DISTRIBUTION OF GLYCOGEN IN THE INTESTINE OF *ASCARIS SUUM*

by

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ABSTRACT

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DISTRIBUTION OF GLYCOGEN IN THE INTESTINE OF ASCARIS SUUM

The intestine of Ascaris suum, which runs between the pharynx and the rectum, has been subdivided into three regions. In each region, data on cell height and thickness of the basal lamella were collected and analyzed. The results were viewed against the function ascribed to the region. The change in cell height and in thickness of the basal lamella between each region appears to occur in concert with the gradation in function of the intestine. Chemical analyses revealed that in fresh male and female worms, the levels of endogenous glycogen are highest in the anterior intestine. In starved worms, the drop in intestinal glycogen occurred mainly in the anterior and was mainly due to the TCA-extractable fraction. Differences in cell height, thickness of the basal lamella and glycogen levels are presented as evidence for regional differentiation in the intestine of A. suum.

ABREGE

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LA DISTRIBUTION DU GLYCOGENE DANS L'INTESTIN DE L'ASCARIS SUUM

Chez l'Ascaris suum l'intestin, qui se situe entre le pharynx et le rectum, fut subdivisé en trois régions. Dans chacune d'elles, la taille des cellules ainsi que l'épaisseur de la membrane basale furent mesurées. Les résultats obtenus ónt été analysés en rapport avec la fonction assignée à la région. La variation dans la taille des cellules et dans l'épaisseur de la membrane basale entre chaque région, semble se manifester en accord avec la gradation des fonctions de l'intestin. Des analyses chimiques ont revélé que chez le mâle et la femelle, le niveau de glycogène est le plus élevé dans la région antérieure de l'intestin. Chez le parasite privé de nourriture, la baisse du niveau de glycogène dans le tissu intestinal s'est manifestée principalement dans la région antérieure de l'intestin, attribuable surtout à une baisse du niveau de glycogène extrait par l'acide trichloroacétique. Les différences au niveau de la taille des cellules, de l'épaisseur de la membrane basale et de la quantité de glycogène sont considérées comme preuve à l'appui de la variation régionale de l'intestin d'Ascaris suum.

Suggested short title:

INTESTINAL GLYCOGEN IN *ASCARIS SUUM*.

DENIS CYR

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"Where there's a will, there's a way." H. Jenlink

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CHAPTER I

INTRODUCTION

The intestine of Ascaris suum is a long, straight, tube-like structure that runs the length of the worm between the anterior muscular pharynx and the short rectum. Unlike the vertebrate alimentary tract, the intestine of A. suum is structurally uniform along its length. The intestine of A. suum is dorso-ventrally flattened and is composed of a single layer of columnar cells resting on a basal lamella. Despite the lack of several cell types and glandular secretions, the intestine of A. suum is nevertheless capable of assuming its role as the principal route for the uptake of nutrients (Fairbairn, 1957; von Brand, 1966). By analogy to the digestive system of other parasites, and vertebrates, the intestine of A. suum has been subdivided into secretory and absorptive regions. Thus, Lee (1965) ascribed secretory functions to the anterior, and absorptive functions to the middle and posterior. Although regional differentiation in the intestine of A. suum has been suspected by a few researchers (Chitwood and Chitwood, 1937; Harpur, 1977), virtually little work has been carried out in this particular area.

The present study was undertaken to determine whether or not there is morphological evidence of regional differentiation. The parameters chosen for investigation were the size of cells, the thickness of the basal lamella and the levels of endogenous glycogen. These were examined in all three regions of the intestine. The rationale for this is that since the intestine of A. suum has no accessory glands and is composed of a single cell type, differences between regions may be located in the structural features of cells. Because the passage of material across the intestine may be related to both the lateral plasma membrane and the basal lamella, morphometric data are analysed and viewed against the function ascribed to the region of the intestine. Endogenous glycogen in each region of the intestine is quantified and, by virtue of its involvement in the secretory and absorptive processes, is associated with the level of cellular activity. In the light of evidence concerning the metabolic inhomogeneity between the acid-extractable glycogen and the glycogen extracted by alkali (Stetten et al., 1958), for each tissue sample the glycogen extracted with trichloroacetic was measured separately from the residual glycogen recovered by alkali.

The differences in cell height, thickness of the basal lamella and levels of glycogen between the anterior, middle and posterior intestine, as well as a differential depletion of the endogenous glycogen in each region, are presented therein as

evidence for the concept of regional differentiation in the intestine of A. suum.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

The present techniques employed in the qualitative and quantitative studies of glycogen have been widely investigated during the last few decades, and have provided a great deal of information on the nature and function of the glycogen molecule. The role of intracellular glycogen as an energy reserve for intestinal parasites has been largely emphasized (von Brand, 1979), although the true nature of glycogen extracted by the reagents currently used has been the subject of controversies during the past years (Stacey and Barker, 1962). Therefore, to critically evaluate the functional significance of glycogen in the three regions of the intestine of Ascaris suum, it is important to review (1) the techniques employed for the study of glycogen, (2) the structural and physiological aspects of the nematode intestine, and (3) the fine morphological and functional variations along the intestine of A. suum.

ESTIMATION OF GLYCOGEN FROM TISSUE SAMPLES

1. Introduction

It has been generally recognized since the time of Pfluger

that improvements in the removal of glycogen from animal tissues requires sophistication of both the extraction technique and the method of determination. The first important source of possible error in glycogen estimations from any tissue relates to the completeness of the extraction where clearly the amount of glycogen remaining in the tissue will depend upon the care taken in the extraction procedure. The second possible source of error relates to the method employed for the quantitative estimation of glycogen, by the use of carbohydrate reagents which must exhibit a certain degree of specificity and precision. It is therefore important to review shortly the routine technique employed, and define precisely the polysaccharide that is being quantified.

2. Carbohydrate Extraction Methods

Alkaline Digestion

Claude Bernard, in 1857, was the first to successfully isolate glycogen from tissue by previous treatment with alkali. Consequently, alkali has been widely employed in the extraction of tissue glycogen, especially since the improvement of Pfluger's method brought forth by Good, Kramer and Somogyi (1933). The use of potassium, rather than sodium hydroxide (Somogyi, 1934) for the preparation of glycogen solutions is preferred in order to avoid the formation of solid sodium soaps and of sodium

carbonate which is sparingly soluble in ethanol. It is generally agreed that the use of potassium hydroxide for quantitative estimations of carbohydrates in tissues is most adequate, however, questions were raised as to the quality of the glycogen. Subsequently, investigations were undertaken to study the extracted glycogen from a qualitative standpoint.

From determinations obtained by light scattering methods, Carroll et al. (1956) reported a greater reduction in the molecular weight of glycogen when hot alkali is used instead of cold trichloroacetic acid. Later, Stetten and Katzen (1961) demonstrated that this reduction in molecular weight is due, at least in part, to a chemical attack on the reducing end of the molecule. In fact, the treatment of glycogen with strong alkali produces a 10- to 100-fold reduction in the molecular weight along with chemical alterations of the molecule (Orrell and Bueding, 1958). Further, dialysis experiments by Carroll et al. (1956) on alkali digests of liver revealed the presence of water-soluble, alcohol precipitable, anthrone-sensitive material other than glycogen which persists even after prolonged heating in alkali. Russell and Bloom (1958) indicated that the residual polysaccharide not extracted from tissue by trichloroacetic acid, but rendered soluble by alkaline digestion, has the properties of glycogen since that material was attacked by alpha-amylase. These researchers also concluded that a portion

of the native glycogen, which Lazarow (1942) earlier suggested to be associated with proteins, is bound in such a manner that the degree of attachment is not disrupted by trichloroacetic acid but is liberated by heating with strong alkali.

Bloom and co-workers (1951) introduced in the literature the distinction between an "easily extractable" and a "difficultly extractable" form of glycogen. By difficultly extractable or "residual" glycogen is meant that polysaccharide which remains in tissues after exhaustive extraction by mild reagents such as water or cold trichloroacetic acid (Stetten et al., 1958). In early studies on rat liver and muscle (Russell and Bloom, 1958) as well as in recent work on the intestine of Ascaris suum (Schanbacher and Beames, 1978), the changes in the total glycogen are accounted for by variations in the acid-extractable glycogen whereas the alkali extracted glycogen remains constant in amount. In view of the difference in response to the changes in total glycogen, comparative studies were carried out to evaluate the use of milder reagents such as trichloroacetic acid (Carroll et al., 1956; Stetten et al., 1956) and cold water (Bueding and Orrell, 1961).

Trichloroacetic Acid Extraction

A second method, which employs trichloroacetic acid (TCA), was outlined by Carroll et al. (1956) and has been widely used

during the last few decades. Due to the appreciable reduction in the molecular weight of glycogen extracted with potassium hydroxide, it is clearly desirable for qualitative studies to obtain glycogen which is minimally degraded so as to approach the molecular weight of the macromolecule in its "native state". Regardless of whether sedimentation or light scattering measurements are employed, the extraction of tissue glycogen with TCA instead of KOH is shown to exhibit an appreciably lower degradation of the polysaccharide and a decrease in the molecular weight by a factor of only 10 to 50 (Bueding and Orrell, 1964).

However, for quantitative analyses, trichloroacetic acid being a mild extracting agent removes only a portion of the total glycogen (Kemp and Kits van Heijningen, 1954) and that which is termed the readily extractable fraction (Bloom et al., 1951). Russell and Bloom (1958) measured the proportion of TCA-extractable glycogen and found it to be in the order of 60-70% and 80-90% of the amount determined by both TCA and KOH for rat liver and muscle, respectively. Nevertheless, Carroll and co-workers (1956) maintained that cumulative recoveries of glycogen obtained by five homogenizations in 5% trichloroacetic acid represented up to 97% of the glycogen present.

As mentioned previously, studies on the quantitative relation, between residual and total glycogen extracted from fed and fasted animals, revealed that the proportion of readily

TCA-extractable glycogen is not constant but varies in a consistent manner with the nutritional state of the animal. These findings, along with the fact that the residual glycogen remained relatively constant in amount at all times, led Russell and Bloom (1958) to believe that the acid-extractable glycogen represents a more physiologically active fraction than that extracted from the residue by alkali. In contrast, the interpretation of results obtained by isotope studies, led Stetten and Stetten (1958) to conclude that the residual glycogen represents the most physiologically active form. They suggested that the residual glycogen, which is presumably protein-bound, is at least in part associated with enzymes involved in glycogen synthesis and breakdown. The resulting controversy, as to which fraction is the most physiologically active, is apparently resolved by a third extraction method which uses cold water to obtain onlya slightly altered glycogen macromolecule.

Cold Water Extraction

In contrast to the two previously mentioned methods of extraction, the cold water procedure has revealed itself to be useful, particularly in studies concerned with the characterization of glycogen (Bueding and Orrell, 1961). However, the cold water procedure is not suitable for quantitative estimations of glycogen since appreciable amounts are lost during the

separation step (Bueding and Orrell, 1964). Nevertheless, from the slightly altered glycogen molecule obtained by the mild extraction, sedimentation analyses have revealed a great polydisperse population of glycogen characterized by more or less two regions of sedimentation coefficients in parasites such as Fasciola hepatica (Bueding and Orrell, 1961), Ascaris lumbricoides (Orrell and Bueding, 1964) and Hymenolepis diminuta (Colucci et al., 1966).

Apart from a greater polydispersity, Orrell and Bueding (1958) found the molecular weight of cold water extracted glycogen to be at least 10 times that of glycogen extracted with cold TCA, and 50 to 100 times that of alkali extracted material. Sedimentation analyses revealed that when the cold water procedure is used instead of the hot alkali procedure, a heavy component is detectable. Furthermore, this heavy fraction of glycogen exhibits changes related to the nutritional state of the organism (Orrell, Bueding and Colucci, 1966).

However, the changes in the sedimentation coefficient spectrum of glycogen induced by starvation most likely result from the mobilization of both the light and heavy components of glycogen. Bueding and Orrell (1961) suggested that the high molecular weight fraction may be produced from, or converted into, the lighter component depending on the level of total glycogen.

3. Glycogen Determination Method

The development of microcolorimetric technique for the determination of glycogen has resulted in a large measure in the replacement of the earlier volumetric (Good et al., 1933) and turbidimetric (Cifonelli and Smith, 1955) procedures. Among the many reagents used in colorimetric tests (Dubois et al., 1956), the anthrone reagent has proved to be excellent for the determination of glycogen.

Anthrone Method

The anthrone reagent, which was first used as a qualitative test for carbohydrates, was subsequently adapted for a quantitative estimation of carbohydrates and glycogen by Morris (1948). Seifter and co-workers (1950) recommended the use of the "direct procedure" for tissue of high glycogen content, in which the alkali digest is directly treated with anthrone reagent. However, for tissue of low glycogen content, the "indirect procedure" is recommended, in which glycogen is first precipitated with ethanol.

Since the anthrone reagent can react with material closely associated with glycogen (Carroll et al., 1956) including hexose phosphate (Mokrasch, 1954), it therefore appears preferable to isolate carefully the glycogen from the digest before colorimetric determination. Nevertheless, following an adequate

extraction procedure, the anthrone method has been reported to exhibit a high degree of specificity and precision (Carroll et al., 1955).

4. Conclusions

It seems likely that the use of hot alkali is suited for quantitative studies of glycogen, whereas the cold water procedure is preferable for a qualitative approach. Moreover, the trichloroacetic acid technique appears to be a good compromise between alkali digestion and cold water extraction, in which the quantitative and qualitative aspects of extraction are to some degree retained.

Because of the relatively high levels of recovery (Carroll et al., 1956) and comparatively moderate alteration of the native glycogen (Stetten and Stetten, 1958), the trichloroacetic method was chosen for our work. However, it is emphasized that the accuracy of each technique resides in both the completeness of the extraction and the quality of the glycogen extracted. Particular attention is therefore given to the use of ethanol in the precipitation of glycogen, and in fact a concentration of ethanol exceeding 66% precipitates virtually all of the glycogen present (Handel, 1965).

The qualitative aspect of the material isolated has been investigated extensively, and therefrom glycogen characterized

from a technical point of view. Handel (1965) proposed glycogen to be defined as material from alkaline digestion, precipitable with ethanol, which reacts with anthrone to give an absorption peak at 620 nm.

For all intended purposes, and so as to be consistent with the methods employed in the present work, glycogen is defined as all anthrone-sensitive material with an absorption peak at 620 nm precipitable with ethanol from TCA filtrates and KOH digests.

MORPHOLOGICAL ASPECT OF INTRACELLULAR GLYCOGEN

Among the various types of branched structures for glycogen which have been deduced from chemical investigations, Meyer and Bernfield's tree-like structure of branching polysaccharide chains is presently the most favored. The minute structure of glycogen is comprised of several hundred units of chains of α-1,4 linked D-glucopyranose residues with α-1,6 inter-chain links. However, because of its size, the branching pattern has not yet been observed in the electron microscope. Nevertheless, with the advent of specific staining for electron optical demonstration of polysaccharides, stained material corresponding to glycogen could be observed.

Independently of physico-chemical studies, electron microscopy has enabled researchers to investigate large particles

located in tissue regions which were known to be rich in glycogen (Fawcett, 1955). In agreement with chemical studies, electron microscope observations revealed two basic types of glycogen particles which differ in particle size.

The first type consists of single particles some 20-24 nm in diameter, which Drochmans (1963) called beta particles. The second form of glycogen, the alpha particle, is a rosette-like unit (100-150 nm in diameter) which presumably consists of closely packed beta particles. Both morphological types are believed to represent the native state of glycogen in the tissues. However, the probable existence of intermediate forms cannot be excluded and it is assumed that the morphologically identifiable forms are the extremes of a graded spectrum (Revel, 1963). This assumption is supported by size measurements of glycogen where both alpha and beta particles were reported to exhibit a wide size distribution (Drochmans and Dantan, 1967). Furthermore, these researchers found that, following exposure to a low pH, the beta particles could be subdivided into smaller gamma particles with approximately the same size as the molecule extracted from tissues following alkaline digestion.

In an attempt to characterize the type of glycogen remaining after alkali treatment, Revel (1963) digested liver glycogen with boiling potassium hydroxide and observed the product under the electron microscope. He reported an accumulation of

small particles similar to beta glycogen which had replaced the larger complex units corresponding to alpha particles.

Morphological observations therefore seem to coincide with results obtained from physio-chemical studies, in that alkali treatment does degrade glycogen to its low molecular weight forms. Nevertheless, additional microscopic examinations remain to be carried out so as to look at the variations in the amount of alpha and beta particles during changes in the nutritional state of animals, including Ascaris suum.

CARBOHYDRATE UPTAKE IN ASCARIS SUUM AND OTHER PARASITES

Experimental examinations of nutrient uptake of Ascaris suum have shown the intestine to be the principal site of absorption despite reports that the cuticle is permeable to water, ions and to certain hydrophobic chemicals (Fairbairn and Passey, 1957; von Brand, 1966; Lee, 1965). In spite of reports that glucose is not transported through the cuticle (Mueller, 1929; Cavier and Savel, 1952), Hobson (1948) working with cuticle scraped free of muscle, maintained that the cuticle is permeable to glucose and other substances. However, Hobson's (1948) results were criticized by Roberts and Fairbairn (1965) : they believed to be questionable since cuticle treated as such was not physiologically normal. Castro and Fairbairn (1969) later

demonstrated that cuticular absorption of glucose in A. suum accounts roughly for 2% of the total glucose consumed. As a result of the small amounts of glucose-^{14}C absorbed via the cuticle, it was therefore concluded that the intestine of A. suum is the chief and perhaps the only route for glucose uptake whereas the cuticle's role in the absorption of glucose is quantitatively unimportant.

On account of its supply of hydrolytic digestive enzymes, the intestine of A. suum appears well adapted for the breakdown and assimilation of nutrients (Rogers, 1940; Carpenter, 1952; Lee, 1962a). In addition to disaccharidases viz., sucrase, maltase (Palma et al., 1970) and trehalase (Feist et al., 1965), the intestine also possesses proteolytic enzymes (Juhász, 1979) as well as esterase (Lee, 1962b) and monoglyceride hydrolase (Beames et al., 1974). However, the intestine of A. suum notably lacks lactase activity and galactose is not transported to any significant extent (Beames, 1971), but lactose is not likely to be found in the intestinal contents of the adult pig (Sanhueza et al., 1968). These facts correspond to the worm's inability to synthesize glycogen from lactose and galactose (Beames, 1971), whereas sucrose, maltose, glucose and fructose are assimilated into glycogen (Cavier and Savel, 1952).

The relatively high specific activities of the digestive enzymes, occurring primarily in the brush border region of the

cell, prompted Gentner and co-workers (1972) to assume the involvement of membrane digestion in the uptake of nutrients. The concept of membrane digestion introduced by Ugolev (1965) is believed to occur in vertebrates and may also exist in lower invertebrates such as ascaroids, since cellular function and structure are similar in both A. suum and mammalian intestine. Unfortunately, this has not been investigated extensively in nematodes. Basically, the process of digestion proposed by Ugolev (1965) consists firstly of luminal hydrolysis, followed by membrane digestion and absorption. Furthermore, it was suggested that parasitic worms infesting the intestine of higher animals are left with only membrane digestion and absorption since previous treatment of foodstuff is done by the host's digestive system. However, this does not necessarily exclude the existence of intracellular digestion in nematodes (Weinstein, 1966; Sood and Sehajpal, 1978) which Riley (1973) diagrammatically represented in his work on Tetrameres fissispina.

Membrane digestion of sugars is nevertheless reviewed as an efficient system, where the hydrolysis of carbohydrates occurring in close proximity to the absorptive surface ensures a rapid and complete uptake of the resulting monosaccharides. This seems to be in agreement with results obtained by Palma and co-workers (1970) from which they concluded that in A. suum, the polysaccharides are hydrolyzed into monosaccharides before any

absorption into the cells occurs. Further support for the breakdown of carbohydrates occurring at the luminal end of cells, stems from the fact that low endocytotic activity was reported in the intestinal cells of A. suum (Van den Bossche and Borgers, 1973) and Cosmocerca ornata (Colam, 1971a).

Experiments on closed sacs of A. suum gut have shown that intestinal absorption of glucose is an efficient process and is capable of operating against a high concentration gradient (Castro and Fairbairn, 1969; Harpur and Popkin, 1973). However, Beames (1971) suggested that in the in vivo situation, there actually exists little or no concentration gradient across the gut of A. suum since glucose concentration in the hemolymph was reported to be low (Fairbairn and Passey, 1957). In the course of being actively transported, glucose does not entirely depend upon its own metabolism for energy to drive its own transport, and presumably, includes the utilization of endogenous carbohydrates. Circumstantial evidence of the involvement of glycogen in the active transport of solutes was brought about by Colucci and co-workers (1966) where, upon subsequent incubation in a glucose enriched medium, severely starved Hymenolepis diminuta converted over twice as much newly incorporated glucose into glucogen than the fed or moderately starved cestodes. Conversely, in A. suum, the absence of glucose in the incubation medium has led to a depletion in glycogen stores, notably in the intestinal cells

(Schanbacher and Beames, 1978).

The amount of glycogen in the intestine is relatively minimal when compared to the levels found in the muscles of A. suum (Roberts and Fairbairn, 1965). The "intestinal" glycogen represents roughly 2.6% of the total glycogen content of the worm (Fairbairn and Passey, 1957) and since approximately one half of the glucose consumed is incorporated into whole body glycogen (Entner and Gonzalez, 1959), it is assumed that a small fraction of the newly absorbed glucose is indeed incorporated into intestinal glycogen. The levels of total carbohydrate extracted from the intestine of A. suum are relatively low, with values such as 47 μmoles/gram (Fairbairn and Passey, 1957), 27 μmoles/gram (Harpur and Jackson, 1976) and 17 μmoles/gram (Schanbacher and Beames, 1978) of the fresh tissue weight. Furthermore, reports indicate that in A. suum, approximately 83% ($^{w}/_{w}$) of the total carbohydrate extracted from the intestine is endogenous glycogen and 17% trehalose (Fairbairn and Passey, 1957; Schanbacher, 1974).

Apart from chemical analyses, numerous descriptive studies on the intestine of A. suum have also shown glycogen in the cytoplasm of the cells (Kessel et al., 1961; Sheffield, 1964; Borgers and DeNollin, 1974; Trimble and Thompson, 1975) (see Figure 1). During observations made on the development of the second stage A. suum larvae, only the rosette-like glycogen was reported in

Figure 1. Electron micrograph showing the alpha particles of glycogen in the cytoplasm of the intestinal cell. Aggregates of glycogen (G) are present in the central portion of the cell above the nucleus (N). X 4,300.

the intestinal cells (Jenkins and Erasmus, 1971; Rubin and Trelease, 1975). Notably, in the second-stage larvae, the glycogen in the yet non-functional intestine is derived from lipid deposits (Rubin and Trelease, 1974; Rubin, 1977) whereas in the adult A. suum, glycogen in the intestinal cells is obtained from the absorbed glucose (Harpur and Jackson, 1976; Schanbacher and Beames, 1978). Both the alpha and beta glycogen have been seen in the intestinal cells of the adult A. suum (Kessel et al., 1961) as well as in the intestine of Ancylostoma caninum (Lee, 1969), Phocanema decipiens (Andreassen, 1968), Trichinella spiralis (Bruce, 1966) and various other nematodes (Wright and Dick, 1972).

Studies on the types of glycogen and their distribution in A. suum as well as in cestodes (Lumsden, 1965) have led to the suggestion that the alpha glycogen represents a storage form, since it is found in cells concerned primarily with carbohydrate storage. On the other hand, the beta or single granule form is believed to be the readily utilizable form, since it predominates in cell types with continual high enery demands. Supporting this view are results obtained by Orrell and co-workers (1964), where the progressive reduction in the relative concentration of high molecular weight glycogen in starving H. diminuta is interpreted as a breakdown of the alpha glycogen.

Because of its relatively simple structure, the intestine

of A. suum has lent itself to numerous investigations for the study of glycogen depletion and the absorption of various sugars. In one such study, Beames (1971) reported that in order to maintain the transport of 3-O-methylglucose across "sac" preparations of A. suum intestine, the presence of exogenous glucose was required in the incubation medium. Therefore, he suggested that the intestinal cells may not be able to mobilize endogenous glycogen for the active transport of sugars, or alternatively, the small supply of endogenous glycogen was rapidly exhausted. Subsequent investigations supported the latter suggestion, in that the endogenous glycogen is indeed mobilized for physiological processes such as the movement of sugars across the intestine (Schanbacher and Beames, 1978). In fact, the incubation of intestinal sac preparations in a glucose-free medium led to a 67% (Harpur and Jackson, 1976) and 100% (Schanbacher, 1974) reduction in the endogenous glycogen within an 80-minute period.

Comparative studies have shown glycogen utilization to differ substantially between helminths kept alive under physiological conditions and those maintained in vivo conditions. Read (1956), for instance, reported differences in the carbohydrate content between two groups of cestodes, where after a 20-hour period, H. diminuta maintained in vivo conditions had utilized 14% more glycogen than worms kept in vitro. Von Brand and co-workers (1968), from their work on Taenia

taeniaeformis, noted that dissimilarities between in vivo and in vitro conditions could possibly lead to physiological differences. Possibly indicative of their occurrence in A. suum is the fact that during the maintenance of live worms in a glucose enriched medium, Van den Bossche and DeNollin (1973) reported no glycogen depletion between worms kept in vitro from 24 to 72 hours, whereas Borgers and DeNollin (1974) discerned a net drop in the amount of glycogen from intestinal cells of worms that had resided in vitro for as little as three hours after collection.

Assessments of the amount of glycogen present in A. suum, as well as its depletion in starving worms, have been carried out with respect to whole body glycogen (von Brand, 1937), muscle glycogen (Harpur, 1963) and glycogen from the intestine (Van den Bossche and DeNollin, 1973). In the light of evidence on regional differentiation along the intestine of A. suum (Lee, 1965; Harpur, 1977), this present study was carried out to determine the levels of glycogen in the anterior, middle and posterior intestine. In addition, subsequent experiments were also carried out to determine if glycogen depletion in starving A. suum occurs preferentially in one region of the intestine. With reports on differences between regions, concerning the levels of enzyme activity (Carpenter, 1952) and the rates of sugar movement across the intestine (Schanbacher and Beames, 1973), an

attempt was made to correlate within each region the amounts of glycogen with levels of secretion and rates of absorption. It is believed that in so doing, a greater understanding of the intestinal physiology of A. suum will result.

STRUCTURAL AND FUNCTIONAL ASPECTS OF THE INTESTINE OF ASCARIS SUUM AND OTHER PARASITES

The intestine of Ascaris suum is dorso-ventrally flattened and is composed of a single layer of columnar cells on a basal lamella. In general, the cells of the intestine of A. suum are similar in appearance to those found in the mammalian intestinal tract. However, the very low mitotic activity (mitotic coefficient value of 0.01-0.02 per 1,000 cells) characterizing the intestine of A. suum (Anisimov and Tokmakova, 1973) is in contrast with the continuous renewal of the mammalian intestinal epithelium (Leblond et al., 1948).

Unlike the vertebrate alimentary tract with its morphologically discernible subdivisions, the intestine of A. suum is structurally uniform along its length, between the pharynx and the rectum. In the adult worm, the intestine is approximately equal in length to that of the worm itself and increases in diameter from 1.5 mm in the anterior to about 3.3 mm at the level of the posterior intestine (Harpur, 1977). Of the several parts comprising the alimentary tract of A. suum, the intestine can be

singled out from the stoma, pharynx, rectum and anus in that it is the only tissue of endodermal origin and is not lined with a cuticle (Chitwood and Chitwood, 1937).

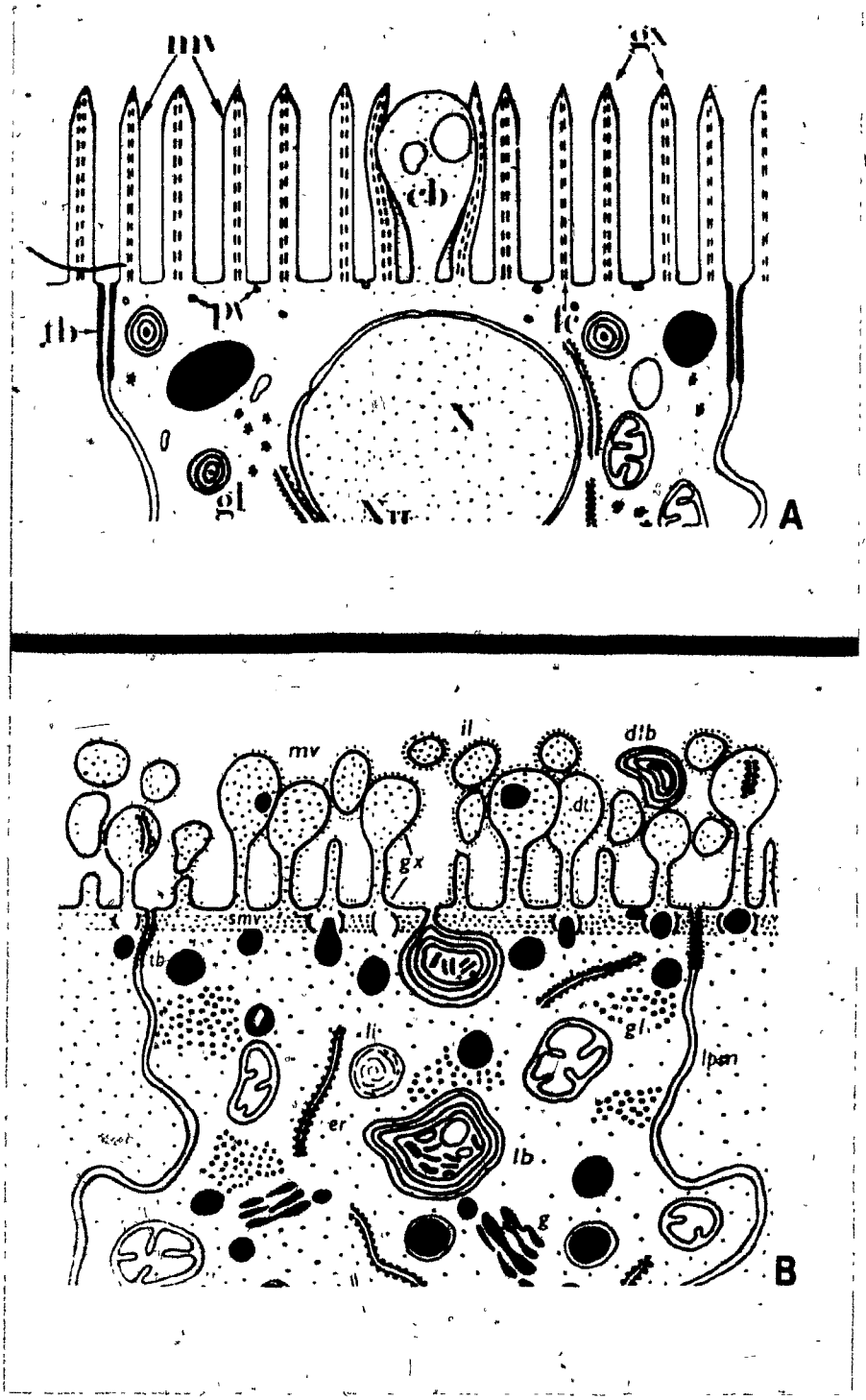
A division of the intestine of A. suum into an anterior, mid and posterior intestine was proposed by Harpur (1977) on the basis of the attachments to the body wall. The intestinal wall of A. suum remains multicellular in all regions of the gut (Kessel et al., 1961; Sheffield, 1964) whereas in the hookworm Ancylostoma caninum for instance, the cells of the mid-portion of the intestine lose their lateral plasma membrane to form a syncytium (Andreassen, 1968). This syncytial structure which is also found in Cyathostoma lari (Colam, 1971b), Haemonchus contortus (Sood and Sehajpal, 1978) and Syngamus trachea (Borgers et al., 1975), possesses all the intracellular organelles as well as the microvillar border characterizing the uninuclear cells of the intestine. The syncytium may be an adaptation of hematophagous parasites, although not all blood feeding parasites have it (e.g. Metastrongylus apri) (Jenkins and Erasmus, 1969).

The microvillar border is almost of universal occurrence in nematodes and is commonly regarded as evidence for absorptive activity. In the intestine of A. suum, for instance, it is estimated that the microvilli increase the surface area by a factor of 75 to 90 (Kessel et al., 1961), whereas in A. canimum and Phocanema decipiens the increase is 35- to 40-fold and about

100-fold respectively (Andreassen, 1968). However, estimates of surface areas from microvilli measurements should be looked upon with some skepticism since the size and shape of the microvilli are known to be affected by the time of immersion in saline as well as the osmolality of the solution prior to fixation (Millington and Finean, 1962). Furthermore, the microvilli from the intestine appear to differ in shape between certain nematodes. For instance, in the intestine of Trichuris suis the microvilli are uniformly slender (Jenkins, 1973) whereas in Metastrongylus apri they are variable and possess largely distended tips (Jenkins and Erasmus, 1969) (see Figure 2) and in Leidynema appendiculata the microvilli are studded with minute vesicles (Feldman, 1972). These alterations of form are somewhat distinct from the more usual distortion caused by vesicle extrusions or blebs observed through the apical plasma membrane in A. suum for example (Sheffield, 1964; Trimble and Thompson, 1975). Nevertheless, this pinching-off of the plasma membrane, which seemingly occursbetween the microvilli or along their sides and ends, are viewed as either a mode of secretory release for digestive enzymes (Kessel et al., 1961) and/or a mechanism for excretion (Borgers and DeNollin, 1975).

In addition to the increased absorptive surface, the brush border also provides a tremendous area for enzyme activity, such as revealed by the disaccharidase activity in A. suum

Figure 2. Semi-diagrammatic representation of the microvilli of the intestinal cell from Trichuris suis (A) (after Jenkins, 1973) and from Metastrongylus apri (B) (after Jenkins and Erasmus, 1969). The symbols are those used by the original authors.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(Gentner et al., 1972). It has been known for years that the apical plasma membrane of a variety of cell types displays a polysaccharide-rich coating, the glycocalyx (Bennett, 1963). This extracellular filamentous material is present in the immediate vicinity of the epithelial cells in A. suum (Sheffield, 1964; Trimble and Thompson, 1975) and presumably acts as a selective barrier (Spiro, 1972), retarding the passage of molecules for enzymatic breakdown (Costerton et al., 1978) and trapping ions as well as small particles (Bennett, 1963; Lumsden, 1973). In addition to the entrapment property of the glycocalyx, it would seem that the adsorption process can also be enhanced by a high turn-over of membrane material within the brush border (Forstner, 1971; Alpers, 1977) as well as possible in situ microvillar movements (Mooseker, 1976).

In contrast to the membrane of the luminal end of the cell, the basolateral plasma membrane is devoid of a microvillar arrangement and glycocalyx coating. Nevertheless, the basolateral plasma membrane remains a site for the movement of ions and water (Machen and Diamond, 1969; Wall et al., 1970) and lipids (Palay and Karlin, 1959) where an estimated 85% of the total cell activity occurs (Schmitz et al., 1973). Interestingly enough, Harpur and Popkin (1973) reported a dilation of the intercellular spaces during the fluid movement across the intestine of A. suum. They concluded that in A. suum, these

instertitial spaces may be part of the route for the movement of water (and probably glucose) from the cytoplasm of the intestinal cells to the pseudocoelom. In support of the conclusion, the extensive and complex infoldings of the basolateral plasma membrane reported in A. suum (Kessel et al., 1961; Sheffield, 1964), Metastrongylus apri (Jenkins and Erasmus, 1969) and Trichinella spiralis (Bruce, 1966) for example, are believed to strengthen the contact between cells (Fawcett, 1955) and may also provide the surface area needed for the volume expansion of the interstitial spaces.

Most investigations have been centered around the intestine of the adult A. suum, but a few researchers have studied the ultrastructure of the intestine in the early larval stages. Jenkins and Erasmus (1971) reported the presence of undifferentiated cells in the anterior part of the second-stage larvae, and showed that these cells would eventually give rise to the intestine. The primordial cells form a column of seven cells which in the larvae taken from the liver, divide along the longitudinal axis. At this 14 to 20 cell-stage, a discernible lumen is formed, but presumably remains a storage organ until the lung stage at which time the intestine appears able to assume digestive functions. Notably, in the lung-stage larvae, the number of cells visible in any cross-section is much less than that found in the adult. Furthermore, these cells are cuboidal and

must therefore increase in size considerably during larval development so as to become the tall columnar cells of the adult stage.

INDICATIONS OF REGIONAL DIFFERENTIATION ALONG THE INTESTINE OF *ASCARIS SUUM* AND OTHER PARASITES

Several parameters can be used to show the existence of morphologically and/or physiologically different regions along the length of the nematode intestine. Morphological evidence for regional differentiation in nematodes is seen with differences in (1) glycogen content, (2) microvillar size and density, (3) abundance of endoplasmic reticulum and Golgi apparatus, (4) cell height and (5) thickness of the basal lamella.

Glycogen appears universally present in the intestine of nematodes, either dispersed in the cytoplasm of individual cells or scattered throughout the syncytial mass, but the amount present seems to differ between certain groups. In the adult blood-feeding *Ancylostoma caninum* for example, the glycogen content of the intestine is low whereas in *Phocanema decipiens*, which feeds on stomach contents, the levels are high with glycogen occupying most of the cell's cytoplasm (Andreassen, 1968). In a comparative study, Andreassen (1968) indicated that the level of glycogen in the intestinal cells could be associated with the mode of feeding or more precisely with the availability of

foodstuff.

Apart from differences between worms, the amounts of glycogen in the intestinal cells also differ depending on the region of the intestine in which the cells are located. In the ascaroid Cosmocerca ornata (Colam, 1971a), the strongyloid A. caninum (Lee, 1969) and three oxyuroids (Anya, 1964), the midgut is shown to contain larger amounts of glycogen than the hind region of the intestine. In the present study, evidence is presented which indicates such differences between regions of the intestine in Ascaris suum.

The size and density of the microvillar border have also been seen to vary along the intestine. In the intestine of C. ornata the size and density of the microvilli are reported to decrease posteriorly, with a microvillar height ranging from 25 μm to 0.5 μm and a surface area increase of 120-fold dropping to a value of 0.5 (Colam, 1971b). Differences in length are also reported between the microvilli in the anterior gut and their counterparts in the midgut of A. caninum, where microvilli of the anterior intestine measure 3 μm high (Miller, 1967) whereas those of the mid-intestine are 8 μm in height (Andreassen, 1968). Despite the fact that the presence of microvilli in general cannot be taken as unquestionable evidence of absorptive functions, the brush border is nevertheless presently regarded as playing an important role in absorption as well as in digestion

(Palma et al., 1970; Gentner et al., 1972; Crane, 1975).

The endoplasmic reticulum appears to be present in the intestinal tissue of most nematodes and has been reported in representatives of ascaroids (Kessel et al., 1961), trichuroids (Wright, 1963; Jenkins, 1973), strongyloids (Andreassen, 1968; Sood and Sehajpal, 1978) and oxyuroids (Feldman, 1972). The Golgi apparatus, in contrast, is not always present, and is apparently absent in the intestinal cells of some nematodes including Trichuris suis (Jenkins, 1973), A. caninum and P. decipiens (Andreassen, 1968), and Capillaria hepatica (Wright, 1963). Apart from differences between worms, in the intestine of Leidynema appendiculata the Golgi complex of the anterior region appears morphologically different from its counterpart in the posterior region (Feldman, 1972). Insofar as its role in the synthesis of secretory products (Bennett, 1970; Bennett and Leblond, 1970) and storage of intracellular lipid (Cardell et al., 1967; Reaven and Reaven, 1977), changes occurring in the Golgi bodies may reflect variations in function.

The intestinal cells of A. suum are columnar in shape (Sheffield, 1964), with a difference in height between the cells of the dorsal and ventral walls and those of the lateral folds of the intestine (Anisimov and Tokmakova, 1973). Moreover, a marked difference in cell height is reported between regions of the intestine in A. suum (Carpenter, 1952). The cells are

tallest in the mid-intestine (200 μm), whereas the cells of the posterior intestine measure 35-56 μm and those of the anterior range from 95 μm to 210 μm. It is noteworthy that the measurements of cell height in the anterior intestine differ between reports, with values of 50 μm and 95-210 μm from Sheffield (1964) and Carpenter (1952), respectively. However, this discrepancy may well be attributed to the difference in treatment of the tissue prior to microscopic examination or simply, differences between individual worms.

Variations in the thickness of the basal lamella along the length of the intestine is reported within some individual worms, such as C. ornata (Colam, 1971a) and L. appendiculata (Feldman, 1972). Unfortunately, no single study on A. suum has revealed such a variation, but data from separate investigations indicate the possible existence of such a difference. From studies by Kessel and co-workers (1961) and Sheffield (1964), the basal lamella from the anterior intestine measures 6.2-7 μm, whereas the basal lamella from the mid-intestine is reported to be 3-4 μm thick (Peczon et al., 1975).

The basal lamella surrounding the intestine of A. suum is subdivided into two distinct sublamina of fine feltwork (Sheffield, 1964) (Figure 3) where peptides, carbohydrates and cholesterol account for 91.4%, 4.9% and 2% respectively of the weight of the basal lamella (Peczon et al., 1975). Despite the

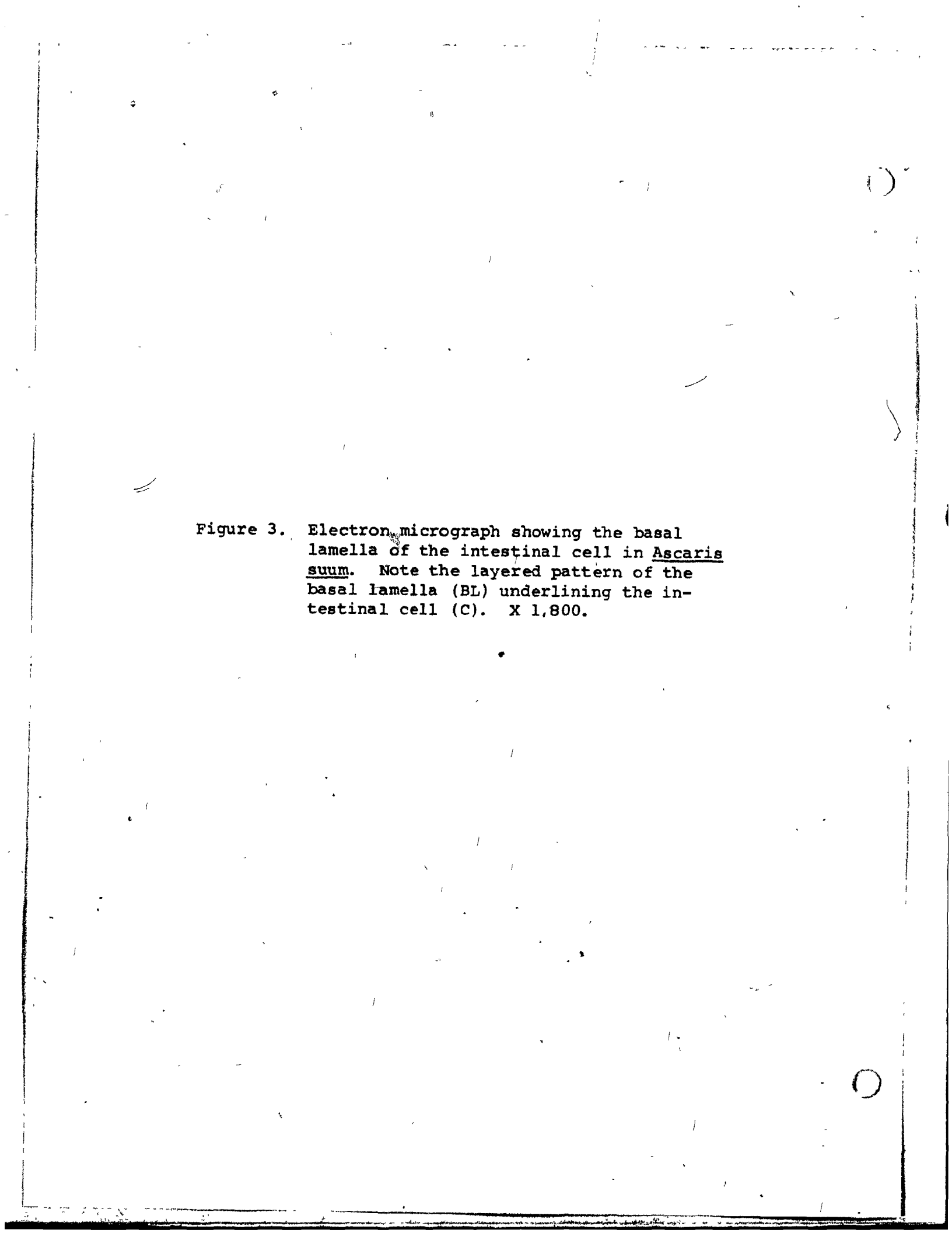
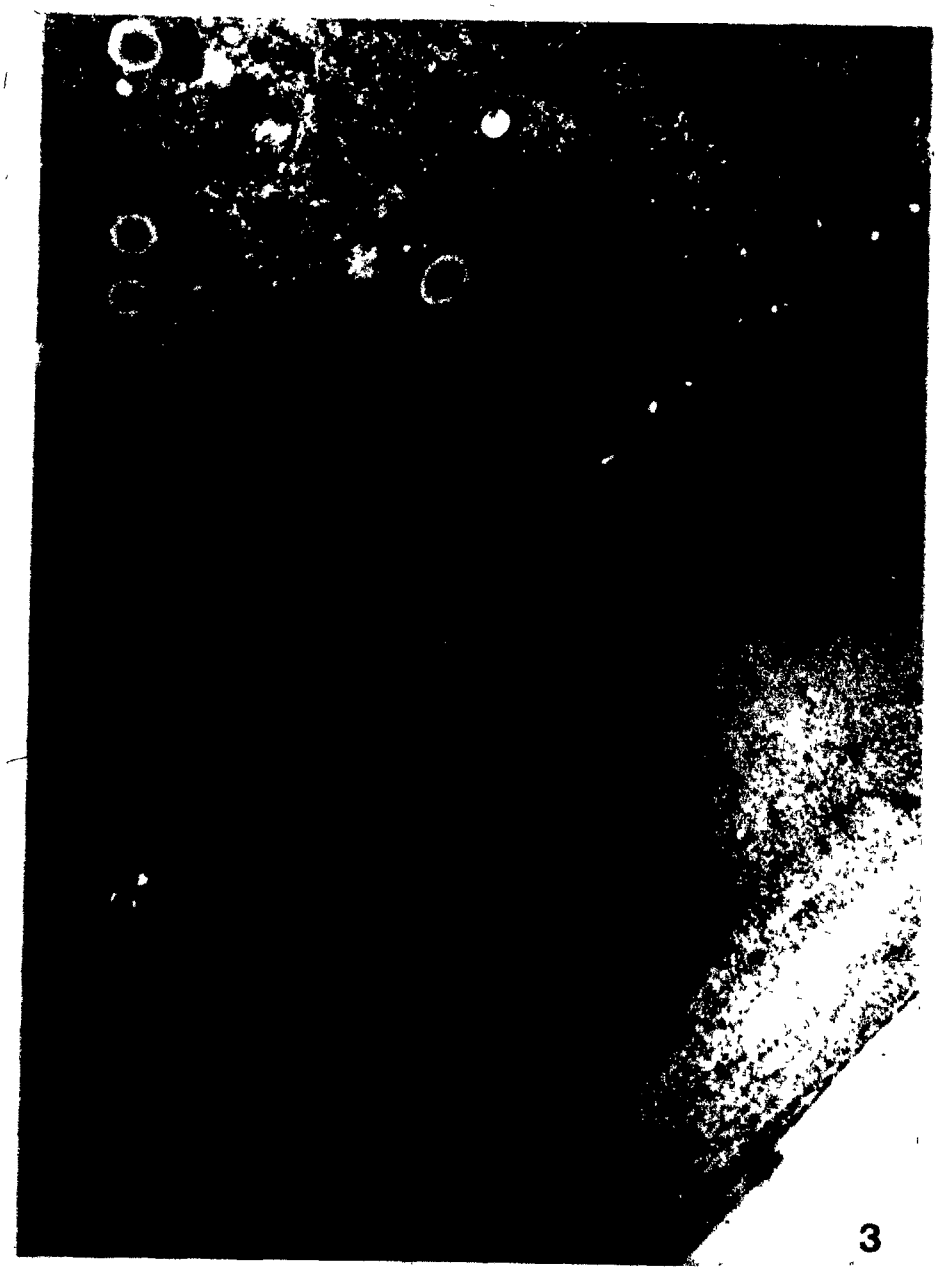


Figure 3. Electron micrograph showing the basal lamella of the intestinal cell in Ascaris suum. Note the layered pattern of the basal lamella (BL) underlining the intestinal cell (C). X 1,800.



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lack of sialic acid and low ratio of disaccharides to heterosaccharides in the intestinal basal lamella of A. suum, the overall structural feature of the basal lamella is analogous to that of vertebrates (Peczon et al., 1977). The basal lamella is generally regarded as a diffusion barrier (Kefalides, 1973), where in the intestine of A. suum the estimated value of the filtration coefficient is 1.14×10^{-12} cm^3/dyne-sec (Donahue et al., 1975). The basal lamella is apparently synthesized by the epithelial cells which it supports (Kurtz and Feldman, 1962) and is commonly found in both the multicellular and syncytial intestine.

Interestingly, the basal lamella covering the syncytial mid-intestine of the hookworm A. caninum is 0.2-0.6 μm thick (Andreassen, 1968), whereas in the multicellular intestine of ascaroids it ranges from 2-3 μm in P. decipiens (Andreassen, 1968) to 7 μm in A. suum (Kessel et al., 1961). This difference between groups of worms, as well as the variation existing along the intestine of some, may well reflect a variability in the absorptive function of the intestine.

Physiological evidence for regional differentiation along the intestine of A. suum includes a variation in both the absorption rate of 3-O-methylglucose across the epithelium and the level of enzyme activity. Following the study by Beames (1971) on the uptake of 3-O-methylglucose by the intestine of A. suum,

Schanbacher and Beames (1973) presented data on its relative rate of movement across the anterior, middle and posterior regions of the intestine. Their results indicated that the movement of 3-0-methylglucose increases progressively from the anterior to the posterior region of the intestine. In fact, the rate of movement across the intestinal wall is some two times greater in the mid-intestine and some three times greater in the posterior intestine than it is in the anterior intestine. Although the rate of movement of the non-metabolizing 3-0-methylglucose is recognized as an estimate of the rate of glucose transport, it has been suggested that the intestinal carriers for glucose differ from those of 3-0-methylglucose since the maximal rate of transport of the latter is approximately twice that of the former (Thompson, 1979).

Also in support of a gradation in function along the intestine of A. suum, is the uptake of vitamin B_{12} (cyanocobalamin) which is reported to predominate in the anterior region (Zam and Martin, 1969). In contrast, the relatively low rate of movement of triglycerides across the intestine of A. suum remains unvaried throughout the length of gut (Beames et al., 1974). However, in view of the low values reported for the movement of triglycerides, it is possible that a significant difference between regions of the intestine may go undetected.

Numerous investigations have been carried out on the

proteolytic and hydrolytic enzymes from the intestine of A. suum, but unfortunately, there is little or no mention of the region of the intestine which is used. A few researchers, however, have taken into account the different regions of the intestine in A. suum, and the results obtained indicate that the anterior intestine is the prevailing region for enzyme secretion. Biochemical determinations of digestive enzyme activity and its distribution in the intestine of A. suum were primarily carried out by Carpenter (1952). Her results indicated that amylase, maltase, proteinase, peptidase and lipase activity predominate in the anterior intestine.

Rhodes et al. (1963) reported the presence of enzyme inhibitors in the intestine of A. suum, which presumably confer a protective role by their action on the host's trypsin and chymotrypsin. Recent work, in which the intestine of A. suum was subdivided into three parts, indicated that both trypsin-inhibitor and chymotrypsin-inhibitor activity occur mainly in the anterior region and decrease posteriorly along the intestine (Juhasz, 1979).

Esterase as well as leucine aminopeptidase activity were determined histochemically in the intestine of A. suum, but little or no difference in activity was observed between regions (Lee, 1962a,b). However, Lee (1962b) has pointed out that histochemical methods are less sensitive to differences in activity than biochemical analyses.

The gradual alteration of the basic morphology as well as the gradation in function suggest that in some nematodes, including A. suum, the intestine is subdivided into regions with more or less distinguishable structural and physiological characters. In the present study, differences in the estimated amount of stored glycogen between the anterior, middle and posterior intestine of A. suum are assumed to be indicative of dissimilarities in the total cellular activity between these regions.

CHAPTER III

MATERIALS AND METHODS

COLLECTION AND TREATMENT

Adult Ascaris suum were collected from the small intestine of infected pigs at a local abattoir. When removed from the intestine, the worms were either immersed in liquid air or immediately placed in a warm saline solution (Harpur, 1963). For a series of experiments, the worms in the latter case were transferred to a fresh saline solution and kept in an incubator at 39°C. Bacterial growth was controlled by changing the saline every five hours. Worms were removed from the saline at 5, 10 and 15 hours after collection and immediately immersed in liquid air. Before dissection, the frozen worms were allowed to thaw at room temperature for several minutes. To expose the intestine, the worms were pinned down and slit longitudinally.

The gut was cut into three parts corresponding to the anterior, middle and posterior intestine, excised and immediately placed in cold saline. To wash out the gut contents, cold saline was gently flushed through with the use of a syringe. The pieces of intestine were then damp-dried on filter paper and the wet weight determined gravimetrically. In order to have

sufficient glycogen for quantitative determinations, it was necessary to combine intestines from three different female worms or five different male worms for each determination.

ANALYSIS OF TOTAL ACID- AND ALKALI-EXTRACTABLE CARBOHYDRATE

The sections of intestine in each tube were sonicated (Blackstone Ultrasonic Probe Model BP2) in 4.0 ml of 5% trichloroacetic acid (TCA) for two minutes and the probe rinsed with 2.0 ml of 5% TCA. Sonication proved to be better than the use of the ultrasonic homogenizer (Polytron) since more anthrone-sensitive carbohydrate was recovered. The homogenate was centrifuged at room temperature for 20 minutes at 900 g. The resulting clear supernatant was decanted into a second tube and the precipitate resuspended in 6.0 ml of 30% potassium hydroxide (Good et al., 1933).

For the precipitation of the TCA-soluble glycogen from the supernatant, 2.3 volumes of 95% ethanol and 0.1 volume of saturated sodium sulfate (Handel, 1965) were added. The mixture was stirred with a Vortex mixer and allowed to stand overnight at 37°C. After precipitation was completed, the solution was centrifuged at 900 g for 20 minutes at room temperature. The supernatant was decanted from the packed glycogen at the bottom of the tube. The glycogen was then dissolved in 3.0 ml

of distilled water. An aliquot of 1.0 ml was taken and the amount of TCA-soluble carbohydrate determined colorimetrically by the anthrone method (Seifter et al., 1950).

The original precipitate was dissolved in 30% KOH by placing it in a boiling water bath for one hour. After removal from the water bath, the volume was adjusted to 10 ml by the addition of distilled water. After thorough mixing, an aliquot of 2.0 ml was pipetted into a second tube, and the acid-insoluble glycogen was precipitated in the same manner as described above.

For glycogen determination, 3.0 ml of anthrone reagent were delivered vigorously to the appropriate samples of glycogen. The mixture was immediately stirred and the tubes then kept in ice-water. All tubes were capped and simultaneously immersed to a depth little above the level of the liquid in a water bath maintained at 80°C (Mokrasch, 1954). After 40 minutes, the tubes were then transferred to the ice-water bath and cooled at room temperature before reading on a Gilford Model 222 and Beckman Monochromator Model DU spectrophotometer at 620 mμ (Morris, 1948).

MICROSCOPIC EXAMINATIONS

Light Microscopy

Sections of the anterior, middle and posterior intestine

were obtained by severing the entire body in 3-5 mm slices. These were fixed in 3:1 ethanol and formalin (McManus and Mowry, 1958), dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. Sections were cut at 7 μm and stained following the Bauer-Feulgen reaction procedure (Humason, 1972). Controls were carried out by digesting some sections with 1% (w/v) solution of diastase at 37°C for one hour. Measurements for cell height and thickness of the basal lamella were taken from the dorsal and/or ventral sides of the intestine.

Transmission Electron Microscopy

Pieces of intestine were fixed in 2% (v/v) glutaraldehyde adjusted to pH 7.2 with 0.1 M cacodylate buffer (Glauert, 1975). After two hours in the fixative, the tissue was washed with two changes of 0.1 M cacodylate buffer and postfixed in 2% (v/v) osmium tetroxide buffered with cacodylate buffer. The tissue was washed again in buffer and dehydration was completed in a graded series of acetone from 50-100%. Embedment was in a mixture of Epon 812[a] and Araldite 502[b] with dodecenylsuccinic anhydride (hardener), dibutyl phthalate (plasticizer) and DMP-30[c] (2,4,6-tri (dimethylaminomethyl)-phenol) (accelerator).

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[a]Fisher Scientific Co., Montreal, Quebec.

[b]Ladd Research Ind., Burlington, Vermont.

[c]Polysciences Inc., Rydal, Penn.

For the cytochemical staining of glycogen, sections were collected on gold grids and stained according to the method of Thiery (1967), with 20 minutes oxidation in 1% periodic acid, 40 minutes in 1% thiosemicarbazide, and 30 minutes in a 1% solution of silver proteinate (Protargol[d]). This technique is referred to as the PA-TSC-Ag protein technique. The sections were examined in a Zeiss EM 9 electron microscope.

Scanning Electron Microscopy

Specimens for scanning electron microscopy were prepared by cryofracturing. Worms were frozen in liquid air and fractured in selected regions with the use of a dull blade. The fragments were immediately transferred to an Edward-Pearse tissue dryer and allowed to dry overnight under vacuum. The pieces of worms were attached by means of a double-face tape to the SEM stub. The specimens were then coated with a thin layer of vaporized gold:palladium alloy and viewed with a Cambridge Stereoscan 600 operated at 15 KV.

STATISTICAL ANALYSIS

All data were subjected to statistical analysis and the significance of the difference between means of two groups was

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[d]Roboz Surgical Inst. Co., Washington, D.C.

determined either by the Students t test or analysis of variance. When found to be significant, differences in means were detected by Duncan's Multiple Range Test (Ferguson, 1971).

CHAPTER IV

RESULTS

MICROSCOPY

To illustrate the distribution and amount of glycogen within the cells, sections were sampled from the anterior, middle and posterior intestine and stained for light microscopy (Figure 4). A qualitative difference in the amount of glycogen-specific stain present in the cells exists between the middle region, and the anterior and posterior regions. The amount of stain in cells from the middle region is smaller than in cells from the two other regions. In this instance, little or no qualitative difference between the anterior and posterior intestine is discernible.

Measurements of cell height from the anterior, middle and posterior intestine are presented in Table 1. The cells of the mid-region are taller than their counterparts in the anterior and posterior regions. The cells are slightly shorter in the posterior region than in the anterior region. Differences between means were tested for significance by analysis of variance and Duncan's multiple range test. The mean cell height of the mid-intestine is significantly greater than the mean values

Figure 4. Light microscope sections from the anterior (A), middle (B) and posterior (C) intestine of Ascaris suum showing the amounts of glycogen. The paraffin sections were treated according to the Bauer-Feulgen method and the reaction product is associated with glycogen deposits (arrow). X 312.5.

Table 1. The height of intestinal cells in the anterior, middle and posterior intestine.

	Intestine			
	Anterior	Middle	Posterior	(±S.E.)
Cell height (μm) (excluding microvilli)	86.73*	119.76	69.89	±5.23
Relative height	1.24	1.71	1.0	

*Each value represents the mean of ten observations ± standard error.

Means not underscored by the same line are different ($P < 0.01$).

of the anterior and posterior regions at the 0.01 probability level. However, at the 0.05 probability level all three means are statistically different, where cells of the anterior and middle regions are respectively 24 and 71% taller than those from the posterior region.

Measurements of basal lamella thickness from the anterior, middle and posterior intestine are presented in Table 2. The basal lamella underlining the cells from the posterior intestine appears thinner than the basal lamella of the anterior and middle intestines. The mean thickness of the basal lamella from the posterior region is significantly less ($P < 0.05$) than the mean values for the other two regions. The basal lamella from the mid-region is however not significantly thinner than that from the anterior region.

Table 2. The thickness of the basal lamella in the anterior, middle and posterior intestine.

	Intestine			
	Anterior	Middle	Posterior	(±S.E.)
Thickness of basal lamella (μm)	7.50*	8.01	5.65	±0.52
Relative thickness	1.33	1.42	1.0	

*Each value represents the mean of eight observations ± standard error.

Means not underscored by the same line are different ($P < 0.05$).

Scanning electron micrographs of the anterior and posterior sections of a worm illustrate the difference in diameter of the intestine between these regions (Figures 5,6). Clearly, the circumference of the intestine is substantially larger in the posterior region than in the anterior region. This agrees with earlier reports on the progressive increase of the intestinal lumen from the anterior to the posterior region (Harpur, 1977). Figures 7, 8 and 9 of the anterior, middle and posterior regions respectively, show the somatic muscles in proximity to the intestine. Notably, the muscle cells are never directly attached to the intestinal wall nor is the intestine connected to the gonads. Interestingly, strands of fibrous material can be seen in close proximity to the basal lamella. In a single experiment where hemolymph was injected into the intestine before

Figure 5. Scanning electron micrograph of the anterior section of Ascaris suum illustrating the size of the anterior intestine. Note the size of the intestinal lumen (|) in the anterior region. X 20.

Figure 6. S.E.M. of the posterior section of Ascaris suum illustrating the size of the posterior intestine. Note the relatively large size of the intestinal lumen (|) in the posterior region. X 20.

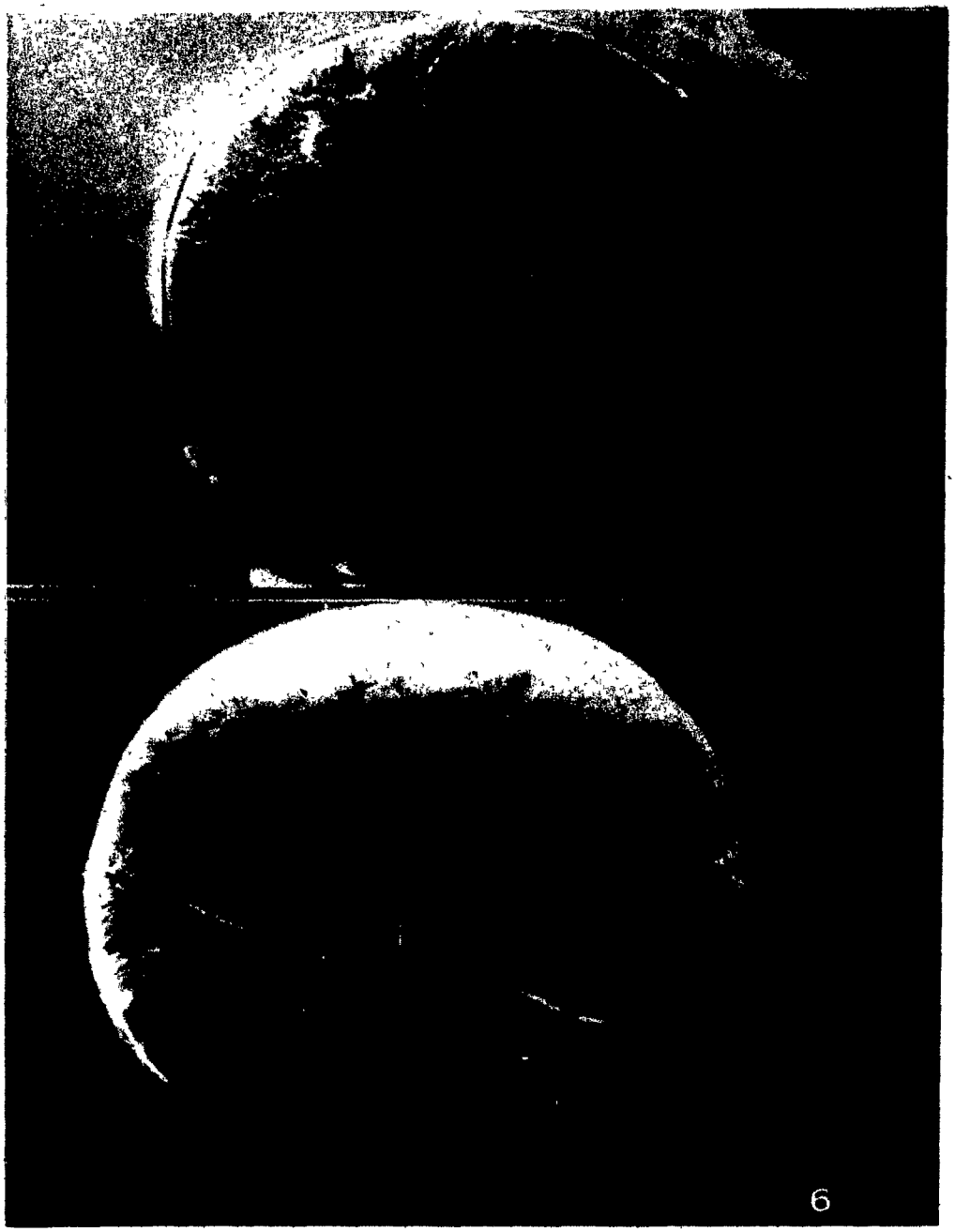
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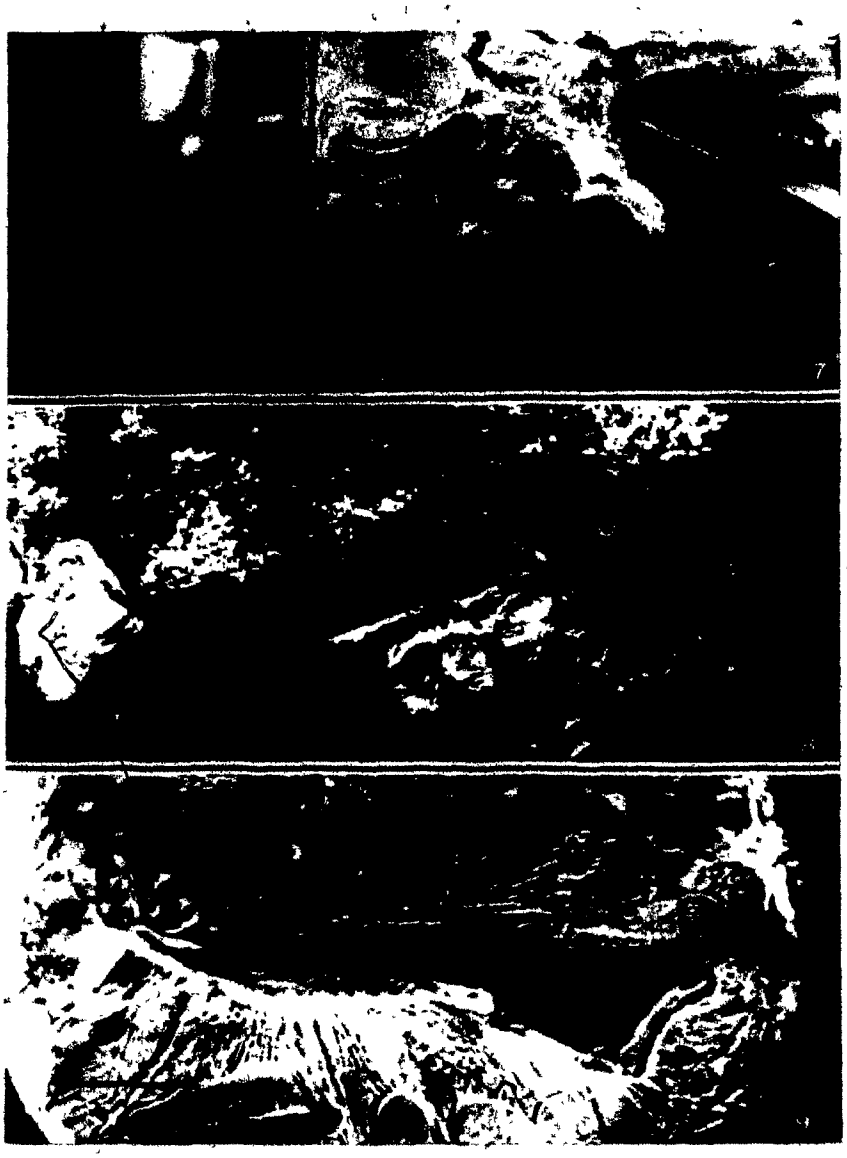
Figure 7. Scanning electron micrograph of the anterior region of Ascaris suum. This illustrates the proximity of the somatic muscles (M) to the anterior intestine (I). (bar size:0.5 mm)

Figure 8. S.E.M. of the mid-region of Ascaris suum. This shows the proximity of the somatic muscles (M) and ovaries (O) to the mid-intestine (I). (bar size:1 mm)

Figure 9. S.E.M. of the posterior region of Ascaris suum. This illustrates the proximity of the somatic muscles (M) to the posterior intestine (I). (bar size:0.5 mm)



freezing, strands appeared in the gut which were very similar to those in the pseudocoel. Thus, the strands shown are partly an artifact.

CHEMICAL ANALYSIS

Amount of TCA-Extractable Glycogen from the Various Regions of the Intestine

Results of the determinations of the TCA-extractable glycogen from the anterior, middle and posterior intestine of female *Ascaris suum* are presented in Table 3. From casual

Table 3. The levels of TCA-extractable glycogen in the anterior, middle and posterior intestine.

	Date	Intestine			±S.E.	n
		Anterior	Middle	Posterior		
Amount of TCA-extractable glycogen (μ moles CHO/g. wet wt.)	9·10·79	8.30	4.14	5.53	±0.84	9
	6·11·79	23.62	6.91	10.95	±4.17	8

n = number of determinations. Each determination was made on a pooled sample of 3 females.

Means not underscored by the same line are different ($P < 0.05$).

inspection of data, it is obvious that the amounts of glycogen, expressed on the basis of tissue wet weight, are markedly larger in the anterior than in the mid-intestine whereas the levels in the posterior intestine are but slightly higher than in the

mid-intestine. The mean level of TCA-extractable glycogen from the anterior region is significantly greater than the mean values of the middle and posterior regions. It is noted however, that the differences between the middle and posterior intestine are not statistically significant ($P > 0.05$).

The amounts of glycogen recovered from all three regions of the intestine by the use of the sonicator, instead of the tissue homogenizer, are presented in Figure 10. With the use of the homogenizer, the mean levels of TCA-extractable glycogen from all three regions of the intestine are somewhat lower than those obtained with the use of the sonicator. In spite of the lower recoveries, the mean level of TCA-extractable glycogen is significantly higher ($P < 0.01$) in the anterior intestine than in the middle and posterior intestines. Furthermore, by virtue of the large number of determinations, the mean level of TCA extractable glycogen in the posterior intestine is shown to be significantly higher ($P < 0.01$) than the mean values of the mid-intestine.

Amount of Alkali Extracted Glycogen Recovered from the TCA Precipitate

Results of the determinations of the glycogen remaining in the protein residues after the removal of the TCA-extractable fraction are presented in Table 4. Inspection reveals that the

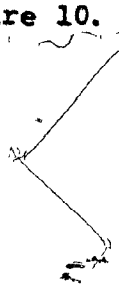


Figure 10. The amounts of glycogen recovered from the anterior, middle and posterior intestine of Ascaris suum. The first column (▨) in each region represents the levels of glycogen recovered with the use of the tissue homogenizer while the second (▩) and third (▤) column of each region represents the levels of glycogen recovered by sonication on two separate collection days.

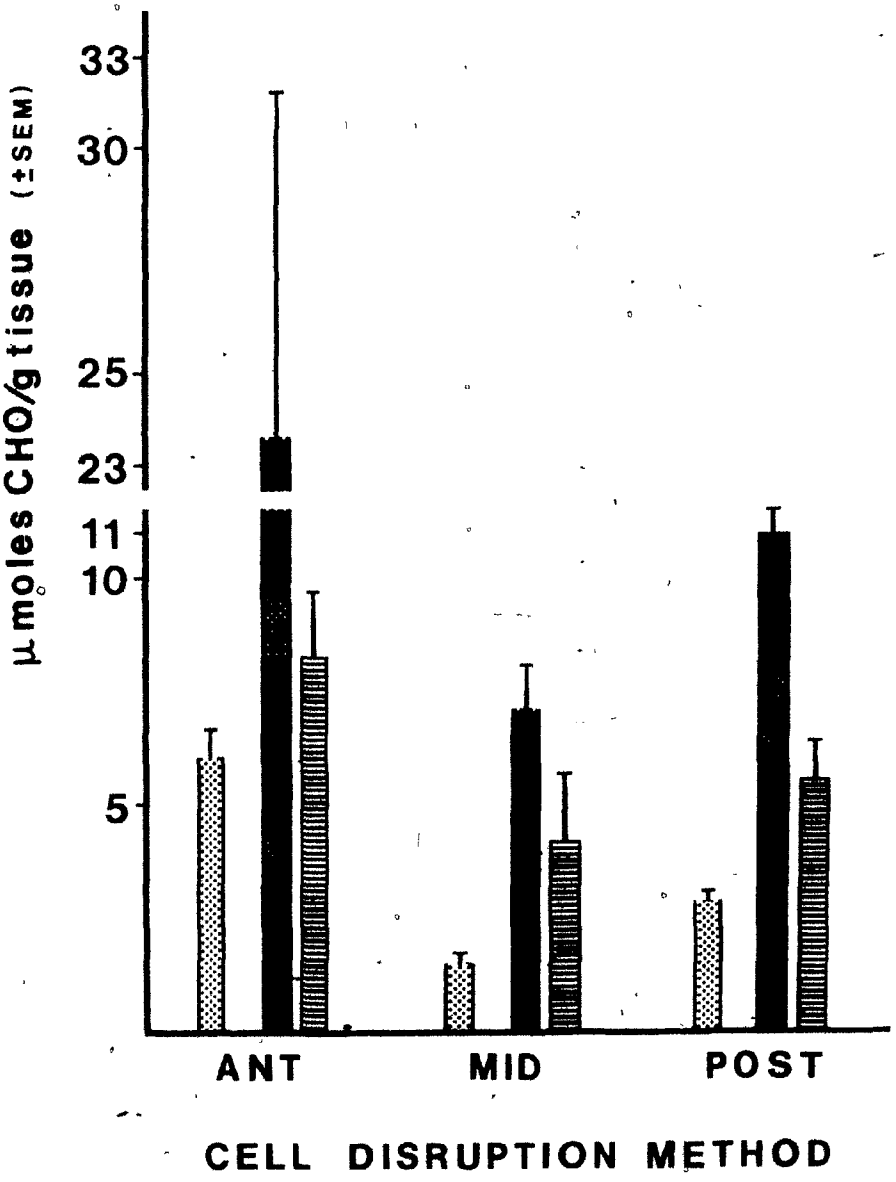

33
30
25
23
11
10
5
µ moles CHO/g tissue (±SEM)
ANT
MID
POST
CELL DISRUPTION METHOD

Table 4. The levels of KOH extracted glycogen in the anterior, middle and posterior intestine.

	Date	Intestine			±S.E.	n
		Anterior	Middle	Posterior		
Amount of KOH extracted glycogen (μ moles CHO/g wet wt.)	9·10·79	10.92	4.83	6.93	±0.82	9
	6·11·79	16.68	6.33	7.07	±2.13	8

n = number of determinations. Each determination was made on a pooled sample of 3 females.

Means not underscored by the same line are different ($P < 0.05$).

amount of glycogen liberated by alkali digestion is largest in the anterior region, lowest in the middle and slightly larger in the posterior region. The mean level of alkali-extracted glycogen from the anterior region is significantly greater ($P < 0.05$) than the mean values of the middle and posterior regions. The posterior region shows a slightly higher mean value than does the middle region. However, the difference between these means is not statistically significant ($P > 0.05$).

Amount of Glycogen Extracted from the Anterior and Middle Intestines of Males

The amounts of glycogen extracted from the anterior and middle intestine of fresh (time = 0 hour) male worms are presented in Table 5. Due to the relatively small size of the posterior intestine in males, pieces are difficult to remove and

Table 5. The levels of glycogen in the anterior and mid-intestine of male worms.

	Intestine			
	Anterior	Middle	(±S.E.)	n
A. TCA-extractable glycogen (μ moles CHO/g wet wt.)	54.86	15.41	±3.38	10
B. KOH extracted glycogen (μ moles CHO/g wet wt.)	27.85	13.23	±1.56	10

n = number of determinations. Each determination was made on a pooled sample of 5 males.

Means not underscored by the same line are different ($P < 0.001$).

consequently no data were collected for this region.

The mean values of the TCA-extractable glycogen and of the alkali extracted glycogen recovered from the anterior are significantly greater than their corresponding counterparts in the middle region ($P < 0.01$).

Looking at Table 6a, the mean levels of TCA-extractable glycogen in the anterior and middle intestine of males are noticeably higher than their corresponding counterparts in females. Differences were tested for significance at the 0.01 and 0.05 probability levels by analysis of variance and Duncan's multiple range test. Although there are only but one set of analysis for males and two sets of analysis for females, the differences between the two populations reveal that the males have more glycogen than females. Results indicate that there is significantly

more ($P < 0.01$) TCA-extractable glycogen in the anterior intestine of males than of females, whereas in the mid-intestine the difference is not significant ($P > 0.05$).

Table 6a. Levels of TCA-extractable glycogen in the intestine of male and female A. suum (μ moles CHO/g wet wt.).

Intestine	Male	Female		(±S.E.)
Date	14·9·79	9·10·79	6·11·79	
Anterior	54.86	8.30	23.62	(±5.95)
Middle	15.41	4.14	6.91	(±3.10)
	(10)	(9)	(8)	

(n) = number of determinations. Each determination was made on a pooled sample of 5 males and 3 females.

Means not underscored by the same line are different ($P < 0.05$).

Inspection of Table 6b reveals that the mean levels of alkali extracted glycogen in the anterior and middle intestine of males are distinguishably higher than the corresponding levels in females. The mean level of KOH extracted glycogen in the anterior intestine of males is significantly greater ($P < 0.05$) than the mean value of the anterior intestine in females. Similarly, there is a significant difference ($P < 0.05$) in the mean values of the mid-intestine between the males and the females.

Table 6b. Levels of KOH extracted glycogen in the intestine of male and female A. suum (μ moles CHO/g wet wt.).

Intestine	Male	Female		(±S.E.)
Date	14·9·79	9·10·79	6·11·79	
Anterior	27.85	10.92	16.68	(±3.02)
Middle	13.23	4.83	6.33	(±1.41)
(n)	(10)	(9)	(8)	

(n) = number of determinations. Each determination was made on a pooled sample of 5 males and 3 females.

Means not underscored by the same line are different ($P < 0.05$).

The Effect of Starvation on the Amounts of Acid- and Alkali-Extractable Glycogen

The changes in the level of glycogen in the intestine of unfed worms kept in warm saline are examined every five hours following collection. The mean levels of glycogen in all three regions were calculated and differences between the means were tested for significance at the 0.05 probability level by Duncan's multiple range test.

Data from the first collection day (Table 7a) show that in the anterior region, the mean level of TCA-extractable glycogen of the "5-hour worms" is greater than the mean values of the 10- and 15-hour worms. However, the difference between the means

Table 7a. Glycogen levels in the intestine of A. suum after removal from the host.

Date of Collection		Intestine	Time (hours) 5	10	15	(±S.E.)
25·9·79	A. Levels of TCA-extractable glycogen (μ moles CHO/g wet wt.)	Anterior	25.62	15.55	15.19	±7.31
		Middle	2.13	2.27	5.19	±1.15
		Posterior	5.17	6.09	7.61	±1.45
	B. Levels of KOH extracted glycogen (μ moles CHO/g wet wt.)	Anterior	14.3	8.89	12.28	±1.99
		Middle	7.71	6.49	8.21	±0.87
		Posterior	9.72	7.54	10.12	±1.41

Each value represents ten determinations. Each determination was made on a pooled sample from 3 females.

Means not underscored by the same unbroken line are considered different ($P < 0.05$).

is not statistically significant ($P < 0.05$). In contrast, the mean level of TCA-extractable glycogen of both the middle and posterior intestines appear to increase with time. The difference between the means over the ten-hour period is not statistically significant in either the middle or posterior intestine.

In looking at the glycogen extracted by alkali, the mean levels of both the anterior and posterior intestine are highest at 5 hours and therefrom drop at 10 hours after collection. By 15 hours, the mean values have increased slightly to reach levels significantly close to those of 5-hour worms. In the mid-intestine, the mean level of alkali-extractable glycogen does not significantly vary over the ten-hour period.

From the second collection day (Table 7b), figures reveal a significant change in the mean level of TCA-extractable glycogen from the anterior intestine, but only between the means of the 5-hour and 10-hour worms. The mean levels from the middle and posterior regions do not vary significantly over the 10-hour period.

In the anterior and posterior intestine, the levels of alkali extracted glycogen do not vary significantly over the 10-hour period. In the mid-intestine, the mean level of glycogen increases significantly at 5 hours, and drops at 10 hours to approximately the 0-hour value. In the posterior intestine, the mean level of alkali extracted glycogen is significantly lower,

Table 7b. Glycogen levels in the intestine of A. suum after removal from the host.

Date of Collection		Intestine	Time (hours) 0	(±S.E.)	5	(±S.E.)	10	(±S.E.)
9·10·79	A. Levels of TCA-extractable glycogen (μ moles CHO/g wet wt.)	Anterior	8.3	±2.41	14.64	±2.29	2.77	±2.41
		Middle	4.14	±1.41	4.67	±1.33	1.93	±1.41
		Posterior	5.53	±1.39	4.64	±1.32	3.18	±1.39
	B. Levels of KOH extracted glycogen (μ moles CHO/g wet wt.)	Anterior	10.92	±1.43	10.88	±1.35	7.5	±1.43
		Middle	4.83	±0.58	6.66	±0.55	4.43	±0.58
		Posterior	6.93	±0.64	7.58	±0.61	5.24	±0.64
(n)			(9)		(10)		(9)	

(n) = number of determinations. Each determination was made on a pooled sample from 3 females.

Means not underscored by the same unbroken line are considered different ($P < 0.05$).

Table 7c. Glycogen levels in the intestine of A. suum after removal from the host.

Date of Collection		Intestine	Time (hours) 0	(±S.E.)	5	(±S.E.)	10	(±S.E.)
6·11·79	A. Levels of TCA-extractable glycogen (μ moles CHO/g wet wt.)	Anterior	23.62	±4.96	7.62	±4.43	5.8	±5.30
		Middle	6.91	±1.61	2.12	±1.44	2.54	±1.73
		Posterior	10.95	±1.69	4.51	±1.51	8.23	±1.81
	B. Levels of KOH extracted glycogen (μ moles CHO/g wet wt.)	Anterior	16.68	±3.34	18.25	±2.99	17.0	±3.57
		Middle	6.33	±0.82	6.22	±0.73	8.4	±0.88
		Posterior	7.07	±1.13	7.95	±1.01	10.06	±1.21
(n)			(8)		(10)		(7)	

(n) = number of determinations. Each determination was made on a pooled sample from 3 females.

Means not underscored by the same unbroken line are considered different ($P < 0.05$).

but only between the 5-hour and 10-hour worms.

From the third collection day (Table 7c), data show a significant drop in the mean level of TCA-extractable glycogen from the anterior, occurring by 5 hours after collection. In the middle and posterior intestine, a significant drop ($P < 0.05$) occurs by 5 hours but thereafter the mean levels increase to reach amounts significantly close to that of the 0-hour worms.

Interestingly, the mean levels of alkali extracted glycogen in all three regions of the intestine do not vary significantly ($P > 0.05$) over the ten-hour period.

CHAPTER V

DISCUSSION

The results of this study indicate that morphological differences exist along the length of the intestine of Ascaris suum. The data obtained show that the height of cells as well as the thickness of the basal lamella vary along the longitudinal axis of the intestine of Ascaris suum. These variations are accompanied by differences in the levels of endogenous glycogen which exist between the anterior, middle and posterior intestine. These differences provide further support for the subdivision of the intestine of A. suum into three parts.

The shortest cells were found in the posterior intestine, whereas in the anterior and middle intestine significantly taller cells were observed (refer to Table 1). These results are in accord with those of Carpenter (1952), and raise the question as to the functional significance of this variation in cell height. In an attempt to answer this question, a search was made for other regional differences.

In our study, the thickness of the basal lamella was measured in the three regions of the intestine. The basal lamella was found to be thinnest in the posterior intestine, whereas

the basal lamellae of both the anterior and middle intestine are significantly thicker by roughly 33 and 42% respectively. It is noteworthy that from two separate studies, the basal lamella from the mid-intestine (Peczon et al., 1975) is much thinner than that of the anterior intestine reported by Sheffield (1964). However, when measurements are made using the same worm, the difference is clearly opposite to that indicated.

With the basal lamella believed to be synthesized by the cell which it supports (Kurtz and Feldman, 1962), it is interesting to note that in the present work, tall cells have a thick basal lamella whereas shorter cells are underlined by a thinner basal lamella. Because a thin basal lamella is presumably advantageous to the transport of material across the intestinal wall to the pseudocoelom (Feldman, 1972), it is interesting to compare the pattern of variation in the rate of transport with cell height and basal lamella thickness.

The rate of movement of 3-0-methylglucose across the intestinal wall is not proportional to the cell height and basal lamella thickness. In fact, the rate of transport of 3-0-methylglucose increases progressively from the anterior region to the posterior region (Schanbacher, 1974). The presence of a thick basal lamella and a low rate of transport of 3-0-methylglucose in the anterior region, as compared to the reverse situation in the posterior intestine, agrees with

Feldman's observations of the hematode Leidynema appendiculata, and seemingly supports the assumption that the basal lamella does affect the transport of material. In pursuing this further, the tall cells from the mid-intestine of A. suum would necessarily have a greater surface area of the lateral plasma membrane than cells from other regions, and by virtue of the involvement of the lateral plasma membrane in the active transport of glucose (Hopfer et al., 1976), the amount of sugar transported across the middle region would consequently exceed that in other regions. However, in contrast to the increased lateral plasma membrane, it could be argued that the significantly thicker basal lamella of the mid-intestine is presumably hampering the transport of sugar to the extent of it being lower than in the posterior intestine.

The analyses for glycogen indicated that the amount of glycogen is maximal in the anterior region, drops significantly in the mid-region and again increases slightly at the level of the posterior intestine. With the reproductive system in close proximity to the mid-intestine, and the role of the former in the production of eggs, it was believed that the large requirements for glycogen and trehalose might account for the relatively low levels of endogenous glycogen which we observed in the mid-intestine. With this in mind, experiments were then carried out where male worms were used instead of females, and from which the

level of glycogen was assessed in the anterior and middle intestine.

Our results show that in males, the level of the estimated total glycogen in the anterior intestine is some 2.8 times higher than in the mid-intestine. This difference is similar to that found in the female worms. The difference in the level of glycogen between the anterior intestine and the mid-intestine could be interpreted in at least two ways. The lower amounts of glycogen in the mid-region could be due to the carbohydrate requirements by the neighboring ovary-uterus and testis-seminal vesicle, in females and males respectively. On the other hand, the significant difference in the amount of glycogen can be an inherent characteristic of the intestine, i.e. a gradation in function along the intestine may account for the regional differences in the level of glycogen. Nevertheless, the difference in the level of glycogen between the regions does not appear to be sex dependent, and whether a similar situation predominates in other nematodes remains at present problematical. Possibly indicative for its occurrence is the fact that differences in the amount of glycogen between regions have been associated with variations in function, in nematodes such as Cosmocerca ornata (Colam, 1971a), Ancylostoma caninum (Lee, 1969) and Leidynema appendiculata (Feldman, 1972).

In contrast to results obtained by Fairbairn and Passey

(1957) from a single determination on the intestine of four male and female worms, our results from several determinations show that the mean level of both the acid and alkali extracted glycogen in the anterior and middle intestine are higher in males than in females. In fact, the amounts of acid and alkali extracted glycogen are significantly larger ($P < 0.05$) in the anterior intestine, whereas in the mid-intestine only the alkali extracted glycogen is significantly greater ($P < 0.05$). The glycogen content of whole worms reported by von Brand (1937) from fifteen determinations was shown to be greater in male than in female A. suum, whereas in a later study, the results obtained by Fairbairn and Passey (1957) revealed the opposite. However, in view of the relatively high levels of glycogen in the intestine of males, the present findings support the extensive analyses made by von Brand (1937).

Von Brand (1937) also reported that in spite of the relatively small size, in male A. suum the glycogen consumption is similar to that of females during the first 24 hours of starvation. In a recent study carried out by Premvati and Chopra (1979), in which the depletion of glycogen was investigated in male and female Oesophagostomum columbianum, the results obtained were similar to those of von Brand (1937). However, Premvati and Chopra (1979) have pointed out that in the relatively small males, the initial decay of glycogen was markedly

more rapid than in females. It can be assumed provisionally that a similar situation occurs in male A. suum, and that the relatively large amounts of glycogen in the intestine of males enable the digestive tract to maintain its integrity during periods of fasting, and in spite of the high metabolism.

The significance of the difference in the amount of glycogen between the regions of the intestine in the female A. suum was investigated by assessing the portion of glycogen extracted by trichloroacetic acid with respect to the estimated total glycogen (i.e. glycogen extracted by TCA and by KOH). In fresh worms, the levels of glycogen extracted by acid as well as by alkali proved to be significantly higher in the anterior intestine than in the middle and posterior intestine. However, in these worms, the ratio of acid-extractable glycogen to the sum total of glycogen extracted by acid and alkali was found to be significantly lower in the mid-region than in the anterior and posterior regions. In the rat liver and muscle, the glycogen extracted by trichloroacetic acid and the glycogen liberated by alkali digestion are believed to represent two metabolically different forms of intracellular glycogen (Bloom et al., 1951; Stetten et al., 1956; Russell and Bloom, 1958). Bearing this in mind, it was particularly interesting to look at the data obtained with the worms starved in vitro.

Induced by either the incubation of the intestine in a

glucose-free medium (Schanbacher and Beames, 1978) or the starvation of worms, alteration of the glycogen content in the intestine of A. suum is largely confirmed to the acid-extractable glycogen fraction rather than to the alkali-extracted glycogen (refer to Table 8). In addition, over a ten-hour period, the drop in the portion of total glycogen extracted by trichloroacetic acid is significantly greater in the anterior intestine than in the middle and posterior intestine, with values of approximately 49, 32 and 23% respectively. In other words, on the basis of the estimated total glycogen, depletion occurred mainly in the portion of glycogen extracted by trichloroacetic acid, and this preferentially in the anterior intestine. Since the anterior intestine corresponds to the secretory portion of the intestine (Lee, 1965), a priori it can be argued that the production of enzymes necessitates a relatively large expenditure of the TCA-extractable glycogen.

In spite of the present lack of data, it can be assumed that in starving A. suum the enzyme production is low. Morphological alterations induced by starvation, such as a reduction in the number of secretory granules and the modification of the Golgi apparatus to a seemingly inactive form (Borgers and De-Nollin, 1974; Borgers et al., 1975), provide circumstantial evidence for a decreased enzyme activity. This follows the involvement of these intracellular organelles in the process of cell

Table 8. Changes in the level of glycogen in the intestine of A. suum during starvation.

Intestine	Glycogen Fraction	Time (hours) 0	10	(±S.E.)	(n)
	TCA (μ moles CHO/g wet wt.)	15.51	4.10	±3.19	(17)
Anterior	KOH	13.63	11.64	±1.79	(16)
	TCA	5.44	2.19	±1.14	(17)
Middle	KOH	5.54	5.79	±0.63	(16)
	TCA	8.02	4.45	±1.18	(17)
Posterior	KOH	6.99	7.35	±0.75	(16)

(n) = number of determinations. Each determination was made on a pooled sample from 3 females.

Means not underscored by the same unbroken line are considered different ($P < 0.05$).

secretion which has been obtained by Bennett and Leblond (1970).

Assuming that enzyme production were to continue despite the absence of ingested polysaccharides and that only intracellular glucose would remain, the process would eventually rely more on the cell's glycogen supply. In turn, the progressive depletion of glycogen would ultimately cause the production of enzyme to drop. If indeed, the decrease in the enzyme production and the depletion of glycogen are closely associated, our results would indicate that the levels of TCA-extractable glycogen best represent the nutritional state of the worm.

Results from sedimentation analyses on the glycogen of Fasciola hepatica and Hymenolepis diminuta indicated that changes in the level of total glycogen induced by starvation were mainly due to variations in the high molecular weight fraction (Bueding and Orrell, 1961). Since the glycogen extracted by trichloroacetic acid has been ascribed a higher molecular weight than alkali extracted glycogen (Stetten et al., 1958), the disappearance of the TCA-extractable glycogen observed in the intestine of A. suum could well correspond to the decrease in the heavy fraction of glycogen observed in the trematode and cestode.

If indeed this is the case, following the assumption that the high molecular weight fraction is converted into a lighter fraction (Bueding and Orrell, 1961), a parallel

situation may well exist in the intestine of A. suum in that during starvation, the TCA-extractable glycogen is converted into a low molecular weight glycogen extractable with alkali. Further, the more glycogen is utilized, the more TCA-extractable glycogen is converted into KOH extracted glycogen, and this apparently occurring preferentially in the cells of the anterior intestine.

It is known that in such helminths as Hymenolepis diminuta (Read, 1956), Taenia taeniaeformis (von Brand et al., 1968) and A. suum (Schanbacher and Beames, 1978), differences in glycogen utilization do arise between worms maintained in vivo and those kept in vitro. In the study of the intestine of A. suum for instance, the importance of working with fresh worms becomes evident since the intestine is most susceptible to changes brought about by starvation (Borgers and DeNollin, 1974) and by drug treatment (Van den Bossche and DeNollin, 1973). It is therefore reasonable to believe that the present results, obtained from worms which were frozen in liquid air upon removal from the host, best represent the true levels of glycogen in the intestine of A. suum.

Present results also indicated, that in starved worms kept alive in warm saline for a ten-hour period, the level of glycogen in the intestinal tissue does not diminish as rapidly as that reported by Schanbacher (1974), with the use of

intestinal sac preparations. In contrast to the in vivo situation, the incubation of intestinal sac preparations in a glucose-free medium resulted in the total depletion of the endogenous glycogen within an 80-minute period. This marked difference in glycogen utilization may be interpreted in at least two ways. The mechanism of glycogen synthesis could be impaired in an intestine which has been removed from the body. On the other hand, the endogenous stores of glycogen in the intestine of unfed worms may be partially replenished by the nearby glucose and trehalose in the hemolymph. Interestingly, Rogers (1945) found the mean concentration of glucose in the hemolymph of unfed worms, kept alive for 24 hours, to increase by 20% over the mean value from fresh worms. As a result, it is possible that glucose from the body fluid remains always available to the intestine, even when ingested glucose is not available. The glucose and trehalose in the hemolymph would be the only available substrates for the synthesis of glycogen in the intestinal cells since glycogen is absent in the body fluid (Fukushima, 1966). This, in turn, would rule out the possibility of having "muscle glycogen" being transferred directly to the intestinal cells.

In addition, Harpur (1963) reported that during the same period of starvation, the glycogen content of muscles decreases by 23% of its initial amount, but since over 90% of the total

carbohydrate is located in the muscle mass, the amount of glycogen in muscles far exceeds that in the intestine at all times. Emerging from this fact, it would seem most probable that the only source of glucose in the hemolymph of unfed worms be the "muscle glycogen." Moreover, the glucose resulting from the breakdown of muscle glycogen, and present in the hemolymph, could presumably be either utilized directly by the intestinal cells or be converted to glycogen in the intestine thereby avoiding further depletion of the endogenous glycogen.

The discrepancy between reports by Van den Bossche and DeNollin (1973) and by Borgers and DeNollin (1974) concerning the amount of glycogen present in the intestine of starved A. suum may be due to the difference in time during which the worms were kept in a glucose-free saline solution. It may be that the glycogen depletion observed by Borgers and DeNollin (1974) occurs up to a certain point, after which time, further substantial decrease is prevented by the glucose from the hemolymph being made available to the intestinal cells.

Studies have revealed that in A. suum, the passage of glucose, fatty acids and vitamin B_{12} across the intestinal wall to the pseudocoelom occurs by active transport (Beames, 1971; Harpur and Popkin, 1973; Beames and King, 1972; Latner and Hodson, 1962). Because the active transport of material as well as the production and transport of secretory products (e.g. enzymes)

require energy derived from the catabolism of endogenous carbohydrate, a possible correlation was looked for between these functions and the levels of glycogen, and this within each region of the intestine. Variations in these three parameters provide sufficient grounds for proposing a structure-function model which agrees with the suggestion that the intestine of A. suum is heterogeneous along its longitudinal axis (Lee, 1965).

The first part of the model which refers to structure, involves the levels of glycogen extracted from the anterior, middle and posterior intestine. The present results indicate that the levels of glycogen significantly drop from the anterior intestine to the mid-intestine, and therefrom increase slightly at the posterior intestine.

The second part of the model which refers to function, involves the levels of enzyme activity and the rates of absorption of 3-O-methylglucose reported by Carpenter (1952) and Schanbacher (1974) respectively. These studies revealed that the level of enzyme activity (i.e. secretion) decreases from the anterior to the posterior and whereas the rate of transport of 3-O-methylglucose gradually increases. It appears very likely that the two extremities of the intestine differ from a functional standpoint in that the anterior intestine is predominantly secretory in function whereas in the posterior intestine, absorption prevails.

However, it should be emphasized at this point, that the little data available on enzyme secretion and rates of absorption do not provide strong statistical support for the structure-function model. Nevertheless, the relationship between structure (i.e. glycogen) and function (i.e. absorption and secretion) is apparent, and can be viewed in at least two ways.

Firstly, the level of glycogen is viewed against the function prevailing in the region of the intestine. In the anterior intestine, where enzyme activity exceeds that in the mid-intestine, the higher levels of glycogen in the former would enable a greater synthesis and transport of secretory products as well as allow the production of enzymes to occur before the next feeding. On the other hand, in the posterior intestine where the rate of absorption of sugars is higher than in the mid-intestine, the larger amounts of glycogen of the posterior intestine would permit more energy to be channeled for active transport.

Secondly, the level of endogenous glycogen is viewed against the combined levels of secretion and absorption in each region of the intestine. Compared with the anterior intestine, the lower levels of glycogen in the mid-intestine can be explained by the two-fold increase in the rate of absorption which presumably outweighs the slightly lower enzyme activity. On the other hand, when compared with the posterior intestine,

the lower levels of glycogen in the mid-intestine can result from a higher cell activity due to the twice as large enzyme activity which seemingly outweighs the approximate 27% lower rate of absorption. When comparing the level of glycogen between the anterior and posterior intestines, the low level in the latter could be explained by its relatively large absorption rate and low level of secretion which would reflect a higher cell activity than the comparatively high level of enzyme activity and low rate of absorption in the anterior intestine.

Although little or nothing has been reported on the actual amounts of energy required for enzyme production and its relationship with the active transport of sugars, it nevertheless seems that the amount of glycogen present reflects the cell's level of activity. The intestine of A. suum has proven itself to be suitable for monitoring these three variables under similar conditions at any one time. It can be assumed provisionally pending final elucidation, that during periods of starvation, some of the glycogen from the muscle mass is broken down to keep concentrations of glucose and trehalose at sufficient levels in the hemolymph so as to always be readily available to the intestinal cells. The intestine would therefore be able to maintain its integrity, with a constant supply of endogenous glycogen to provide energy for intracellular activities, e.g. secretion and absorption.

CHAPTER VI

SUMMARY

Microscopic examinations were carried out to investigate morphological differences between the anterior, middle and posterior intestine of Ascaris suum, and relate these with the gradation in function of the intestine. Our results show that, along the length of the intestine, there are variations in the cell height, in the thickness of the basal lamella and in the levels of glycogen.

The cells are some 24% taller in the anterior intestine and some 71% taller in the mid-intestine than in the posterior intestine. The basal lamellae of the anterior and middle intestine are some 33 and 42% thicker, respectively, than the basal lamella of the posterior intestine. First, these findings indicate a positive correlation ($r = +0.87$) between the size of cells and the thickness of the basal lamella. Second, and more important, the change in the combination of cell height and thickness of the basal lamella between each region of the intestine seems to occur in concert with the gradation in function of the intestine of A. suum.

Chemical analyses revealed that the levels of glycogen in

female worms are markedly higher in the anterior intestine than in the middle and posterior intestine. Since similar results were obtained in males, the difference in the level of glycogen between the anterior and middle intestine is therefore not sex dependent, and is very likely to be an inherent characteristic of the intestine. Further, in contrast to an earlier report, the present results indicate that the level of glycogen in the intestine is higher in males than in females. The small size of males, with presumably a higher metabolic rate, may explain the larger glycogen reserves.

When live worms were kept in a glucose-free medium, the levels of glycogen decreased in all regions of the intestine, but dropped most significantly in the anterior region. This decrease in the glycogen content, induced by starvation, was mainly due to the drop in the level of acid-extractable glycogen. It is suspected that the acid-extractable glycogen corresponds to the storage form of glycogen whereas the alkali extracted glycogen represents the readily utilizable form (Stetten et al., 1958). With this in mind, interpretation of our findings in starved worms leads to the assumption that in the anterior intestine much of the endogenous glycogen is utilized mainly for the production of enzymes in prevision of a meal. In the middle and posterior intestine, however, the stored glycogen is presumably used primarily for the absorption of nutrients, the

decrease being much less during periods of fasting.

In comparison with results obtained from the use of intestinal sac preparations incubated in a glucose-free medium, the depletion of glycogen in the intestine was shown to be much less rapid in starving worms. The failure to demonstrate a marked depletion in the glycogen content may be attributed to the ability of the intestine to utilize the readily available glucose and trehalose in the hemolymph. The functional significance of having this constant supply of glucose, is presumably to help the intestine in maintaining its integrity during periods of fasting.

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