal membranes of the several epithelial cells with which it may be in contact.

Cuticle (Figs. 81-83)

The cuticle is 7.5-8.0 microns in thickness with little variation. The external surface is somewhat irregular, the inner surface less irregular and closely applied to the apical membrane of the epithelial cells. In general the cuticle is composed of a highly compacted, finely granular, moderately electron-dense material. This cuticle is very homogeneous with no prominent substructure, no pores, or fibrillar structure. External to the cuticle are prominent clumps of an unknown material which is electron-opaque even without the sections being stained with uranyl acetate-lead citrate. The outer layer of the cuticle is a 130 A trilamellar sheet. The outer and very thin inner layers of this sheet are electron-dense.
Below this thin layer is a zone of about 720 A thickness resembling the greater thickness of cuticle in texture but being more compact and more electron-dense. The inner aspect of the cuticle is closely applied to the epithelial cell membranes occasionally penetrating into slight indentations of the cell surfaces. The cuticular material may penetrate a short distance into the intercellular space between the cells. The cuticle would appear to be an intimately associated extracellular product of the epithelial cells. Fine filaments extend into the epithelial cell cytoplasm from patches on the apical cell membranes suggesting sites of attachment between the epithelial cells and the cuticle.

**Epithelial Cells** (Figs. 84-93)

**General**

The epithelial cells are cuboidal in shape, about 6 to 7 microns in height, with regular apical and basal surfaces. The lateral surfaces of the cells are highly interdigitated and attached to one another by junctional complexes. Each cell contains a centrally placed nucleus embedded in a cytoplasm rich in detail. Occasional large irregular vesicles apparently devoid of contents are seen in these cells (Figs. 80). Filament bundles course between the apical and basal surfaces apparently inserting on each of these surfaces and providing a cytoskeleton for support of the general cuticular organization. Each epithelial cell contains glycogen particles, numerous vesicles, an occasional lipid droplet, a supranuclear centriole pair, mitochondria, rough endoplasmic reticulum and several Golgi complexes.

**Junctional Complexes** (Fig. 85)

These intercellular junctions have the conformation typical to cells which are attached by septate desmosomes. The two 70 A trilamellar
membranes are separated by a rather constant gap of about 170 A. Occasion-
al sequences of cross-bridges typical of septate desmosomes are seen al-
though many of these bridges appear to have been lost during fixation.
The intercellular material appears slightly more dense than the cytoplasm
of the epithelial cells.

Nuclei (Fig. 86)
The nuclei are rounded, slightly irregular in shape and measure 2-3
microns in diameter. The nuclei are delineated by a nuclear envelope,
two irregularly parallel membranes creating a perinuclear cisterna of
varying widths. Numerous ribosomes stud the cytoplasmic surface of the
nuclear envelope and the outer membrane of this envelope is in frequent
continuity with elements of the rough endoplasmic reticulum. Frequent
nuclear pores, each bridged by a thin amorphous septum, traverse the nuclear
envelope. The nuclear ground substance is finely flocculent and contains
scattered peripheral and central patches of heterochromatin. Each nucleus
contains one centrally placed compact nucleolus. The nucleoli are typical
in appearance consisting of a central pars amorpha surrounded by a
nucleolenema consisting of 150 A granules embedded in an amorphous matrix.

Inclusions
The filament bundles coursing throughout the cytoplasm consist of
close parallel arrays of 50 A straight filaments. Occasional patches of
filamentous material are also observed.

Glycogen particles are distributed into two size ranges--compact
α particles of about 300 A diameter and β particles in rosette form
ranging up to 850 A in diameter.

Vesicles ranging from round 300 A diameter structures up to large
irregular structures 1 micron or more in diameter fill the cytoplasm.
These large vesicles often appear devoid of any contents but at other times contain glycogen particles and other cytoplasmic constituents resembling the heterolysosomes of many cell types.

**Organelles**

Each epithelial cell contains a centriole pair located between the nucleus and the apical cell membrane. The centrioles are typical in appearance and oriented with the long axis of one perpendicular to the long axis of the other forming a T-shape. Each member of the pair measures about 0.29 micron in diameter and 0.37 micron long. A 725 A central vesicle is located in the center of each centriole.

The mitochondria in the epithelial cells of *E. bicaudatus* are ovoid in shape, measuring up to 1.4 microns long and are distinctive in appearance. Each mitochondrion is classically and very clearly organized. Each organelle is delineated by an outer limiting membrane. Separated from this outer membrane by a space of about 100 A is an inner limiting membrane occasionally infolded into the center of the organelle to form cristae. The mitochondrial matrix is of low density and contains electron-opaque granules of about 150 A diameter.

One or more Golgi complexes reside in a supranuclear position in each epithelial cell. Each Golgi complex is about 1.0 micron in profile and consists of a small number of rounded or slightly flattened cisternae and numerous 400-550 A round vesicles associated with all aspects of the complex. The cisternae on one face of a complex are devoid of any apparent contents and somewhat swollen while those on the other face contain a moderately electron dense material and are more compact. The vesicles associated with this face may contain a similar material or may appear somewhat empty and be coated on their cytoplasmic surface with a layer of
amorphous material.

Irregular profiles of rough endoplasmic reticulum are common in the cytoplasm of these cells. Ribosomes of 150 A diameter dot the cytoplasmic surface of the membranes of this organelle.

Other Cytoplasmic Features

A few characteristic vesicular structures with a moderately dense granular matrix occur in each epithelial cell (Figs. 90 and 91). These structures are reminiscent of the microbodies or peroxisomes common to vertebrate kidney and liver. It is of interest that microbodies are now being found in the cells of other organisms, for example the protozoan Tetrahymena (Mueller, 1966), and appear to be organelles of wide phylogenetic distribution.

The cytoplasm of an occasional epithelial cell will contain a large number of ovoid membrane bound "secretory granules," many measuring over 1.5 microns in length (Fig. 92). These granules are associated with Golgi complexes measuring up to 1.0 microns or more in profile, considerably larger than is normal to most of the cells. Other epithelial cells may contain one or two small similar granules. It is not known whether these cells which were noted by Shapeero (1962) represent a special population of epithelial cells or merely a functional state common to all of the cells.

Basal Region (Fig. 93)

The filament bundles coursing through the cytoplasm of the epithelial cells appear to insert into patches of dense material associated with thickenings of the basal membrane. Similar patches of dense material are found on the extracellular side of these membrane thickenings. The basal lamina is thick measuring up to 0.35 micron and appears to consist
of filaments of a mucous-like material running parallel to the basal membrane of the epithelial cells. The connective tissue layer contains numerous fibrils of indeterminable length and up to 1.2 microns diameter resembling collagen with a characteristic banding pattern.

Cestoda

Prochristianella penaei (Figs. 94 to 96)

The principal features of the cestode absorptive surface have been clearly defined by Lumsden (1966). Those features are briefly reiterated here using micrographs of *P. penaei* as illustrative material. The cestode is covered by a synctial epidermis which consists of two major components. The outer aspect of the tegument forms a continuous cytoplasmic layer enclosing the helminth. The surface membrane of this tegument is greatly elaborated in surface area being formed into numerous digitiform microvilli. No organelle other than mitochondria are found in this outer region. The nuclei and most of the other organelles are located in cytoplasmic pockets sunk into the sub-tegumental region which are connected to the outer aspect of the tegument by a number of constricted passages. This organization is clearly depicted in Fig. 96. Included in the perinuclear cytoplasm are numbers of free ribosomes, rough endoplasmic reticulum, mitochondria, Golgi complexes and occasional lipid droplets.

Rotifera

Asplachna brightwelli and Philodina acuticornis odiosa (Figs. 97 to 100)

The surface of the body wall of the rotifer has been described by Koehler (1964, 1966). Certain details of these findings are reviewed and illustrative micrographs of body wall and stomach are presented to facilitate comparisons with acanthocephalan surface structure. The features
of the syncytial rotifer integument bare an amazing resemblance to those of the acanthocephalan body wall. External to the surface membrane of Asplachna is a thick crinose surface coat. A less well-developed surface coat is evidenced on the surface of Philodina. The external surface area of the body wall of the rotifers is elaborated by the presence of distinct surface crypts, the crypts of Asplachna being precisely bulb shaped, those of Philodina being more irregular in shape. A complex terminal web analogous to that in Moniliformis is found in both rotifers. Not only is the outer aspect of the terminal web differentiated into a more dense layer, but an elaboration of the terminal web forms an annulation in close apposition of the membrane of the necks of the surface crypts.

Many rotifers have been taxonomically placed on the basis of being "loricate" or having a thick cuticle, Philodina being an example. In studying Philodina it becomes clear that the "lorica" is actually a thickened cytoplasmic terminal web. A analogous structure contributed to the mistaken impression that acanthocephalans had a cuticle.

The rotifer body wall contains glycogen particles (noteably both α and β particles in Asplanchna and apparently only β particles in Philodina), mitochondria, Golgi complexes, rough endoplasmic reticulum and various vesicles. Also apparent in the body wall of Philodina are filament bundles coursing through the cytoplasm which insert into the terminal web on one end and into patches of dense material on the inner aspect of the basal membrane associated with desmosomes.

The absorptive surface of the stomach of Philodina is also elaborated by means of surface crypts (Fig. 100). Of interest is the fact that the terminal web surrounding these structures actually forms a crystalline basket around each of the crypts (Koehler, 1966: Mattern and Daniel, 1966).
GENERAL DISCUSSION

Although the organization of the acanthocephalan body wall is characteristic of this group, no cytological features have been described in this study which, in general, are not found in other eucaryotic cells. Many of these points of structure have recently been confirmed by Wright and Lumsden (1969). As mentioned previously the absorptive surface of Moniliformis dubius has many elements in common with the absorptive surface of the vertebrate intestinal epithelial cell -- a distinctive polysaccharide surface coat, a means of elaboration of the surface area of the absorptive surface and a cytoskeleton supporting this increased surface area.

The major features of the Streifenzone are termed "surface crypts" rather than "pore canals" as used by other authors. The term crypt defined as "a slender pit" seems more appropriate than the term canal which is anatomy usually applies to a tube or duct. As calculated these surface crypts provide a forty-fourfold increase in the surface area of a twenty-one day old male worm. This figure may be compared to similar computations by Bequin (1966) of the elaboration in the area of the absorptive surfaces of three species of cestodes (3.1 to 6.1 times), the columnar epithelial cells of the moust intestine (26 times) and the intestinal epithelial cells of Ascaris (126 times). The lanthanum experiments clearly demonstrate the continuity of the lumen of the surface crypts with the milieu exterieur. This continuity previously has been affirmed by Edmonds and Dixon (1966) who reported that they were observing pinocytosis.
The filamentous cytoskeleton which provides a structural basis for the surface crypts and occupies such a large proportion of the Streifenzone is called a terminal web on the basis of its resemblance to the similar structure in the ectoplasmic zone of intestinal epithelial cells (see Fawcett, 1966; Cardall et al., 1967). The terminal web in these cells shows a histochemical predominance of protein (Puchter and Loblon, 1958). Crompton (1963) obtained similar histochemical results for this region of the acanthocephalan body wall. Such cytoskeletons as detected by a specific histochemical technique (successive treatment with tannic acid, phosphomolybdic acid and amino black) have been demonstrated in a large number of cell types of Leblond et al. (1960). These "cell webs" are arranged in a characteristic manner in any given cell type and are suggested to have a function in shaping the cells. Apparently the combination of the outer sheet of the terminal web and the plasma membrane is the region of the acanthocephalan body wall which over the years has been reported as a "cuticle". It must be reiterated that the acanthocephalans do not possess a structure analogous to the cuticles of such groups as the nematodes, annelids or arthropods.

A substantial body of evidence has been presented demonstrating that extracellular carbohydrate-containing materials are a common feature of the surfaces of cells (see Revel and Ito, 1967; Cook, 1968a, b). These materials have been termed collectively the "glycocalyx" by Bennett (1963). Ito (1965) has introduced the term "surface coat" to apply to the surface-associated "fuzz" on the absorptive surface of the mammalian intestinal epithelial cell. To term the extracytoplasmic component of the acanthocephalan outer surface the "surface coat" is merely to broaden Ito's definition to include the carbohydrate-containing constituent of
all specialized absorptive surfaces. It must be realized that "coat" is a misnomer in that the extra-cellular materials are now considered to be a part of the periphery of cells, not transient layers or coverings on the cells such as goblet cell mucous (Cook, 1968b). The body wall of _M. dubius_ has two clearly delineated extracytoplasmic carbohydrate-containing components -- the surface coat and the basal lamina.

Wright and Lumsden (1968) have demonstrated that the surface coat of _M. dubius_ is a polyanionic carbohydrate-containing substance. In the current study the surface coat binds lanthanum salts and colloidal thorium dioxide. The lanthanum generally stained the filaments of the surface coat and also occurred in scattered patches along its outer fringes. Such lanthanum staining was first reported by Doggenweiler and Frenk (1965) in various nervous tissues. A similar patchwork pattern of staining occurs in the region of the intestinal brush border of the mouse (Overton, 1967). Lesseps (1967) found that the extracellular lanthanum-staining material of embryonic chick cells could be digested by phospholipase C. He speculated that this material could contain lipid, protein and/or carbohydrate. Recent work indicates that the surface coat of cells is composed of glycoprotein (Benedetti and Emmelot, 1967, 1968; Rambourg and Leblond, 1967; Cook, 1968b). Definitive work on the basal lamina of the glomeruli of bovine renal cortex by Spiro (1967a, b, c) clearly indicates that this portion of the glycocalyx is composed of glycoprotein.

The unusual sieve-like effect displayed by the surface coat of _M. dubius_ during en bloc thorotrast staining was not obtained by Wright and Lumsden (1968) and remains unexplained. Even though the colloidal thorium particles were generally excluded from the surface crypts during
en bloc staining, some material in a portion of these structures did bind thorium micelles to epoxy thin sections. More interesting was the binding of thorotrast over structures termed vesicles which contain a material morphologically reminiscent of the surface coat. It is tempting to speculate that these vesicles function in the transport of the surface coat from its sites of synthesis to the surface of the helminth. Current evidence implicates the Golgi complex as the site of synthesis of such carbohydrate-containing compounds (Neutra and Leblond, 1966, 1969; Rambourg et al., 1969); however, no distinct association was observed between these vesicles and the Golgi complexes in the body wall of *M. dubius*. The role of these vesicles is a problem which should be pursued further.

It is interesting that the surface coat was not stained with ruthenium red as would be expected from studies on other tissues (for example see Groniowski et al., 1969). Positive ruthenium red staining of the membrane of the surface crypt may be used to further differentiate this membrane from the membrane of the outer surface. Luft (1965) showed that ruthenium red binds polyaacids of a high charge density. Staining was more intense for acid mucopolysaccharides than for silicate acid-containing compounds. Ruthenium staining was limited to the outer leaflet of the surface membranes of intact cells (Luft, 1966; Kelly, 1967). The membrane of the surface crypts distinctly was stained on both the lumenal and cytoplasmic leaflets.

*M. dubius* provides an ideal model system for studies on the composition, biosynthesis and functional significance of the surface coat and this surface coat should be particularly accessible to isolation. It should be remembered also that helminths in the vertebrate intestine are notably resistant to the digestive and immunological mechanisms of the host... The reason may well be the presence of this carbohydrate-containing coat.
Positive cytochemical evidence for acid phosphatase activity (Goldfischer et al., 1964) establishes the presence of lysosomes in the body wall of M. dubius. Lead phosphate precipitation in the surface crypts appears to confirm the observation of Rothman (1967) of acid hydrolase activity in these structures. The morphological diversity of lysosomal types in the body wall cytoplasm was unexpected but only limited information of their functional significance was obtained. Generally the lysosomes appear to be heterolysosomes involved in the digestion of phagocytized or pinocytized materials (see DeDuve and Wattiaux, 1966). None of the lysosomes were involved in autolysis or digestion of normal components of the body wall cytoplasm. The small type I lysosomes were not involved in digestion and because of their predominant occurrence in the assumed region of pinocytotic activity are thought to be primary or virgin lysosomes.

One of the more interesting parts of this study were the experiments on protein uptake. First, recall that the neck regions of the surface crypts contain a small meshwork of fine filaments. The lanthanum experiments emphasized these filaments by causing them to appear in negative image. Thorium dioxide micelles of a diameter of more than 85 Å were excluded from the surface crypts during en bloc staining of fixed material even though thorotrust staining of epoxy thin sections indicated the presence of polyanionic substances in the crypt lumens and ruthenium red staining indicated the presence of polyanionic components in the membranes delineating the crypts. Thorium dioxide micelles and ferritin molecules were also excluded from the surface crypts when worms were incubated in vitro in the presence of these large molecules. Only questionable evidence was obtained for the actual uptake of a few small thorium micelles in spite
of the fact that both thorium and ferritin brought about a noticeable change in the contents of the crypt lumens and an apparent increase in lysosomal activity in the Streifenzzone. In contrast, the 3,3'-diaminobenzidine reaction product, indicating the presence of horseradish peroxidase, filled many of the surface crypts and occurred in numerous lysosomes in the region of the crypts after the in vitro-incubation of worms with this protein molecule. It must be concluded that the fine filaments in the neck region of the surface crypts are acting as a molecular sieve restricting entrance into these structures of molecules larger than a certain size. Recall that the thorium dioxide micelles ranged from less than 85 A to greater than 300 A. Only a few of the smaller micelles bypassed the neck of the crypts. Ferritin is a large protein with a molecular weight approaching 500,000 and a diameter of 110 A (Farrant, 1954; Harrison, 1959; Bruns and Palade, 1968). Horseradish peroxidase is much smaller having a molecular weight of 40,000 and a diameter estimated from 25-30 A (Karnovsky, 1967) up to 50 A (Clementi and Palade, 1969). Thus the sieves in the necks of the surface crypts admit particles with a maximum diameter of 80-85 A and the practical maximum size probably being somewhat less than this. This system of molecular sieves is comparable to the theorized small pore system of mammalian capillaries (Grotte, 1956; Landis and Pappenheimer, 1963). These small pores are believed to have a maximum diameter of 70-90 A. Interestingly, the capillary small pores apparently consist of filamentous sieves interposed in extracellular passages through or between endothelial cells with the morphology of the sieve and the passage being peculiar to the location of the endothelial cell (Karnovsky, 1967; Clementi and Palade, 1969). The size and mesh of the sieves, not the size of the particular passages, would appear to convey the specificity
to the small pore system. No explanation can be offered for the apparent entrance of a mixture of thorotrast and carbon particles into the surface crypts of M. dubius during the experiments of Edmonds and Dixon (1966). Carbon particles which are reported to have diameters from 200-500 A (Biozzi et al., 1953) as well as thorium micelles would be expected to be excluded from entrance into the crypts.

Pinocytosis is considered to be a co-ordinated sequence consisting of three stages: a reversible binding to the cell surface or specifically to the surface coat, the subsequent non-reversible metabolically linked passage of the material into the cell and, lastly, a complex series of morphological and chemical events within the cell (Nachmias and Marshall, 1961; Gosselin, 1967; Brandt and Freeman, 1967). With M. dubius all three of these stages have been demonstrated to some extent. All of the tracer molecules in the morphological studies were bound to the surface coat. The horseradish peroxidase in addition gained entrance to the surface crypts and is assumed to have been pinocytized being subsequently found in lysosomes in the Streifenzone. Because ferritin and thorotrast molecules were excluded from the surface crypts and generally were not pinocytized, it may be assumed that the crypt membranes are the site of pinocytosis (unless the size of these molecules alone would preclude uptake). M. dubius offers an ideal system for studies of pinocytosis. By utilizing molecules of greater than 85 A diameter and thus preventing entrance into the surface crypts, the binding step in pinocytosis can be isolated and examined without the need of metabolic blocking of the subsequent steps. The rate of pinocytosis by M. dubius is of some interest but the experiments on uptake of labeled protein were so beset by artifacts that no speculations can be made in this direction.
Of more interest is the question of why *M. dubius* has the facility of pinocytosis by its absorptive surface. Is this phenomenon an ancestral feature which has been retained or does pinocytosis play a major role in the economy of the worm? Read and Kilejian (1969) have recently shown that *Hymenolepis diminuta* (Cestoda) which occupies the same environment (i.e. the rat small intestine) as *M. dubius* undergoes a diurnal migration in the intestine which appears to be related to the feeding habits of the host. This perhaps allows the tapeworm to follow the bolus of food in the host intestine and to take advantage of the normal feeding and digestive rhythms of the host. Independent studies by Threadgold (unpublished) and Lumsden (unpublished) have shown that *H. diminuta* lacks the ability to pinocytize or digest extracellular protein molecules. *M. dubius* is generally found attached to the intestinal mucosa by its thorny anterior end. Perhaps *M. dubius* does not migrate as does the tapeworm and requires pinocytosis and limited extra-cytoplasmic digestion in the surface crypts to best take advantage of its environment.

The organization of the body wall of *Priapulus* bears little resemblance to that of the acanthocephalans. A distinct chitinous cuticle (Carlisle, 1959; Shapeero, 1962) lies over a cuboidal epithelium. The older contention that the priapulids are pseudocoelomate (Lang, 1953) is questioned by recent reports of a nucleated peritoneum (Shapeero, 1961, 1962). The priapulids molt in a manner resembling that of arthropods. (Note that Cable (1965, personal communication) has recently reported possible molting by an acanthocephalan.) The organization of the priapulid epidermis closely resembles that of the arthropod (see Smith, 1968; Locke, 1968), but there appear to be a number of structural differences between the priapulid and arthropod cuticles (see Noble-Nesbitt, 1968; Neville et al., 1969).
As with the priapulids, the structure of the cestode tegument argues against a close relationship to the acanthocephala; however, when the structure of the acanthocephalan and rotifer body walls is compared, one is quickly impressed by a number of common structural features. The most obvious of these is the surface elaboration of the syncytial body walls by means of crypts, a feature unique to these two groups. (The surface of the coenocytic epidermis of the digenetic trematodes is elaborated by means of indentations; however, these bear little resemblance to the surface crypts of the acanthocephalans and rotifers.) In both cases, the surface crypts are supported by a cytoplasmic terminal web, a feature which has contributed to the mistaken idea that the members of both of these taxa possess cuticles. The terminal webs are tripartite having distinct outer regions, more extensive inner regions surrounding the crypts and a differentiated cytoplasmic annulation encircling the neck of each surface crypt. Also in common are the bundles of cytoplasmic filaments coursing between the inner and outer surfaces of the body walls of these two groups. These filament bundles appear to insert into the terminal webs.

To examine one structural feature of these four taxa is not considered sufficient to offer definitive data regarding the relationships involved. In fact my feelings about such an inquiry are amply expressed by Chitwood (1940)—

"Even to enter a discussion of..... relationships invites criticism and focuses a spotlight on one's ignorance of zoology. It is difficult for a scientist to obtain sufficient knowledge to speak with accuracy concerning his specialty and whether or not he can do so outside his specialty remains to be seen."

Other arguments have been raised supporting a close relationship
between the Acanthocephala and Rotifera. Von Haffner (1942) considered
the ligament strand as a greatly reduced digestive tract and pointed out
that a similar reduction of the gut to a strand occurs in male rotifers.
He also felt that the nephridial and reproductive systems enter a common
cloaca represented by the bursa in the Acanthocephala. Von Haffner (1950)
argued for a common invagination of the anterior part of the body by
specific retractor muscles and a common segmentation of the egg. Only
Remaine (1963) has taken Von Haffner's arguments seriously in recent years.
Interestingly the crypts of the rotifer stomach are not only similar in
morphology but also in function to those of the acanthocephalan surface.
Koehler (1966b) has demonstrated the involvement of these structures in
the uptake of large molecules.

This study should encourage not only the examination with the electron
microscope of other systems in the Acanthocephala, Rotifera, Priapulida
and Cestoda for clues as to phylogenetic relationships but should also
interest investigators in the groups about which we know even less -- the
Gastrotricha, Kinorhyncha and Nematomorpha. Contrary to the statement
of Van Cleave (1941), we now perhaps have the means to determine the
relationships of the Acanthocephala.
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LIST OF ABBREVIATIONS

An, Annulation  n, Nucleolus
B, Hypodermal bulb N, Nucleus
BL, Basal lamina  P, Pseudocoele
C, Surface crypt  S, Streifenzone
Ce, Centriole  SC, Surface coat
Cu, Cuticle  T, Tegument
Ep, Epidermis  TW, Terminal web
ER, Endoplasmic reticulum  v, Vesicle
f, Filaments  V, Vesicle (large)
F, Filzfaserschichte  Lysosome types
G, Golgi complex  1, Round
gly, Glycogen  2, Flattened
L, Lipid  3, Univesicular
Lu, Lumen  4, Multivesicular
M, Mitochondrion  5, Lysosome
mt, Microtubule  
Mu, Muscle fiber  

Figures 1 and 2 Phase-contact photomicrographs of cross-sections of the trunk wall of Moniliformis dubius (16 day old male).

Figure 1 Phase optics stress the cytoplasmic continuity of the coenocytic trunk wall. Neither partitioning cell membranes nor any delineation of true layers may be seen. Numerous lipid droplets are apparent. These lipid inclusions often appear to be coalescing to form larger aggregations. Internal to the trunk wall is the basal lamina and a connective tissue layer, circular and longitudinal muscle layers and the pseudocoel. x 620.

Figure 2 The outer region of the trunk wall depicted in this photomicrograph is relatively free of lipid droplets. Longitudinal furrows in the helminth surface as shown here are relatively common. The Streifenzone is apparent but structural details are too minute to adequately resolve with the light microscope. x 2150.
Figure 3 *Moniliformis dubius* (40 day old male). A relatively low power electron micrograph of the Streifenzone (S) and a portion of the Filzfaserschicht (F). The Streifenzone is characterized by numerous vesicular structures whereas the Filzfaserschicht contains lipid droplets, a felt-work of fibrous material and large numbers of electron-opaque glycogen particles. The large numbers of glycogen particles which were found in this particular worm are somewhat unusual. × 25,800.
Figure 4 Moniliformis dubius (21 day old female). This micrograph depicts the major features of the Streifenszone. The worm is coated with a surface coat (SC) of a fine filamentous nature. Numerous surface crypts (C) appear as large scattered vesicular structures with elements occasionally appearing to course to the surface of the helminth. The crypts are separated by patches of moderately electron-opaque material (\#). Mitochondria (M), glycogen particles, microtubules, and other cytoplasmic details are evident in the inner portion of the Streifenszone. Bundles of fine cytoplasmic filaments (f) extend between this region and the deeper cytoplasm of the body wall. × 34,350.
Figures 5 and 6 Moniliformis dubius (21 day old male). Examples of varying conformations of the surface crypts (C). These slender pits in the surface of the worms serve to elaborate the total membrane area of the absorptive surface. A single crypt may have one, two, (Fig. 6) or possibly more connections to the milieu exterieur. Generally, the crypts have a rough conical shape with a broad base tapering somewhat to a narrow neck. Although the membranes delineating two pits come into close apposition, there is no evidence that the basal regions of these structures interconnect. A finely flocculent material fills the surface crypts. Occasionally small vesicular or myelinated profiles can be seen in the crypts. These are apparently small protrusions of the crypt wall into the lumen which appear to be isolated as a result of the plane of section. Figure 5, x 30,000. Figure 6, x 27,000.
Figures 7 and 8  Moniliformis dubius. The external region of the Streifenzone at a high magnification. The carbohydrate-rich surface coat (SC) is composed of loose layers of fine filaments and tiny clumps of a mucous-like material. No intrusion of this coating into the surface crypts is evidenced. The trilamellar surface membrane lies over a thin cytoplasmic zone which is of low electron density.

Figure 7 About 500 A beneath the surface membrane lies a dense band which merges with a slightly less dense underlying mass (TW). This accumulation of very fine electron-opaque cytoplasmic filaments is referred to collectively as the terminal web. A small condensation of these filaments forms an annulation (An) around the neck of each surface crypt. The combination of the surface membrane and the outer dense band of the terminal web is probably the feature that has been referred to by light microscopists as the cuticle. (21 day old female), x 68,400.

Figure 8 The terminal web forms a matrix permeated by large pores through which the surface crypts extend. A thin cytoplasmic layer lies between the membrane of the surface crypt and the fine filaments of the terminal web. A distinct interface often is apparent at the meeting of this cytoplasm and the terminal web. Microtubules (mt) and filament bundles extending from the deeper regions of the body wall often extend along the crypt membrane through this cytoplasmic sheath. (14 day old male), x 68,400.
Figure 9  *Moniliformis dubius* (14 day old male). The surface membrane. This trilamellar membrane shows no asymmetry as is common to the membranes of many absorptive surfaces; however, a layer of material from the surface coat is closely associated with the outer electron-opaque leaflet of the membrane (arrows). Little detail can be perceived in the cytoplasmic layer immediately subjacent to the surface membrane. x 153,000.

Figures 10 and 11  The arrows delineate the approximate extent of penetration of morphologically recognizable surface coat into a surface crypt. The filamentous annulation around the constricted neck of the crypt is apparent. A loose meshwork of material can be seen to congregate in this neck opening at the level of the annulation. The surface membrane is continuous with the crypt membrane but a transition in thickness from about 115 Å to 95 Å occurs around the level of the annulation. Fig. 10 (21 day old male), x 76,500. Fig. 11 (14 day old male), x 153,000.

Figure 12  A high magnification view of a portion of a surface crypt. A slightly flocculent material is seen in the crypt (C) but no luminal coating on the surface crypt membrane is apparent. The luminal leaflet of the trilamellar membrane appears to be more electron-opaque than the cytoplasmic leaflet. (14 day old male), x 153,000.
Figure 13 Moniliformis dubius (21 day old male). A tangential section of the outer Streifenzone. Note the pubescent nature of the surface coat (SC). Each annulation (An) stands out as a ring-like structure around a neck of a surface crypt. The diameter of the necks may be as small as 120 Å (arrow). The filamentous nature and continuity of the various elements of the terminal web are quite apparent. A cytoplasmic sheath of about 650 Å thickness surrounds each crypt (C). x 57,750.
Figures 14 and 15 *Moniliformis dubius* (21 day old male). Tangential sections through deeper portions of the Streifenzone. The principal formed elements in the cytoplasmic sheath surrounding each crypt are microtubules (arrows), glycogen particles (gly) and various membrane-bound vesicles.

Figure 14 A section from the middle region of the Streifenzone. x 57,750.

Figure 15 A section from the inner region of the Streifenzone depicting the inner aspects of the surface crypts and terminal web. x 57,750.

Inset Cross-section. When two surface crypts come into contact, the cytoplasmic leaflets of each of the trilamellar membranes appear to fuse in a fashion similar to that seen in the myelinated Schwann cells lying around axons. x 79,800.
Figures 16 and 17 Moniliformis dubius. Two more micrographs of the inner aspect of the Streifenzone. Numerous examples of a regular interface between the cytoplasm and the terminal web can be seen.

Figure 16 The inner extent of the terminal web (#). This region of the trunk wall abounds in cytoplasmic detail including mitochondria, glycogen particles, microtubules, small vesicles, profiles of what might be endoplasmic reticulum and occasional lipid droplets (L). (14 day old female), x 36,750.

Figure 17 The bundles of filaments (f) which extend from the trunk wall into the cytoplasmic sheath surrounding each surface crypt (C) are particularly evident in this micrograph. (21 day old male), x 36,750.
Figures 18 to 22  *Moniliformis dubius*. Cytoplasmic features common to the inner aspect of the Streifenzone. A group of vesicular structures which are termed lysosomes on the basis of cytochemical evidence are designated numerically according to structure.

Figure 18  This micrograph covers an area extending from the region of the base of the surface crypts (C) to the felt-like fibers of the Filzfaserschicht (F). Small Golgi complexes (G) and occasional lipid droplets (L) occur in this region. Type 3 lysosomes have a finely granular matrix and a univesicular structure. This appearance might be achieved by sectioning through a type 2 lysosome (Fig. 21). (14 day old male), x 41,250.

Figure 19  An example of a small spherical type 1 lysosome with a finely granular matrix. Mitochondria (M) in the cytoplasm of *M. dubius* typically have few infoldings of the inner limiting membrane to form cristae. Small ovoid vesicles (v) containing a loose filamentous material are common throughout the trunk wall cytoplasm but occur in greatest numbers in the region of the surface crypts. Evidence suggests that these vesicles are not lysosomes. (21 day old male), x 66,000.

Figure 20  Two more examples of type 1 lysosomes in close proximity to a surface crypt (C). (14 day old male), x 66,000.

Figure 21  Vesicular structures with a finely granular matrix which are flattened or pinched in at the center are termed type 2 lysosomes. (14 day old male), x 66,000.

Figure 22  Multivesicular bodies are considered to be type 4 lysosomes. The small internal vesicles typically appear to contain little material. (21 day old male), x 66,000.
Figure 23 *Moniliformis dubius*. Absorptive surface of the cystacanth. The cystacanth body wall shows much less cytoplasmic detail and less diversity in structure than the adult. The carbohydrate-rich surface coat (SC) is very thick and is composed of fine, tightly packed filaments and beads. A zone which is analogous to the Streifenzone is present but the prominent surface crypts of the adult are poorly developed. The Filzfaserschicht (F) is thin and its fibrils are closely packed. x 30,800.

Inset The structure of surface details at a higher magnification. A layer of the surface coat is closely applied to the outer leaflet of the trilamellar membrane. The small pits in the surface of the cystacanth are structurally reminiscent of the adult surface crypts. The terminal web forms a distinct annulation around the neck of these pits. x 60,000.
Figures 24 and 25: Moniliformis dubius. The surface morphology of 1 day old worms is indistinguishable from that of older worms. Clearly, the major events in differentiation of the absorptive surface must occur during the first 24 hours after introduction of the cystacanth into the rat host. Two examples of these changes are shown.

Figure 24 One hour after oral infection of the rat, the juveniles are in the small intestine and attached to the mucosa. Already surface crypts (C) are well developed and the fibrils of the Filzfaserschicht have begun to disperse. Surface coat morphology still resembles the cystacanth with occasional disruptions. x 46,950.

Figure 25 At six hours after infection the Streifenzone is more clearly organized. Note the concentration of surface coat in the necks of the surface crypts (C). x 46,950.
Figures 26 and 27 Moniliformis dubius (14 day old male). The Filzfaserschicht or fibrous felt layer of the body wall.

Figure 26 The outer region of this zone. This portion of the body wall is characterized by the presence of large fibrils woven into a felt-work. Mitochondria, lipid droplets (L), glycogen, vesicular structures and profiles of what might be smooth endoplasmic reticulum abound in this zone. x 36,000.

Figure 27 The surfaces of lipid droplets (L) are usually indistinct and are closely associated with large numbers of glycogen particles. The lipid is of fairly homogeneous appearance and has a low affinity for osmium tetroxide. x 36,000.
Figures 28 and 29  *Moniliformis dubius*. Filzfaserschicht.

Figure 28  Even at a high magnification little structural detail about the fibrils of the Filzfaserschicht is revealed. Note the apparent substructure of the glycogen particles. (14 day old male), $\times$ 72,000.

Figure 29  The closely packed fibrils are intermingled with large numbers of glycogen particles. The somewhat limited organization of *M. dubius* mitochondria can be seen. An occasional crista can be found penetrating the dense granular mitochondrial matrix. (21 day old male), $\times$ 29,000.
Figure 30 *Moniliformis dubius*. Cystacanth Filzfaserschicht. The fibrils in the cystacanth are ordered and tightly packed with frequent cytoplasmic glycogen particles between the fibrils. × 44,400.
Figures 31 and 32 Moniliformis dubius. The inner four-fifths of the body wall has been designated the Radialfibrillenschicht by Meyer (1932-33).

Figure 31 A general view of the cytoplasm of this region. Lipid droplets (L) occupy a large portion of the volume of the Radialfibrillenschicht. Many droplets are quite large and would fill many times the area depicted in this micrograph. Mitochondria (M), glycogen particles and well organized Golgi complexes (G) are abundant. The structure designated as a type 5 lysosome occurs only in this portion of the body wall. Small vesicles containing a loose filamentous material are common in this region. (21 day old male), x 42,000.

Figure 32 The name of this region is based on large numbers of radially oriented bundles of fine filaments. These filament bundles appear to form structural bridges between the inner and outer surfaces of the body wall in much the same fashion as the spokes of a wheel. (21 day old female), x 49,500.
Figure 33 *Moniliformis dubius*. Large nuclei (N) are found in the basal region of the body wall. These nuclei are very irregular in shape and contain a number of apparent nucleoli (n). Few patches of recognizable heterochromatin are found. The nuclear envelope is typically penetrated by nuclear pores (arrows). Large numbers of mitochondria are often clustered in the cytoplasm of the Radiarfibrillenschicht. (6 hour old worm), x 25,300.
Figure 34  Moniliformis dubius (21 day old male). Profiles of what clearly may be termed granular endoplasmic reticulum (ER) were found only in the perinuclear cytoplasm. Note also the multivesicular body (4) and the frequently occurring vesicles (v). The thin nuclear envelope is frequently traversed by nuclear pores (arrows). Concentrations of a fine material occur around these pores. The nuclear ground substance (N) is rich in electron-opaque granules embedded in a finely flocculent matrix.  x 51,000.
Figures 35 and 36  *Moniliformis dubius* (21 day old female). Nucleolar fine structure.

Figure 35  This somewhat compact nucleolus is typical in appearance. The fine closely packed granular material in the center is the pars amorpha. The peripheral nucleolonema consists of many 150 A granules embedded in a finely-textured matrix. The typical ribbon-like nature of the nucleolonema is not apparent.  x 45,900.
Figure 37 Moniliformis dubius (14 day old male). The inner surface of the body wall. The membrane of this surface is elaborated into numerous intertwining thin channels. Golgi complexes (G), glycogen, vesicles, and type 5 lysosomes occur frequently in this region. The radial filament bundles (f) of the body wall appear to anchor on the basal membrane at sites resembling half-desmosomes. Internal to the body wall is a basal lamina (BL) of fine amorphous material. The underlying connective tissue contains many thin fibrils which appear to be beaded along their length and hollow in cross-section. x 36,000.
Figures 38 and 39. *Moniliformis dubius*. The continuity of the lumen of the surface crypts of the Streifenzone with the *milieu exterieur* is clearly demonstrated by fixing the helminths with solutions containing lanthanum salts. Unstained sections.

Figure 38. The lanthanum lightly stained the surface coat with heavy clumps adhering to its outer fringes. x 37,500.

Figure 39. Two surface crypts are almost entirely filled with the electron-opaque lanthanum. x 114,700.

Inset. Generally no lanthanum was found in the neck region of the crypts (as above), an exception being shown here. The lanthanum acts as a negative stain to demonstrate the mesh-work of fine filaments located in this neck region.
Figures 40 and 41 Moniliformis dubius. Micrographs of the absorptive surface of specimens which have been stained en bloc with Thorotrast. The surface coat apparently has acted as a sieve retaining the larger thorium micelles at its outer surface and allowing the smaller particles to pass inward. Thorium micelles are bound directly to the individual filaments and beads of the surface coat (note Fig. 41). The thorium particles appear to be excluded from the surface crypts. Uranyl acetate-lead citrate. x 47,250.
Figures 42 and 43. To determine whether thorium-binding material is present in the surface crypts, Epon thin sections were stained with Thorotrast.

Figure 42. Thorium micelles bind to the epoxy sections in large numbers over the region of the surface coat (SC). The thorium binding over a number of surface crypts (C) is well above background indicating some polyanionic material in these structures. Unstained. × 43,500.

Figure 43. Heavy thorium binding occurred over the structures which have previously been identified as vesicles. This is suggestive that the material in these vesicles is a polyanion, possibly the same material which constitutes the surface coat. Unstained. × 43,500.

Figures 44 and 45. Micrographs depicting the detailed morphology of these vesicles (v) in proximity to the surface crypts (Fig. 44) and in the deeper cytoplasm (Fig. 45). These are vesicular structures composed of a single trilamellar membrane and containing a concentration of a filamentous material reminiscent of that material constituting the surface coat. Uranyl acetate-lead citrate. × 87,000.
Figures 46 to 48 Moniliformis dubius. The absorptive surface of specimens which have been fixed in solutions containing ruthenium red.

Figure 46 The membrane of the surface crypt is stained with the ruthenium-osmium complex; however, no staining of the surface coat nor surface membrane was detected. Occasional clumps of the stain were found on the surface coat. Otherwise unstained. x 80,100.

Figure 47 When thin sections of ruthenium treated tissue are stained with uranyl and lead salts, the trilamellar appearance of the surface crypt membrane is greatly enhanced. x 80,100.
Figures 49 and 50  Moniliformis dubius. Thin sections of tissue which has been incubated for acid phosphatase activity utilizing sodium β-glycerophosphate as the substrate.

Figure 49  A general acid phosphatase activity is demonstrated in the surface crypts, possibly associated with the crypt membranes. Unstained. x 42,000.

Figure 50  Lysosomal activity in the deeper tissues. These structures appear to be multivesicular bodies which have enzyme activity in their matrixes but not in the small internal vesicles. Unstained. x 42,000.
Figure 51  *Moniliformis dubius*. Acid phosphatase localization. An unstained section of control tissue. This tissue was incubated in a medium lacking substrate but processed in an otherwise identical manner. x 42,000.
Figures 52 and 53 *Moniliformis dubius*. Acid phosphatase localization (substrate, sodium β-glycerophosphate). The precipitated lead phosphate appears to be concentrated on the membranes of the surface crypts. The small vesicular structures in the region of the crypts containing the reaction product (arrows) are considered to be type I lysosomes. Uranyl acetate-lead citrate. x 39,000.
Figures 54 and 55 *Moniliformis dubius*. Acid phosphatase localization (substrate, sodium $\beta$-glycerophosphate).

Figure 54 Further examples of localization in the surface crypts and adjacent lysosomes. The Thorotrast-binding vesicles (v) are free from acid phosphatase activity. Uranyl acetate-lead citrate. x 29,000.

Figure 55 Acid phosphatase activity in type 5 lysosomes located close to the inner surface of the body wall. These lysosomes produce a reaction product which is restricted to their periphery and contain fine mucous-like filaments in their lumens. Note the generalized cytoplasmic precipitation of lead phosphate. This appears only in the inner portion of the body wall and appears to be a specific reaction. Uranyl acetate-lead citrate. x 39,900.
Figures 56 to 59 *Moniliformis dubius*. Ferritin uptake experiments. Uranyle acetate-lead citrate.

Figure 56 An accumulation of ferritin and mucous-like material occurs in patches on the outer aspects of the surface coat. x 60,900.

Figures 57 to 59 As a result of the ferritin incubations, electron-opaque concentrations of material frequently occur in the surface crypts. These concentrations occur only rarely in controls or material prepared for morphological study. The density of these small clumps preclude any possible identification of ferritin molecules. x 60,900.
Figures 60 to 65: Moniliformis dubius. Ferritin uptake experiments. Corresponding to the appearance of dense concentrations in the surface crypts, similar dense accumulations became frequent in the various lysosomal types in proximity to these crypts. These densities do not occur in the lysosomes of controls nor samples prepared for morphological study. Again these concentrations were too dense to allow ferritin identification although certain small particles are suggestive of the protein molecule. Shown are examples of these accumulations in type 1 (Figs. 60 and 61), type 2 (Fig. 62), type 3 (Fig. 63) and type 4 (Figs. 64 and 65) lysosomes. Note that the dense material is located among the small vesicles in the multivesicular bodies. Uranyl acetate-lead citrate. x 66,000.

**Figure 66** An accumulation of thorium dioxide and mucous-like material occurs in patches on the outer aspects of the surface coat as in the ferritin experiments. x 54,000.

**Figure 67** A mucous-like accumulation on the outer aspect of the surface coat. This might be a particular response of the surface coat to protein molecules in the milieu extérieur. Note the dense particles attached to the surface of the crypt. x 132,000.
Figures 68 and 69 Moniliformis dubius. Thorotrast uptake experiments. As a result of the Thorotrast incubations dense concentrations appeared in the surface crypts. Controls and material prepared for morphological study only rarely have these densities in the crypts. Particles which might be identified as thorium dioxide micelles are observed in these clumps. Uranyl acetate-lead citrate. x 43,500.
Figures 70 to 73. *Moniliformis dubius*. Thorotrast uptake experiments. As in the ferritin experiments, dense accumulations became frequent in the various lysosomal types found in the region of the surface crypts. These densities do not occur in controls nor in samples prepared for morphological study. Some small particles seen in these dense accumulations might be identified as thorium micelles. Scattered thorium particles were found at a low frequency in these lysosomes when observing unstained sections. Depicted are examples of these dense accumulations in type 1 (Figs. 70 and 71) and type 4 (Figs. 72 and 73) lysosomes. Uranyl acetate-lead citrate. x 66,000.
Figure 74  Moniliformis dubius. Horseradish peroxidase uptake experiments. The electron-opaque reaction product of the diaminobenzidine reagent localizing the peroxidase fills the surface crypts. Only a limited amount of reaction product was found associated with the surface coat. The protein tracer is apparently accumulated in the surface crypts. Unstained. x 34,500.
Figures 75 and 76. Moniliformis dubius. Horseradish peroxidase uptake experiments.

Figure 75 Localization of peroxidase in the surface crypts. Unstained. x 37,200.

Figure 76 Control. Worms were incubated in a medium containing no peroxidase but otherwise processed in a manner identical to other peroxidase samples. Unstained. x 37,200.
Figures 77 to 79 Moniliformis dubius. Horseradish peroxidase uptake experiments. The reaction product was found in numerous small vesicles which are considered to be lysosomes in the proximity of the surface crypts. It is apparent that all of the lysosomal-type structures do not contain peroxidase (Figs. 78 and 79). The peroxidase containing bodies may be secondary lysosomes formed by the fusion of pinocytotic vesicles containing the protein with primary lysosomes. Uranyl acetate-lead citrate. x 38,400.
Figure 80. *Priapulus bicaudatus*. The structure of the surface is depicted in a low power electron micrograph. The simple cuboidal epithelium (Ep) is covered by a thick extracellular cuticle (Cu). The epithelial cells rest on a basal lamina, a connective tissue layer and underlying muscle fibers. x 10,800.
Figure 81 *Priapulus bicaudatus*. Details of cuticular structure. The cuticle is composed of a highly compacted, finely granular material and except for a thin outer region is very homogeneous in structure. Bacterial cells were occasionally associated with the outer surface. X 19,450.
Figures 82 and 83 *Priapulus bicaudatus*. Details of cuticular structure.

Figure 82 The outer aspect of the cuticle. External to the cuticle were clumps of a material which is electron-opaque even without uranyl acetate-lead citrate staining. The outer cuticular layer (arrows) is a thin apparently trilamellar sheet. Internal to this layer is a 500 Å granular zone of appearance similar to the deeper cuticular material but noticeably more compact and electron dense. x 42,000.

Figure 83 The inner aspect of the cuticle rests against the apical membranes of the epithelial cells. This cuticle would appear to be an intimately associated extracellular product of the epithelial cells. Fine filaments extend from patches on the epithelial cell membrane into the cytoplasm of these cells suggesting structures resembling half-desmosomes. x 42,000.
Figure 84 Priapulus bicaudatus. The general features of two epithelial cells are seen. These cells are roughly cuboidal in shape with highly interdigitated lateral surfaces. Each cell has one large centrally positioned nucleus (N) and a wealth of cytoplasmic detail including mitochondria (M), glycogen, endoplasmic reticulum and numerous vesicles. A centriole pair (C) and one or more Golgi complexes are found in a supra-nuclear position. Bundles of fine filaments appear to extend between the apical and basal surfaces of the cells. Some tissue damage from fixation is evident. x 21,300.
Figure 85  *Priapulus bicaudatus*. The interdigitating surfaces of two or more epithelial cells. This particular type of conformation is characteristic of junctions involving septate desmosomes although only suggestions of cross-bridges are found here. An examination of a number of micrographs (for example Fig. 81) shows that these junctions do involve septate desmosomes. x 42,000.

Figure 86  Details of the epithelial cell nucleus and perinuclear cytoplasm. The nucleus (N) is delineated by a nuclear envelope which is frequently traversed by nuclear pores (arrows) each having a thin septum separating the cytoplasmic and nuclear ground substance. The cytoplasmic surface of the nuclear envelope is studded with numerous ribosomes in a manner similar to rough endoplasmic reticulum. The nuclear ground substance contains numerous clumps of mostly peripherally scattered heterochromatin. The nucleolus (n) is compacted having a dense central pars amorpha and surrounded by a granular nucleolonema. x 27,300.
Figure 87  Priapus bicaudatus. Details of the epithelial cell cytoplasm. The cytoplasm contains mitochondria (M), alpha and beta glycogen particles, profiles of rough endoplasmic reticulum, patches of filamentous material (f) and numerous vesicles (v). Large vesicles (V) often contain glycogen particles. A Golgi complex (G) and a centriole pair (Ce) lie between the nucleus (N) and the apical cell membrane. x 41,250.

Inset  Another section of the same centriole pair shows a central vesicle (arrow) in one member of the pair. x 41,250.
Figures 88 and 89: Details of the apical cytoplasm of two epithelial cells. Mitochondria (M) display an easily discerned profile. The moderately dense mitochondrial matrix often contains dense granules of varying sizes. These Golgi complexes (G) are typical in appearance. The Golgi complexes are always located in proximity to the apical cell membrane and might be suspected to be involved in maintenance of the cuticle. The nature of the structure V is undetermined. This could be a portion of an adjacent cell which has been isolated by the plane of section. $x \sim 41,250$. 
Figures 90 and 91. *Priapulus bicaudatus*. A few of these vesicular structures resembling the microbody (or peroxisome) of vertebrate liver and kidney occur in each epithelial cell. x 66,000.

Figure 92. The cytoplasm of an occasional epithelial cell is filled with membrane-bound granules associated with a Golgi complex. An occasional small granule of similar appearance may be found in the other epithelial cells. Whether this is indicative of a particular population of cells in the epithelium or simply a functional state common to all of the epithelial cells is not clear. x 28,500.
Figure 93 *Priapulus bicaudatus*. The basal region of the surface epithelial cells. A nucleus (N) and an adjacent large electron-lucent vesicle can be seen in the right hand portion of the micrograph. The numerous bundles of fine filaments (f) appear to anchor in patches of dense material on the basal membrane (arrow). Subjacent to the membrane is a basal lamina (BL) composed of fine filaments. Collagen-like fibrils occur in the connective tissue space. Note the thin extracellular coat on the underlying circular muscle layer. x 33,000.
Figures 94 and 95. Prochristianella penaei. Micrographs depicting the cestode tegument. The absorptive surface is thrown into numerous digitiform microvilli. The cytoplasm contains numerous secretory granules, mitochondria and microtubules. Neither other organelles nor lateral cell membranes are found in this outer portion of the tegument. The tegument rests on a basal lamina, a fibril containing connective tissue matrix and underlying circular muscle fibers. x 36,000.
Figure 96 Prochristianella penaei. The cestode tegument is arranged syncytially with the nuclei and most of the cell organelles located in cytoplasmic regions sunk into the subtegumental zone. The various elements of this organization are shown in this micrograph. The tegumental cytoplasm is connected to the perinuclear cytoplasm by a number of constricted passages. The nucleus (N) lies in a cytoplasmic pocket containing numerous free ribosomes, rough endoplasmic reticulum, mitochondria, Golgi complexes (G) and lipid droplets (L). x 25,300.
Figure 97 Asplancha brightwelli. Detailed view of the integument. The rotifer body wall bears an amazing resemblance to the absorptive surface of acanthocephalans. External to the surface membrane is a thick crinose surface coat (SC). Elements of this surface coat are closely applied to the outer leaflet of the trilamellar surface membrane. The area of the absorptive surface is greatly increased by hypodermal bulbs (B) comparable to the acanthocephalan surface crypt. The surface coat appears to extend into this bulb. A thick filamentous terminal web (TW) adds structural rigidity to this absorptive surface. A portion of this terminal web forms a thickened collar or annulation around the neck of the hypodermal bulb. The body wall contains mitochondria (M), a Golgi complex (G), and numerous glycogen particles. The underlying pseudocoel (P) is filled with various materials. (Micrograph courtesy of Dr. J.K. Koehler). x 57,000.
Figure 98 *Philodina acuticorius odiosa*. Detailed view of the integument. Philodina is an example of a rotifer which has been placed into a taxonomic niche on the basis of having a lorica or thick external cuticle. As it turns out, the "lorica" is not a cuticle but rather an elaboration of the cytoplasmic terminal web (TW). The annulation encircling the neck of the hypodermal bulb (B) is apparent. Note the peculiar nature of the surface coat and the large Golgi complex in the body wall. The internal membrane of the body wall often closely approaches the basal membrane of the bulbs but no continuity between the pseudocoel (P) and bulb lumen has been observed. (micrograph courtesy of Dr. J.K. Koehler--fig. 7, Koehler (1966) constitutes a portion of this micrograph). x 60,000.
Figure 99  Philodina acuticorius odiosa. The body wall is attached to underlying muscle fibers (Mu) by distinct desmosomes across the pseudocoel (P). These desmosomes are connected to the terminal web (TW) of the body wall by bundles of fine filaments (f). A similar cytoplasmic skeleton connecting the terminal web and inner membrane of the body wall is found in Moniliformis. (micrograph courtesy of Dr. J.K. Koehler) x 69,000.
Figure 100 Philodina acuticorius odiosa. Details of the luminal surface of the stomach. The surface area of the rotifer stomach is elaborated by means of numerous crypts (C), the lumen of the crypts being continuous with the lumen (Lu) of the enteron. A portion of the terminal web (TW) surrounding these surface crypts is elaborated into a crystalline basket. Note also the thickened sheet of the terminal web immediately subjacent to the surface membrane. (Micrograph courtesy of Dr. J.K. Koehler) x 42,000.