SHORT-CIRCUIT CURRENT AND SOLUTE FLUXES ACROSS THE GUT EPITHELIUM OF *ASCARIS SUUM*

By

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 1977

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ACKNOWLEDGMENTS

I must confess that there was, at times, a sense of uncertainty as to the outcome of this work. But now, I am happy that the end has come and the work is finished. I suppose that if there had been no uncertainties as to the outcome, I would have less personal pleasure and pride in my work. I can not say that I overcome my sense of uncertainty with a grim determination and an iron will. More accurately, I was able to do my work in an atmosphere with the qualitites of tolerance, understanding, trust and mutual interest. This kind of atmoshpere promotes a light-hearted approach to the work, amiable friendships, and good research. If these qualities can be liken to a fine optical lens, then Dr. Calvin Beames is its designer, grinder, and polisher. No one has ever had a more conscientious or finer teacher.

The other members of my committee have all been very helpful to me. Dr. John Sauer has always been available to discuss my work and to share his ideas. Dr. Bill Edwards shared both his knowledge of pharmacology and his social amenities, freely. Dr. John Venable had to leave before my work was completed; however, his patient instructions in my early work was a joy that is not soon forgotten. Dr. Olin Spivey graciously joined my committee relatively late, but

since then, he has been very helpful in guiding me around some theoretical pitfalls.

Besides the many good people that are found "down the hall" willing to share their thoughts, there are two people in the laboratory which have been tolerant, understanding, and generally a lot of fun. Ms. Rhonda Gamble has added a feminine touch to the laboratory which compliments her professional aspiration. Dr. Manus Donahue has expressed lyrical qualities in what he does which infects people around him and, at times, the whole department. Rhonda and Manus have been good and close companions in work and in play.

Finally, I must give credit to whom it goes to most of all - Rosa Lee, my wife. When uncertainties would overtake my thoughts, she would console me. I am glad to share with her the pleasure of knowing that the work is done and that new adventures lie ahead.

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CHAPTER I

INTRODUCTION

The large parasitic roundworm *Ascaris lumbricoides* infects over half billion human beings throughout the world (Stoll, 1947). A physiological variety, *Ascaris suum*, infects swine and costs the swine industry over $200 million annually (Levin, 1968). *Ascaris* infection manifests itself most commonly as pneumonia which occurs when the worm larvae migrate via the lungs to the intestinal tract (Levin, 1968). Secondary pathogenic conditions develop when errant larvae migrants move to other organs such as the lymph nodes, spleen, liver, kidney, or brain where they may induce an inflammatory reaction and subsequent dysfunction of that organ. As adults living in the saml1 intestine, *Ascaris* may seriously compete with their hosts for nutrients, irritate the mucosal lining, obstruct the intestine, occlude bile and pancreatic ducts, and perforate the intestine wall causing peritonitis (Chandler, 1966).

Recently, researchers have become increasingly aware of the incidence of cross infections in humans from other host ascarids. Human diseases caused by the larvae of *Toxocara canis* (dog), *Ascaris suum* (swine), *Parascaris equinum* (horse) have been reported (Chandler, 1966).

In light of the importance of this nematode to both our health and our economics, an understanding of the worm's ability to obtain nutrients from its host would be invaluable for development of rational drug therapy and suitable prophylaxis. In the adult worm, nutrients are absorbed across the worm's intestine. This organ has both an absorptive function and an excretory function. The apparent anatomical simplicity of intestine belies the many functions which it must be capable of accomplishing. The gut of Ascaris absorbs a number of organic compounds and secrets a variety of volatile fatty acids which are products of its metabolism (Fairbairn, 1957; Harpur, 1969). The underlying mechanism(s) by which materials are moved across the intestine is not clearly understood. Results of several investigations with in vitro preparations of the intestine suggest that a carrier mediated mechanism exist by which specific sugars are moved into and across the intestine. These carriers are located in the apical region of the epithelial cells. The process requires energy that is derived from carbohydrate metabolism, and it is capable of moving certain sugars against a concentration gradient. The process is sensitive to phlorizin, and appears to be partially dependent upon the presence of Na^+ in the luminal perfusate. There is a net flux of fluid and Na^+ from the luminal to the pseudocoelomic side of the intestine during the transport of glucose, and the bulk of this flow appears to occur via the intercellular spaces of the epithelium. In the above re-

spects the absorption of sugars by the worm's intestine is similar to the absorption of sugar by the intestine of vertebrates. There are, however, some very interesting and significant differences between the absorption of sugars by the intestine of Ascaris and the absorption of sugars by the intestine of vertebrates. Galactose is not transported in significant amounts by the worm's gut, and it does not compete with the transport of 3-0-methyl glucose (Beames, 1971; Schanbacher, 1974). Ouabain does not appear to inhibit the transport of the sugars by the intestine of Ascaris. Mebendazole (an anthelmintic that is very effective against nematodes) inhibits sugar absorption by the worm, but it does not inhibit sugar transport by the vertebrate intestine. (Borgers et at, 1975; Van den Bossche, 1972). The movement of ions across the vertebrate intestine is well documented (Clarkson, 1967; Crane, 1969; Schultz, 1973; Ussing et al, 1974). The net movement of Na^+ ions across the intestine of vertebrates results in the formation of a potential difference such that the serosal surface is electrically more positive than the mucosal surface. Contrary to this, it has been reported (Harpur and Popkin, 1973) that Ascaris demonstrated a electrically negative pseudocoelomic surface.

The purpose of this investigation was to further our understanding of the transport processes utilized by the gut of Ascaris. In particular, the investigation was oriented towards describing the electrical characteristics of Ascaris intestine and relating the results with what is generally

understood in similar epithelia. The study was initally limited to the several electrical parameters and their relation to the flux of solutes which are thought to be the major constituents of its natural environment, and it was directed towards the active processes. Subsequent to this, further information was sought to describe the function of structures unique to this epithelum. In other words, the study was then directed towards the passive processes.

The methods used in this investigation were dictated by the need for handling the tissue and simultaneously measuring several parameters. A tissue chamber was designed with the hope that it would cause a minimum of trauma to the tissue. The chamber featured water jackets to maintain temperature, gas ports to aerate to facilatate the circulation of the perfusing solutions, electrode ports to allow the positioning of recording electrodes close to the tissue, current electrode and sampling ports to the perfusing solutions. Electronic instruments were designed to automatically monitor the transmural potential and enforce the short circuit conditions.

The results indicate that the intestine of Ascaris is able to actively generate a transmural potential which is not the result of any singular process. The unique morphological features of the gut epithelium, appear to be important in determining the orientation of the observed potential. The only obvious restrictions of the lateral extracellular spaces, which is functionally referred to as the "shunt" pathway, are

the apical junctional complex and the basement membrane (basal lamella). The permeability characteristics of the apical junction complex and the basement membrane (basal lamella) at either end of the extracellular space influences the net distribution of charged particles across the "shunt" pathway. The formation of ion gradients within the "shunt" results in the formation of diffusion potentials and the orientation of transmural potential such that the luminal surface is electrically positive. The nature of the "pump" which creates the ion gradients is suggested by determinations of the uptake of Na^+ and glucose. In these determinations, the coabsorption of Na^+ and glucose across the luminal membrane was demonstrated both isotopically and electrically. The existance of the Na^+ - glucose coupled transport is consistent with the concept that coupled transport is depended upon the preexistance of a Na^+ gradient across the mucosal cell membrane. Such a process is indirect evidence for the existence of a Na^+ ATPase or "pump". The formation of ion gradients within the "shunt" pathway and formation of a Na^+ gradient across the luminal surface may be one and the same process. However, the possibility that this nematode can transport other ions such as H^+ and volatile fatty acids precludes any simple conclusion.

CHAPTER II

LITERATURE REVIEW

In recent years, there have been substantial gains in our understanding of the transport processes that is associated with the absorptive epithelium. These gains were initiated by the use of tissue models such as frog skin and toad bladder. Such models have led to certain hypotheses on the mechanism of the transport system associated with gut epithelia. This section reviews the hypotheses concerning intestinal absorption, how absorption is studied, and what is generally known about Ascaris absorption.

Absorptive Processes

There are generally thought to be five absorptive processes: (1) mass flow through pores; (2) diffusion, which does not involve any specific structures; (3) facilitated diffusion, which involves a specific structural relationship; (4) active transport, which involves both a structural relationship between the membrane permeating molecule and a source of energy; and (5) pinocytosis (Read, 1966). Of these five processes, active transport and pinocytosis are the most intriguing since they are so intimately related to the living process. That is, they are in some way coupled to

the metabolic energy of the cell. Active transport is more completely understood than pinocytosis and appears to involve three steps: (1) the binding of a solute to the receptor site on the membrane, (2) translocation of the coupler across the membrane, and (3) coupling of the transport process to the metabolic energy producing systems of the cell (Oxender, 1972).

There is no one generally accepted explanation for the energy coupling in active transport. Recent reviews have listed a few of the major observations and thoughts on energy coupling (Oxender, 1972). In Escherichia coli, the addition of PEP and ATP to the incubation media does not stimulate amino acid uptake. Consequently, it is thought that oxidative phosphorylation is not responsible for the energy. Mitchell (Oxender, 1972) has proposed that active solute transport may be accomplished by local chemosmotic potentials across membranes. Along with this idea, Pavlosva and Harold suggest that protein gradients within the membrane are the energy transducers (Oxender, 1972).

Another concept which follows Mitchell's proposal is Kaback's "Transport carriers" (Oxender, 1972). In this theory, the carriers are electron transfer intermediates between dehydrogenases and cytochrome b_1. The reduced form of the intermediate carriers have a low affinity for the substrate. Oxidation of the carriers during the electron transfer provides the energy to convert the low affinity form to the high affinity form. This concept of "transport carriers"

is supported in part by the observation that in E. coli, amino acids and sugar transport appear to be coupled to D-lactic acid dehydrogenase. Other evidence indicates that in Staphylococcus aureus, lactose and glucose-6-phosphate transport are linked to α-glycerophosphate dehydrogenase.

Once the "metabolic" energy is transduced in establishing a concentration gradient for a particular solute (i.e., sodium ions), the solute's gradient can in turn be the force for the transport of other solutes (i.e., glucose) (Oxender, 1972). The evidence for this is based upon the observation that a number of sugars and amino acids are closely coupled with Na^+ transport in the direction of the Na^+ electrochemical gradient.

Thermodynamics Review

It is the intention of this section to touch upon the pertinent concepts of thermodynamics which are felt to be important for an understanding of transport processes. In particular, there are thermodynamic concepts which help to differentiate passive processes from the active process associated with the movement of materials across membranes. This review is intended not to be complete nor to show derivations or proofs of the concepts given. The intention is to relate fundamental precepts of thermodynamics to the energetics of solute flow in terms of direction, energy source, and coupling with other flows.

General Concepts. The first law of thermodynamics states that the change in the internal energy of a system is the sum of the heat gain by the system and the work done on the system (Pure and Applied Chemistry, 1970). That is:

$$dU = dQ + dW$$

where

U is the internal energy

Q is heat gain by the system

W is the work done on the system

The work expression can be in terms of mechanical work, PdV; electrical work, Ψde; or chemical work, $\mu_i dn_i$. The work done on a system, then, can be expressed as the sum of these terms:

$$dW = PdV + \Psi de + \mu dn$$

This concept of work is important in that it describes what is required for a process to occur. At constant temperature, T, and pressure, P, the reversible (minimum) work done on a system other than pressure-volume work is equal to the change in free energy of the system. Therefore, the change in free energy in a process is equal to the minimum work (other than P-V work) required for the process to occur:

$$\Delta G = W_{min,P}$$

If the $\Delta G>0$, then $W_{min,P}>0$, and the process would require an input of work. However, if $\Delta G<0$, then $W_{min,P}<0$, and the process will occur spontaneously. In other words, if a system contains enough free energy, the process may occur without an input from outside the system.

When energy is put into a system, not all the energy is

used as work energy, some is represented by heat energy. The relationship between the heat gain of a system and temperature change has been termed entropy (S). $Q_{rev}/T=S$. This term represents the energy not available to do work. Thermodynamics does not explain why this happens; however, it is generally thought that entropy is a measure of disorder. That is, randomness tends to replace order, and an improbable arrangement tends to shift toward a more probable one (Christensen, 1972). If the change in entropy is greater than dQ/T (i.e., $dS>dQ/T$) in one direction of a process, an irreversible change will occur in that direction.

From the foregoing a relation between entropy and free energy can be derived. First, however, a transformation must be defined. In many chemical reactions and living processes the pressure remains constant. Consequently, the work done on such a system may exclude any work due to pressure changes. All work except pressure is symbolized by W_p. So the following parallel relationship can be stated:

$$\Delta U = Q + W$$

$$\Delta H = Q_p + W_p$$

where

ΔH represents the change in enthalphy or, in other words, the change in ΔU minus $P\Delta V$.

Knowing enthalpy, Q can be substituted for $T\Delta S$, giving:

$$\Delta H = T\Delta S + W$$

Solving for W gives:

$$W = \Delta H - T\Delta S$$

If this W represents the minimum amount of work necessary for a process to occur and that $\Delta G = W_{min,T,P}$, we can substitute the expression to give:

$$\Delta G = \Delta H - T\Delta S$$

This defines the relationship between free energy and entropy. The free energy measures the ability of the system to do work other than pressure-volume work. So that the free energy is measured by both the electrical and chemical work (Curran, 1967):

$$\Delta G = -\Psi de - \mu dn$$

From this relationship it can be seen that free energy can be related to a change in the chemical quantity (n) of the solute. The quantity which allows us to relate the concentration of a substance and free energy is the value termed chemical potential (μ). This measures the change in the free energy in the system for a change of one mole in the amount of a solute of type i (Chritensen, 1972). The formalized statement is:

$$\mu = \frac{\partial G}{\partial n}\Big)_{T,\ P,\ n_{j \neq i}}$$

This term allows the determination of how much useful work (free energy) might be obtained from a difference in its value for two phases or how much work would be necessary to create such a difference (Christensen, 1972).

If the solute carries a charge such as an electrolyte, the free energy is equal to the electrical and chemical work as stated above. The free energy (G) that is associated with

both kinds of work, electrical and chemical, is commonly referred as the electrochemical potential:

$$\tilde{\mu} = \mu + z F$$

Or, in other words:

$$dG_{T,P} = \tilde{\mu} dn$$

It is this relationship which allows us to determine directly how much useful work might be obtained from the difference in concentration of an electrolyte in two separate phases (Curran, et al., 1967). That is:

$$dG_{T,P} = (\tilde{\mu}^a - \tilde{\mu}^b) dn$$

where (a) and (b) are the two phases. The two phases can be thought of as the solutions on either side of a plasma membrane or a tissue membrane. Diffusion of a substance will occur across a membrane in the direction in which its free energy (electrochemical potential) distribution will be uniform throughout (Christensen, 1975).

Energy Coupling. In the preceding section it was shown that classical thermodynamics can determine the distribution of forces which can drive a reversible process. Nonequilibrium thermodynamics, on the other hand, can be used to determine how the energy arising from the flux of one solute can serve to drive the flux of a second solute (Christensen, 1975). This is referred to as coupled transport. The unique feature of coupled transport processes is that not all the energy that is furnished by the flux of the first solute is utilized by the flux of the second. Consequently, the rever-

sal of the transport process cannot restore the original condition (Christensen, 1975). This is referred to as an irreversible process.

Since some of the energy in a coupled transport process is not utilized in work, internal entropy of the system is increased. That is, the rate of entropy production is a function of the fluxes that occur and the forces that produce them:

$$\frac{dS}{dt} = \Sigma J\ X = \Phi$$

where

J is the i^{th} type flux

X is its conjugate force

Interestingly, the flux of a solute may be affected by other fluxes present in the system. The degree of coupling between any two fluxes, J_i and J_j, is given by $L_{ij}X_j$ where X_j is the conjugate force of the j^{th} flux. Thus the net flux J_i is given:

$$J_i = \Sigma_j\ L_{ij}\ X_j$$

where

L is the coupling coefficient that expresses the effect of force X_j on the flux J_i

An extension of this concept proposes that $L_{ij} = L_{ji}$. This is known as the reciprocity theorem of Onsager (Curran, 1967).

The thermodynamic evaluation of a coupled transport process has been attempted by utilizing the entropy production equation stated above (Curran, 1967). That is:

$$\Phi = J_A\Delta\bar{\mu}_A + J_B\Delta\bar{\mu}_B = \Sigma JX$$

where

A is one solute

B is another solute

$\Delta\bar{\mu}$ is the electrochemical potential across the membrane

For the process to occur, the value Φ must be positive. The coupled transport mechanisms that have been evaluated are the Na^+ - glucose and Na^+ - Amino acid mechanisms. It is generally thought that glucose and amino acid transports are driven by the energy inherent in the asymmetric distribution of Na^+ across the cell membrane (Curran, 1967). Empirical tests have generally given positive values for Φ for the Na^+ - glucose mechanism but not for the Na^+ - amino acid mechanism. The difficulties of determining the chemical potentials for the Na^+ - amino acid mechanism have not allowed for the successful evaluation of Φ (Meares, 1973).

The question arises how is the energy coupled from one flow to another? Unfortunately, thermodynamics does not address itself to this question. Nonequilibrium thermodynamics, though not directly concerned with such an answer, has proven to be of great value in the conceptual arguments of how a process may occur than in the actual quantitative correlation of fluxes and forces (Meares, 1973). Christensen (1975) has proposed a transport model which may explain the mechanism of energy coupling. This model visualizes a protein oligomer assembly extending across, or nearly so, the plasma membrane in which substrate on either side may make occasional con-

tact. One substrate which represents the energy source due to its high electrochemical potential on one side would attach itself to an active site; this attachment results in a conformational change for the receptor sites for the other substrates, and in a reorientation of the oligomer from one surface to the other. The resulting change in position leads to a change in the affinity of both active sites and results in the flow of both substrates in the same or opposite direction.

Active Transport. There have been various attempts to give an all inclusive definition of active transport. One definition states that the movement of solutes depends on the activity or energy change of another system (Conway, 1954). Rosenberg's (1954) definition is more specific: active transport brings about the net transfer of a solute against its electrochemcial potential difference.

The Rosenberg definition has two important drawbacks (Curran, 1967). The movement of a substance could be in the direction of its electrochemical gradient but be greater than predicted. This would certainly indicate an energy input other than the preexisting electrochemical potential. Another drawback to the definition is that the movement of a substance i may be coupled to another force even where there is no electrochemical gradient of substance i. That is, there may exist a flux due to a coupling with other flows as defined by the following relationship:

$$J_i = \Sigma L_{ij} \Delta\mu_j$$

where the flux of i is coupled to the flux of j even when $\Delta\mu_i = 0$.

Kedem (1961) has proposed a definition which circumvents the difficulties of previous definitions by making an assumption. She assumes that the energy which accounts for an active process is derived from the metabolic processes of the tissue. Therefore, active transport is simply the interaction between the transmembrane flow of a solute and a metabolic reaction. Such an interaction could be shown by demonstrating an increase in metabolic activity during transport, or the slowing or stopping of the transport process by the use of metabolic inhibitors (Kotyk, 1970).

The Kedem definition describes the qualitative nature of transport. It tells us whether a transport process is active or passive. The quantitative nature of transport is determined by measuring the influx and outflux of a solute across a membrane. Preexisting conditions across the membrane may substantially influence the flux of the solute and alter the flux ration from 1. The factors that influence the flux ratio are those forces which alter the value of the electrochemical potential of a solute. Ussing has formulated the relationship of the forces to the flux ratio in the following equation:

$$\frac{J_i^a}{J_i^b} = \frac{\Delta\mu i^a}{\Delta\mu_i^b} = \frac{c^a y^a}{c^b y^b} \cdot e^{\frac{-zF}{RT}(\Psi^a - \Psi^b)}$$

where

c^a and c^b are the concentrations of the solute on either side of the membrane

y^a and y^b are the activity coefficients of the solute

$\psi^a - \psi^b$ is the potential difference across the membrane

As it can be seen, if the concentrations of the solute are made the same across the membrane and the potential difference held at zero, the flux ratio across the membrane should be 1. However, if there is a discrepancy in this value, it is assumed that forces in the membrane are acting on the flux of the solute (Ussing, 1951). The correction factor which makes J_i^a/J_i^b equal to μ_i^a/μ_i^b is equal to the force which causes the discrepancy. It is equal to the force of active transport. This force is:

$$E_i = \frac{RT}{zF} \ln \frac{J^a}{J^b}$$

Ion Pumps

There are at present two schools of thought as to the functional organization of living cells. The first, the membrane theory, visualizes the cell as containing water and solutes in a aqueous solution and in a free state. The mechanism which maintains nonequilibrium ion distributions across the cell is termed the "ion pump" and is associated with the cell membrane. The second theory, termed the association - induction hypothesis, maintains that the cell is a complex protein-aqueous fixed charge system in which water

molecules exist as polarized multilayers, and the activity of the solutes within the cell are essentially the same as in the environment. Consequently, according to the association-induction hypothesis, a "pump" is neither necessary nor does it exist (Ling, 1969). Contrary to this hypothesis, the preponderance of experimental evidence indicates that water appears to freely enter and leave cells (Kolata, 1976). And when cells are lysed, the intercellular water is freely available as solvent. In RBC's, 92% of the water is free (Christensen, 1972).

The isolation of the brush border (microvilli) from the intestines of the guinea pig and hamster have demonstrated a Na^+-K^+ ATPase activity (Schultz, 1967). Fractionation of bovine brain have yielded a partially purified Na-K ATPase protein having a molecular weight of 94,000 (Oxender, 1972). It seems that the Na^+-K^+ ATPase may be part of a Na^+-K^+ transport mechanism responsible for the maintenance of the high intracellular K^+ concentration and low intracellular Na^+ concentration characteristic of the tissues (Schultz, 1967).

In addition to the Na^+-K^+ pump, there appear to be other types of ion pumps. In tadpoles, Na^+ can accumulate from Na_2SO_4 in the incubation media even though SO_4^{-2} is not absorbed. Since the tadpole excretes NH_4^+, it is thought that there is an exchange of Na^+ for NH_4^+ or Na^+ - NH_4^+ exchange pump (Alvarado, 1970). In the turtle, the net ion movement across the urinary bladder is equal to the uptake of Na^+ when the anions Cl^- and HCO_3^- are absent. In studies on the

acidification mechanism of the turtle bladder, it was observed that the pCO_2 of the mucosal fluid decreased, suggesting that the active absorption of HCO_3^- lowers the pCO_2 of the mucosal fluid (Gonzalez, 1967). From the above observations, there appear to be several ion pumps working independently.

Along with the concept that ions are pumped into or out of cells is the concept of net ion distribution throughout the tissue. This distribution or ion movement across the epithelium appears to take the form of two pathways. The transcellular pathway involves the movement of ions across at least two cell membranes arranged in series. The other pathway is referred to as an extracellular pathway or "shunt" pathway. This pathway is in parallel with the transcellular pathway and circumvents it.

It appears that many absorptive epithelia possess both pathways, but one pathway may predominate over the other, giving the tissue its particular absorptive characteristics. In those tissues in which the transcellular pathway predominates, the tissue is characterized by a high transepithelial electrical potential difference (PD), high resistances, and low hydraulic conductivities. Examples of this pathway are isolated frog skin and toad urinary bladder. In tissues where the shunt pathway predominates, the tissues are characterized by low transepithelial PD's, low resistances and relatively high hydraulic conductivities. Examples are the small intestine, proximal renal tubules, and gall bladder (Schultz, 1973).

Na^+ and Non-electrolyte Absorption

The Na^+-K^+ pump found in the cell membrane establishes a Na^+ gradient decreasing from the outside to the inside of the cell. The functional role of the lower Na^+ concentration within the cell has been linked to the uptake or absorption of non-electrolytes such as sugars and amino acids. Presently, there are two major hypothesis which describe how the Na^+ concentration gradient might function to cause the uptake of a non-electrolyte. The Crane hypothesis is based upon the idea that a carrier within the mucosal border forms a ternary complex with the exogenous Na^+ and non-electrolyte. This complex then transverses the membrane and releases the Na^+ and non-electrolyte within the cell. Subsequently, the Na^+ is removed via the Na^+ - K^+ pump and the non-electrolyte distributes itself according to its activity. The second hypothesis is known as the Csaky hypothesis. Its central idea is that intracellular Na^+ is required for the coupling of metabolic energy to the carrier system. Support for this hypothesis is based on the observation that removal of extracellular Na^+ diminishes non-electrolyte absorption. Presumably, due to a depletion of intracellular Na^+ pools (Schultz, 1967).

Much evidence has been accumulated which indirectly supports the Crane hypothesis. The observation that a metabolically poisoned tissue will continue to take up a non-electrolyte would suggest that energy is not directly coupled to non-electrolyte absorption (Crane, 1969). Mathematical

models suggest that once the ternary complex (Na^+ - non-electrolyte-carrier) moves to the inner surface of the cell membrane, the high K^+ concentration allows K^+ to compete with Na^+ for a cation binding site on the carrier. Subsequently, the affinity of the carrier for the non-electrolyte is reduced and the non-electrolyte is dissociated into the cell sol (Schultz, 1967).

Transepithelial Permeability

It has been observed that small inorganic ions such as K^+, Na^+ or Br^+ do not permeate across the uninterrupted bimolecular membranes of phospholipids unless associated with a carrier molecule (Bangham, 1972). And even when these ions are moved across a membrane, it is usually exchanged with an equivalently charged ion. As a result, there is no or very little net charge segregation or potential across the membrane. Consequently, it is thought that the electromotive forces generated across the cell membrane of epithelial cells have little direct influence on the transepithelial potential. It is, therefore, believed that the high conductant extracellular pathway or shunt pathway predominantly influences the transepithelial potential (Frizzell, 1972).

The observation that increasing the width of the lateral spaces of the gut epithelium does not signifcantly increase tissue conductance or permeability to small water soluble molecules suggest that the lateral spaces themselves do not act as the main barriers of the shunt pathway. Since the only obvious restriction of the lateral spaces in these gut

epithelia is the tight junctions, it was proposed that the tight junctions constitute the principle barrier to the transmural movement of ions throught the shunt (Schultz, 1972).

If the tight junction functioned as the only barrier to ion movement, the epithelium would act as one membrane instead of a two membrane system such as that made up of the mucosal cell membrane and the serosal cell membrane. Barry (1970) using the gall bladder as a model tested to see if it was a one membrane system. He reasoned that the change in PD across a single membrane caused by a given change in salt composition in one bathing solution depends upon the salt composition of the opposite bathing solution. The change in PD resulting from a change in the mucosal solution would depend upon the serosal solution composition. If, on the other hand, the measured PD were the sum of two PD's in series, then the drop in PD resulting from a change in mucosal bathing solution composition would be initially independent of the serosal composition (Barry, 1970). The resulting changes in PD's with change in cation concentration in the presence of a non-permeating anion and constant total cation concentration is a simple function of their permeability ratio. That is, the change in potential can be related to the concentration change by the constant field equation of Hodgkins and Katz (Hoshiko, 1973). From such measurements, it was concluded that the tight junction is permeable to small ions and water and appears to provide the principle re-

sistance barrier of the extracellular pathway. Also, it appears to be permselective for cations and imposes a 2.5 fold restriction on Cl^- permeability compared to Na^+ (Frizzell, 1972).

Surface Charge

The reason why the selectivity of the tight junction for cations and the ability of the ion pump mechanism functions effectively may be related to the electrically charged sites on the membrane. Fixed positive charges on a membrane give rise to a depleted cation and an enriched anion population; hence, a reduced chance for cation transfer. A fixed negative charge would bring about a reversed situation (Bangham, 1972). The degree of affinity of a cation for a given anionic site may be determined by the difference in the electrostatic free energy obtained by the pairing of anions and cations at a distance of closest approach and the free energy lost by the dehydration of those ions. The differences in the affinity of two cations for the same anionic site would then be determined by the differences in the free energy of hydration of the two cations and their ionic radii (Bangham, 1972).

The nature of the tight junction which would make it permselective for cations was thought to be related to sialic acid content. Sialic acid appears to be largely responsible for negative surface charge of the glycocalyx membranes. As the membranes of adjoining cells become opposed at the tight junction, the sialic acid content is much reduced as dis-

closed by electrophoretic studies (Frizzell, 1972). The implication is that the permeability characteristics of the tight junction is not determined by the surface charges found on the membrane.

Water Transport

Along with the concept that the shunt pathway may very well influence the transmural potential of epithelial tissues, the shunt appears to be the mechanism responsible for isotonic fluid transport. It is thought that solute is transported into the apical portions of the lateral intercellular spaces which results in a local region of hypertonicity. This provides the driving force for water flow through the tight junction and cell interior into the lateral spaces (Frizzell, and Schultz, 1972; Clarkson, 1967). The resulting increase in water volume in the lateral spaces causes the spaces to swell many times from their original size. This phenomena has been observed in a number of different tissues (Diamond, 1964).

Measuring Ion Current

Short Circuit Current

An electrical potential develops across most absorptive epithelia, and, in most instances, is due to the net movement of sodium ions. This information is derived from quantitative determinations that are carried out by using ^{22}Na and ^{24}Na and single, double and triple label radioisotope counting techniques to measure the forward and back flux of the test

compounds across the membrane. Initially, the potential which develops from the transport of sodium by this system is reduced to zero by applying an external current, termed the short circuit current (SCC). The measured current (usually in μA) is compared to the net flux of the ion(s) under investigation. In frog skin experiments, the net flux of sodium is equivalent to the observed SCC.

As ions are pumped across a membrane, a PD is generated due to the resistance of the membrane. That is, the observed voltage difference across the membrane is related to the ion current and the resistance of the membrane as defined by Ohm's Law (V = IR). The current that is generated through the membrane can be measured by short circuiting the membrane via a pathway which would allow the current to move to the other side through a lower resistance than the membrane resistance. The current through this external pathway would be equal to:

$$I_e = \frac{EMF}{R_p + R_e}$$

where

I_e is the external current

R_e is the resistance of the external pathway

R_p is the resistance of the internal EMF or pump

The current through R_e is the same as R_p but in opposite direction and is referred to as the SCC. If R_e is near zero, then the $I_e = \frac{E}{R_p}$. Since in reality R_e will never be zero and

there exists shunt(s) pathways across the tissue with its own resistance (R_s), then:

$$SCC = I_s + E_e$$

since

$$R_e = \frac{R_e R_s}{R_s + R_e}$$

$$= E \frac{1}{R_p + \frac{R_e R_s}{R_s + R_e}}$$

simpified:

$$SCC = \frac{R_s + R_e}{R_p(R_s + R_e) + R_s R_e} \times E$$

The difficulty with using this relationship is that the values for R_s and R_p must be determined before the SCC can be calculated. To overcome the difficulty of determining the R_s and R_p, a current can be sent through the membrane of such strength and direction that it reduces the observed PD to zero. Exactly how this applied external current works is probably best explained by the analysis by Karger (Watlington, 1970).

In those situtaions in which the bathing media resistance is significant, a more complex mathematical relationship must be considered. There are various techniques which allows for the error introduced by the electrodes (Rothe, et al 1969; Ussing and Zerahn, 1951). Certainly, the easiest procedure to use is the automatic electrical feedback compensation as described by Rothe (1969) or the three electrode procedure as described by Harvey (personal communication).

However, these special procedures can be avoided if the measuring potential electrodes are placed very near to the membrane in order to reduce the bathing media volume and, consequently, its additive resistance.

In determining the SCC of a membrane, other conditions may exist which might significantly introduce erroneous results. The existence of a membrane diffusion potential would obscure the actual measurements of the currents by the active components of a membrane. The procedure to follow in avoiding diffusion potentials is to place identical solutions on the two sides of the membrane (Watlington, 1970) and to have the two solutions on either side of the membrane well stirred during the SCC procedure. If the two solutions cannot be made the same, correction has to be calculated. The net ion flux and the rate of electrical charge transfer can be calculated by using Fick's law:

$$J_i = P_i \ (a_i^A - a_i^B)$$

where

J_i is the net flux of ion i with a permeability P and a chemical activity a_i in compartments A and B

The effects of the SCC procedure, may or may not be injurious to the tissues. Fully shorted skin from the frog has shown a swelling of the first living cell layer just underneath the stratum corneum (Voute, 1973). Presumably, the swelling is due to the accumulation of solutes in the intercellular spaces and the resulting movement of water into these spaces due to osmosis. There appears to be no injury to the

tissue due to the swelling. However, in other types of membranes, swelling may very well be great enough to injure the tissue. One possible procedure to assess such damage during an experiment would be to plot the change in tissue resistance against time between the open circuit and closed circuit condition. Significant differences would possibly indicate damage due to swelling.

Ussing Chamber

There are generally two kinds of electrical clamping procedures. One procedure involves maintaining a constant voltage or potential across a membrane. This procedure is known as voltage clamping. Another procedure maintains a constant current across a membrane. This is known as current clamping. The method described in the preceding section dealt with the voltage clamping procedure, and this section will review some of the practical considerations in accomplishing the voltage clamping procedure and the measurement of the current necessary to clamp a membrane - the short circuit current.

The apparatus generally considered ideal for the SCC procedure was designed by Ussing (1951) and has become known as the Ussing chamber. There have been many modifications made on the original Ussing chamber to suit the particular tissue and experimental conditions, but the essential features of the chamber are the same. The tissue is placed as a membrane separating two physiological solutions. Two KCl-Agar bridges open on either side of the membrane. These bridges make contact with two KCl-Calomel electrodes with which the

PD across the membrane is measured using a high impedence potentiometer. A pair of current electrodes are placed in the two solutions on either side of the membrane. The current electrodes are attached to an external EMF such as batteries or a well regulated power supply circuit. By adjusting the voltage between the current electrode, a current can be passed through the membrane to drive the PD of the membrane to zero (Ussing, 1951).

In designing a chamber, consideration must be given to the membrane and current electrode geometry. The placement of the electrode should be such that the current density through the tissue is nearly the same so as not to give rise to differences in the voltage drop across the membrane. If the electrode is considered a point source of current and is x distance from the center of a round tissue which has a radius of r, then the current must pass through a greater distance to reach the periphery of the membrane by $\sqrt{r^2+x^2} - x$. The current density would be less at the periphery than at the center. Therefore, when the center of the tissue is clamped at zero, the periphery would still have a slight potential. To minimize this error, the distance x of the electrode from the membrane should be as great as possible (Ussing, 1951), or the area of the electrode should be increased.

Another consideration that should be made is the voltage drop due to the resistance of the bathing fluid between the potential measuring bridges and membrane when clamping

the tissue. This problem is emphasized when using a tissue of a relatively low specific resistance. In such a case, the voltage drop due to the bathing media may be of the same order of magnitude as the spontaneous potential of the membrane (Rothe, 1969). There are several ways to minimze this error. One way would be to place the agar bridges to the potential measuring electrodes as close to the membrane as possible. This would decrease the solution volume on either side of the membrane and, consequently, reduce the secondary voltage drop. Another procedure would be to compensate for the voltage drop by increasing the SCC. That is, if the resistance of the layer of saline between the bridges and membrane are known, then the voltage drop due to a given current can be readily calculated and the SCC adjusted to give an observed PD of the negative value of the potential. Thus the tissue would be clamped at zero (Ussing, 1951). A procedure which can be used which automatically corrects for this type of error is the three electrode method (Harvey, 1973). In this procedure, a second Agar-bridge-Calomel electrode setup is placed on the same side as one of the reference electrodes used to measure the PD of the tissue at a distance from this reference electrode equal to the distance between the two tissue measuring electrodes. The PD between these two electrodes on one side of the membrane is inverted and summed with the third electrode on the other side of the membrane. The sum automatically subtracts the necessary correction. The only precaution that needs to be made is that the bathing

solution on either side of the membrane have the same specific resistance and that the bridge tips be equally spaced.

There are basically two methods by which the SCC can be applied. The traditional method has been to apply a voltage across the membrane by using a battery and a potentiaometer and manually adjusting the voltage driving the transmural potential to zero. Alternately, automatic electrical circuitry detects the membrane PD then delivers enough current to zero the membrane. The automatic circuitry essentially depends on a negative feedback loop which includes the tissue region (Menninger, 1960).

The automatic SCC procedure has advantages over the manual procedure. It has a much greater time resolution so that it follows rapidly changing events. It also allows one to maintain the continuous voltage clamp without having to continuously monitor the potential.

Aside from its obvious advantages, the automatic procedure must attend to the same precautions as with the manual procedure. In addition, there are errors which are peculiar to the automatic circuitry. If there is significant impedence loading of the imput electrodes by the differential amplifiers or significant cable capacitance, there could develop a significant electrode current and a resulting error in the SCC. Reactive coupling of the clamped region directly to ground other than that of the grounded electrode may result in erroneously measured currents (Menninger, 1960).

Edge Damage

One of the intuitive questions one asks concerning the placement of a living membrane in the Ussing type chamber is to what extent does damage accrue due to the physical compression around the edge of the tissue? Dobson (1968) found using frog skin, which is a rather thick tissue, that a 1 cm^2 area of tissue showed a 22% damage area. However, this damage had no effect on the SCC eventhough the damaged area acted as a shunt pathway and lowered the spontaneous PD. Several precautions can be employed to decrease the edge damage. One is to decrease the edge to surface ratio. Since the edge length is equal to $2\pi r$ and the surface area is given by πr^2, by increasing r, the ratio of circumference to area will decrease. Another approach is to carefully design the clamping surfaces to minimize damage to the clamped tissue. A good practice is to supply the surfaces with "0" rings which tend to reduce the squeezing of thick tissues and causing secondary compression due to the displacement of tissue not actually under surface compression.

Absorptive Processes of Ascaris

One of the advantages of working with Ascaris is that it is readily available and can be maintained in the laboratory fo several days. Another advantage is that it is large enough to be easily handled (Griechus, 1966). Consistent with its size is the size of its intestine. The size of this organ would certainly seem to indicate that it was used

for absorptive purposes as do other animals with comparatively sized intestines. However the intestine was not considered the only absorptive surface until recently, when the role of the worm's cuticle was examined (Read, 1966). Apparently the cuticle can absorb a few small ions and anthelmintic hydrocarbons (Read, 1966); however, the low molecular weight nutrients such as glucose cross only the intestine (Castro, 1969; Read, 1966).

Anatomical Features of Ascaris Gut

The gut of *Ascaris* extends the full length of the worm, from mouth to anus. Its intestine wall consists of a single layer of tall columnar epithelial cells about 90 μ in height (Kessel, 1961; Sheffield, 1964). There are some general features of these epithelial cells which can readily be recognized under the light microscope. The luminal surface of the intestine cells have a bacillary or microvilli layer. On the pseudocoelomic side, the intestinal cell is attached to a basal lamella or basement membrane by a series of tortuous infoldings of the cell membrane. Within each cell is found an extensive network of mitochondria in the shape of long strands, clumps of small granular inclusions, and a basically situated nucleus. Just beneath the bacillary layer is found a region that extends into the cell about 3μ called the terminal web. This area is characterized by the absence of any apparent cytoplasmic organelles (Kessel, 1961).

The only surface by which the gut can absorb substances from the lumen is its microvillous surface. These microvilli

are similar to the ones of other absorptive gut epithelia. The conformation of the microvilli is thought to be a means by which the surface area can be effectively increased. Kessel (1961) estimated that there is approximately 3,000 microvilli per cell and that each microvillus is 6μ long and 0.1μ in diameter. With this information it was calculated that the microvilli presented a surface area for each cell of 13,000μ^2. This is an increase in the gut epithelium surface area of about 90 times over that of a smooth surface.

Electron micrographs reveal that the microvilli's outer surface is covered with a glycocalyx and unit membrane. Tubular filaments apparently make up its central core which extends into the terminal web as rootlets (Van den Bossche, 1973; Kessel, 1961). This arrangement is very similar to that of mammalian intestinal epithelium.

The gut epithelium of Ascaris appears to be a continuous sheet with no pores between cells that would allow the free passage of small organic molecules. Studies using horseradish peroxidase have shown that the microvilli became uniformly covered with reaction product. The reaction product continues over the entire luminal surface and was stopped at the point where the cells join together in terminal bars (Van den Bossche, 1971).

These terminal bars have sometimes been referred to as demosomes. However, the structures which have been traditionally referred to as desmosomes appear to have more filaments attached at the inner leaflets of the unit membrane

and a greater space between the outer leaflets. Similar to other desmosomes, however, the plasma membrane just below the terminal bar become interdigitated with the adjacent cell (Kessel, 1961). Just below this is found large intermittant intercellular spaces (Van den Bossche, 1973; Kessel, 1961).

At the opposite pole of the intestinal cell and attached to it is the basal lamella. Under the light microscope, this structure appears as a continuous, homegenous sheet which stains slightly basophilic. The basal lamella is about 6-7μ thick (Kessel, 1961). It is assumed that it arises as a secretory product of the epithelial cells. Due to its structural integrity and tensile strength, the basal lamella may act as a supporting apparatus for the epithelial cells and a guide for cell regeneration (Ross, 1968). Since there appear to be no pores and it is a relatively thick layer, it may be selectively permeable to certain solutes or colloids (Donahue, 1976). The role that the basal lamella may have in determining the rate and specificity of absorption of particles may be far greater than that of a nonselective, inert membrane.

Absorptive Process of Ascaris

At present, there appears no definite answer as to how this anaerobic nematode maintains itself through its absorptive processes. Certain comparisons with other animals with similar anatomical features have been made in order to derive

tentative hypotheses and answers. However, the empirical data would seem to be contradictory to the comparisons that have been made, or would suggest that a different physiological mechanism exist in Ascaris. The following discussion reviews some of Ascaris gut physio-chemical profiles.

Some enzyme activities found in vertebrates microvilli are also found in the microvilli of Ascaris. Dissacharide hydrolases are prominant components of the microvilli. Also an enzyme that hydrolyzes 5' AMP at pH7 is found. This enzyme is similar to that which appears in rat and mouse intestine (Van den Bossche, 1973). Unlike vertebrate intestine which contains high levels of alkaline phosphatase, Ascaris intestinal epithelium has a strong acid phosphatase (AcPase) activity (Van den Bossche, 1973). This activity appears to be bound only to the microvilli, lysosomes, and secretory granules (Borgers, 1970). Speculation as to the function of the acid phosphatases has been that they are related to extracellular digestion and absorption of macro-molecules (Van den Bossche, 1973). Incubation of the gut tissue with 0.01M NaF inhibits the acid phosphatase activity. Presumably, the metabolic machine that forms the secretory granules is inhibited as is the depostion of the acid phosphatase enzyme at the brush border (Borger, 1970).

Borger (1974) has recently made observations which suggest that the secretory granules seen under the electron mi-

croscope are important in the formation of the digestive enzymes and absorption by the microvilli. He observed that the granules formed by the Golgi apparatus are morphologically identical to those granules found in the terminal web and near the microvilli. All these granules, Golgi granules and terminal web granules, stain identically when using silver-proteinate. The enzymes which may be released by these granules to the microvilli are the esterases, leucine amino-peptidase, disaccharidases, monoglyceride hydrolase, besides the acid phosphastases discussed above.

There are several disaccharidase enzymes found associated with the brush border (Gentner, et al., 1972). The disaccharidases include sucrase, maltase, trehalase, and palatinase. Maltase activity appears to be greater than the other enzymes. Notably, the enzyme for lactose digestion was found to be absent. Presumably, this lack was the cause for hogs fed on milk and milk by-products to resist ascarid infections. Gentner, et al (1972) suggests that the disaccharidases location on the microvilli may be advantageous in that the products of hydrolysis could be coupled to the carrier enzymes of the plasma membrane.

Of the important monosaccarides that have been studied, glucose and fructose are absorbed much more rapidly than galactose and 3-0-methly glucose (Sanhueza, 1968). Because

of its greater absorption rate and its more general availability to the worm, much attention has been paid to glucose. After some effort, glucose was shown to be absorbed against a high concentration gradient (Sanhueza, 1968; Castro, 1969). Furthermore, glucose uptake was partially inhibited by phlorizine, a known inhibitor of glucose uptake in mammalian intestinal absorption (Sanhueza, 1968). Schanbacher, (1974) has shown that 3-0-MG absorption is partially dependent on Na^+ within the incubation medium. Presumably, these observations indicate that glucose is actively taken up by the gut of Ascaris and possibly cotransported with Na^+.

Whenever a metabolite such as glucose is shown using tracer techniques to be taken up by a tissue, the question arises as to whether the observed activity is that of glucose or some product of its metabolism. The answer to this particular question is of crucial importance in the characterization of the process as active or passive. One technique that is used to clarify the mechanism is the substitution of 3-0-methylglucose, a glucose analogue, for glucose in the incubation media. This analogue is absorbed but not metabolized by most tissues (Schultz, 1964). Using this technique, it has been shown that 3-0-methyl glucose is taken up by the gut tissue of Ascaris, but the absorption requires glucose in the media (Beames, 1971). It appears that the glucose is required by the cell order to supply the energy for the active uptake of 3-0-methyl glucose (Schanbacher and Beames, in press).

Besides carbohydrates, the gut can transport lipid materials. Beames (1974) estimates that triglycerides move across the gut epithelium at only one percent of the rate of glucose absorption. Monoglycerides absorbed into the tissue are apparently converted mostly into free fatty acids which then move out of the cells into the pseudocoelom. The gut epithelial cells have demostrated a certain degree of specificity for cholesterol which they can accumulate preferentially over B-sitosterol (Beames, 1974).

As previously mentioned, the gut of Ascaris contains a strong acid phosphatase activity. Of the total activity found in the gut tissue, 80% is found associated with the brush border (Borgers, 1974). Phosphatases are a general class of enzymes which catalyze the hydrolytic cleavage of phosphate bonds. This hydrolytic cleavage results in the removal of phosphate from certain orgaic phosphates such as hexophosphates, glycerophosphates, and nucleotides (Harpur, 1973). Borgers (1973) tested the specificity of the acid phosphatase (AcPase) found in the intestine of Ascaris with the following compounds: G-6-P, AMP, ADP, IDP, TPR, and ATP. The results were similar using the different test compounds, suggesting that the Ascaris enzyme was a non-specific AcPase.

The non-specific nature of the AcPase activity and its ability to act as an adenosine triphosphatase may be implicated in the active cation transport mechanism. Such enzyme activity has been found in a variety of tissues and its presence in the brush border of the small intestine of

mammals is consistent with the presence of a mechanism for active Na^+ extrusion from the cells. It may be part of a K^+ - Na^+ transport mechanism responsible for the maintenance of the high intracellular K^+ concentration and low intracellular Na^+ concentration characteristic of other tissues (Schultz, 1967). Indeed, there is direct evidence that Ascaris can transport Na^+ ions. Using sac preparations of Ascaris intestine Harpur (1973) observed that there was a net movement of Na^+ from the mucosal side to the pseudocoelomic side. However, these observations were confounded when the reverse movement was observed using inverted gut preparations. Harpur was unable to give an explanation for these observations.

Desiring to study transmural flux more closely, researchers have attempted to adopt the Ussing methodology to the study of Ascaris absorption. Fisher (1962) used a Ussing chamber to measure the bidirectional flux of various substances across Ascaris gut tissue. Possibly because of the technical problems involved, no conclusion as to the results were presented. Another attempt was made by Harpur(1973). His results indicated that there was a small but variable positive potential on the mucosal side of the intestine. Since this is similar to that of the gall bladder (Barry, 1970) he concluded that the transport mechanism involved was probably the same.

Hemolymph

One of the traditional methods by which a homeostatic mechanism can be detected is by comparing the chemical consti-

uents of body fluids with the concentration of those constituents in the environment in which the animal lives. If there is a component that is found to be at a different concentration, then a regulatory mechanism may be suspected. However, if the animal lives in an environment which is near physiological suitability, there would be little or no indication nor need for such a regulatory mechanism. Hobson (1952) measured the composition of Ascaris hemolymph and the composition of the intestinal contents in which the nemotodes lives. Table I shows the results of his measurements. Subsequent measurements by others have verified the results (Harpur, 1974).

It appears that the Na^{+} and K^{+} concentrations of the hemolymph are variable, and as Hobson noted, these variations did not seem to be dependent upon the variation in the external media. Furthermore, the Ca^{++} and Mg^{++} concentrations were relatively constant and not affected by their concentrations in the external media (Hobson, 1952). It is interesting to note that the hemolymph from in vivo worms had an osmolarity that was hypotonic to the environment. However, when the worms were kept for seven hours in a physiological solution, the hemolymph became hypertonic (Harpur, 1965). It would appear that some type of regulatory mechanism was changed. It has been observed that Cl^{-} content of the hemolymph was closely related to the Cl^{-} content of the holding media when there is no glucose in the media (Hobson, 1952); however, when glucose is included, the altered chloride con-

TABLE I

ELECTROLYTE COMPOSITION OF
WORM AND PIG*

	Na	K	Ca	Mg	Cl	P
Intestinal Fluid of Pig (mM)	124	26.7	4.5	5.5	61	24.2
Body Fluid of *Ascaris suum* (mM)	129	24.6	5.9	4.9	53	17.0

*From A. D. Hobson (1952)

centration was prevented (Harpur, 1965). These findings suggest that an energy requiring mechanism may be operating.

Adult ascarids are anaerobic and produce a variety of volatile fatty acids as waste products of their carbohydrate metabolism. The acids that are known to be produced by Ascaris are: succinic, tiglic, 2-methyl valeric, n-valeric, 2-methylbutyric, propionic, and acetic (Harpur, 1969). These acids are found in relatively high concentrations in the hemolymph and are subsequently removed via the feces to the environment. Even though these acids are present, the pH of fresh hemolymph has been measured to be 6.713 (Harpur, 1974). This value is near the pH of water at 38°C, so that the hemolymph may be considered to have a neutral pH. These results suggest that the worm is able to effectively regulate and eliminate these acids via its intestine.

One of the important functions which the hemolymph has is probably to circulate nutrients from the intestine to the rest of the worm's tissues. Analysis has been conducted to determine the types of nutrients that are predominant in the hemolymph. Early studies state that the hemolymph contained less than 1% of reducing sugars (Fairbairn, 1957). The predominant sugar was found to be trehalose. More recently, Griechus (1966) examined the content of the hemolymph for reducing sugars in unstarved worms and found that it contained about 1%. It has been proposed that after glucose is absorbed by the epithelium it is rapidly converted to trehalose. This would minimize the work required to retain glucose in the

hemolymph. However, it was determined that muscle, integument, ovaries and uteri do contain trehalose, but the intestine contains very little trehalose (Fairbairn, 1957). Furthermore, the gut does not produce trehalose (Read, 1966). Glycogen was found to be absent from the haemolymph (Fukushima, 1966). Neutral glycoproteins have glucosamine and galactose amine as component sugar was detected in the hemolymph (Fukushima, 1966). The significance of this compounds in the heamolymph has not been determined.

Ascaris Feces

The formation of feces by the worm apparently is the result of two processes. The first process is the engulfing of liquid food material which simply passes along the whole length of the intestine and out the anus. The second process involves the absorption and excretion of material out of and into the lumen of the intestine by the intestinal cells. The ability of the intestinal cells to significantly alter the composition of the fecal contents has been assayed by Harpur (1965). He found that the worm was unable to produce a hypertonic or even an isotonic feces even though there were substantial amounts of excreted nitrogen and fatty acids. The worm produces large amounts of fatty acids which appear in the feces by a process of excretion and not simply by an ultrafiltration process. This conclusion was based on the observation that there was a negative correlation for the movement of propionic acid into the lumen and that there was an

absence of an increasing concentration gradient towards the anus (Harpur, 1969). This last observation has been taken to suggest that there was an enteropseudocoelomic circulation of fatty acids and nutrients. That is the anterior muscle tissue, which are the more active muscles, formed fermentation products which entered the gut lumen, were reabsorbed by the posterior intestine wall, converted to volatile fatty acids, and then excreted.

In Vitro Effects

Within a few days after being removed from the pig, Ascaris becomes motionless, flaccid, pale and generally morbid; then, the worms begin to die one by one. The survival time can be increased by frequent changing of the saline holding media, but after ten days or so the worms are useless for experimentation. It appears that the intestine plays an important role in maintaining the worm. Of the morphological changes that the worm undergoes in vitro, the gut epithelium shows the earliest changes (Borger, 1974; Griechus, 1966). After several hours in holding media, the worms intestine showed the following changes: there was a considerably drop in glycogen content, disappearance of the secretory granules from the terminal wall and Golgi regions, the complete absence of AcPase activity in the Golgi saccules, and the formation of cytoplasmic blebs at the microvillus border. Furthermore, it was observed that the transport of glucose across the strips of Ascaris intestine was much slower in worms that

were more than four hours old (Fisher, 1962). After seven hours, the osmolality of the hemolymph changes (Harpur, 1965 and 1974). In fresh worms, the osmolality is about 330 mOsm; after 7 hours, the osmolality increases to 350 mOsm. These changes are reflected in similar changes in the feces.

The observed "blistering" mentioned above, may be caused by the osmolarity effect of the keeping medium. Working with toad bladder, DiBona (1973) observed blistering of the serosal surface when the solution on the mucosal side was hyperosmotic to that of the serosal side. He reasoned that solute molecules diffuse into the intercellular junctional complexes from the mucosal solution and cause water to move osmotically from the cells and lateral intercellular spaces into the junctions. The increased volume within the junctions produced the blistering. This in turn alters the physiological role of the juntional complex. These junctional barriers are considered to be the major resistant element to the movement of water and solutes through the extracellular space or shunt. Deformation with blistering causes a lowering of resistance and any permselectivity that may exist. If the comparison between toad bladder blistering and that of Ascaris gut is valid there would be reason to expect a diminution in the ability of the gut epithelium of Ascaris to maintain its body fluid concentration, resulting ultimately in the death of the worm.

Coupled Transport in Other Parasites

It is generally believed that there are similarities of biological processes between various parasitic helminths (Saz, 1972). One of these processes is the coupled transport of Na^+ and glucose. In a recent review, a number of parasitic helminths were listed which have indeed been shown to display a Na^+-glucose coupling (Pappas, 1972). Those species were Hymenolepis diminuta, Taenia crassiceps, Taenia taeniaformis, and Calliobothriu verticillatum (Cestoda). In all these species, the influx of glucose showed first order dependence on Na^+ concentration in the medium. As has been stated before, the Na^+-non-electrolyte coupled absorption processes may very well be wholly dependent upon pre-existing Na^+ gradients formed by an active Na^+ - K^+ pump mechanism (Crane, 1969). The existence of such a pump mechanism has not been demonstrated in any parasitic helminth. The reason may be its rather low activity in anaerobic animals. The observation that other helminths show a Na^+ dependency suggests that Ascaris may very well show a similar dependency. However, coupled transport mechanisms are not easily demonstrated. In some cases, the coupled absorption may be obscured. In Staphloccoccus typhimurium, the cotransport of Na^+ and melibiose was obscure, apparently by the rapid re-extrusion of Na^+ (Oxender, 1972).

Biochemistry of Ascaris

Carbohydrate Metabolism

Analysis shows that the radionuclide labeled glucose enters into every major fraction of Ascaris. The activity is highest in glycogen and volatile fatty acides (Entner, 1959). Apparantly, half of the glucose is converted to glycogen. The other half is distributed into glycolytic intermediates and volatile fatty acids. The randomization of carbons in acetate suggested that the pentose-phosphate shunt has a small contribution in the metabolism of the worm. Langer (1971) was able to show the presence of the enzyme transaldolase, along with other enzymes of the Pentose Cycle, which makes it highly probable that there is an active pentose cycle in A. suum. Unlike mammalian tissue, the phosphoenol pyruvate (PEP) formed during glycolysis could not significantly be converted to pyruvate, due to the low activity of pyruvate kinase (Saz, 1969). In its place, the enzyme PEP carboxykinase converts PEP into oxaloacetic acid. This enzyme, therefore, functions in the fixation of CO_2, which would explain the earlier observation that CO_2 was required for glucose uptake (Sanhueza, 1968). Once formed, the OAA was found to be reduced to malate, which entered the mitochondria. After entering the mitochondria, one half of the molality of malate was reduced to pyruvate and the other half was reduced to succinate, which in turn gave rise to acetate and propionate. These last two compounds apparently acted as precursors for the

volatile fatty acids. The reduction of malate, through the intermediate fumarate, gives rise to the esterification of inorganic phosphate into ATP. The P_i/malate ratio was measured to be approximately 0.5 (Saz, 1971). Figure 1 summarizes these findings of the carbohydrate pathway in Ascaris as it is currently understood (Beames, 1971). It is apparent from this figure, that the mitochondria normally do not use oxygen. The ascaris mitochondria are anerobic. In comparison with the size of mammalian mitochondrial cristae, the cristae of Ascaris mitochondria are much reduced (Kessel, 1961). This probably reflects the lack of electron transfer system enzymes known to exist in the aerobic mitochondrion.

The lack of the need of oxygen in the catobolism of carbohydrates does not preclude a need for oxygen in other pathways. Apparently oxygen is required for the synthesis of hydroxyproline by Ascaris. This amino acid is a constituent of the worm's cuticle (Saz, 1972). The presence of an oxygen carrying heme found in the hemolymph further suggests that there is some physiological role for oxygen (Fairbairn, 1957).

Interestingly, the carbohydrate catabolism of Ascaris is not unique. There are a variety of parasitic helminths which are similar pathways. Table II lists some of these varieties. It would seem that besides their metabolic similarities, the parasites may have other similarities, especially in their absorptive processes.

Hormones

The active transport of materials across a membrane is

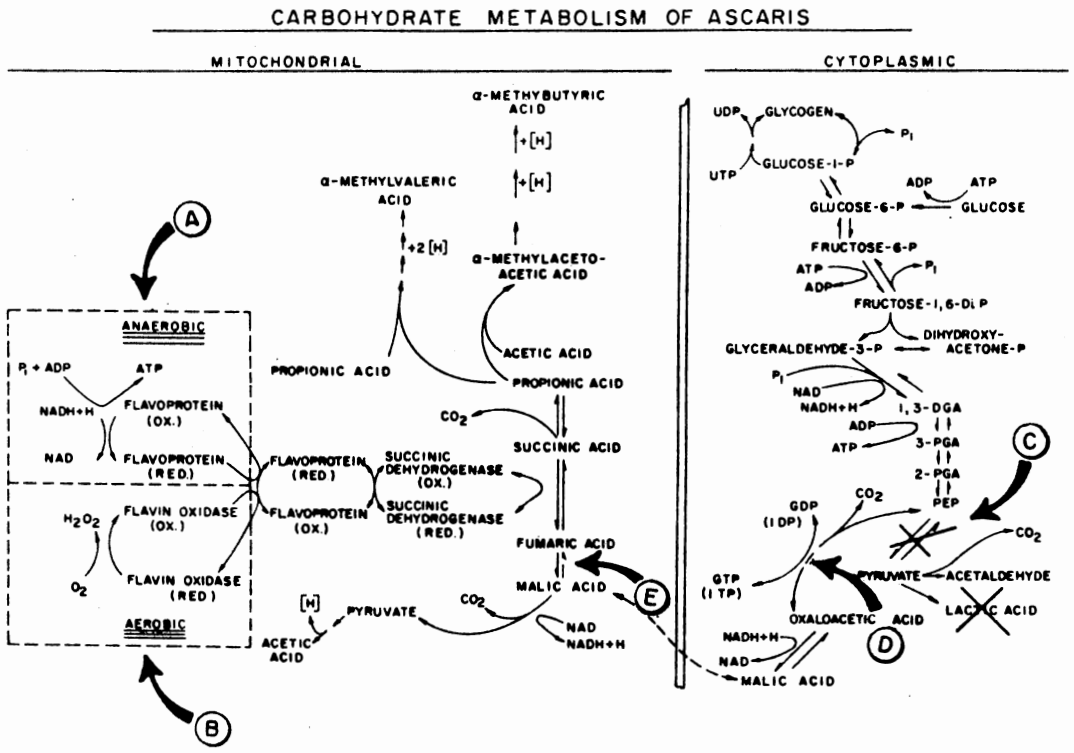

Figure 1. Carbohydrate Metabolism of Ascaris suum Taken from Beames (1971).

TABLE II

PARASITES WHICH MAY HAVE SIMILAR BIOCHEMISTRY AS *ASCARIS**

Nematodes	Trematodes
Heterakis gallinae	*Fascila hepatica*
Trichuris vulpis	
Trichinella spirals	
Syphacia muris	
Dictyocaulas virviparus	
Cestodes	**Acanthocephalsa**
Hymenoiepis diminuta	*Moniliformis dubis*
Moniezia expansa	
Edinococcus granulosus	
Tainia taeniaformis	

*From H. J. Saz, 1972

dependent upon the energy supplied by the metabolic machinery. In turn, the substrates from which this energy is derived is based on what is absorbed. For A. suum, the diet is rich in carbohydrates, and consequently it has been postulated that carbohydrates are the most important energy source for the parasitic worms (Read, 1961). In mammals, carbohydrate metabolism is mediated through hormones. Such a regulation may also exist in parasites, but of course, the hormones could be significantly different. The metabolic hormones of invertebrates are different from mammalian metabolic hormones in that the mammalian hormones seldom have any effect on invertebrate metabolism (Mansour, 1967). One metabolic hormone which may display evolutional non-specificity is serotonin. When this hormone and glucose are supplied to Ascaris suum, the worm becomes more active (Harpur, 1964). There is a 75% increase in the worm's contraction rate. When either glucose or serotonin alone is supplied to the worm, there is no significant change in the contraction rate. The underlying mechanism by which serotonin acts is suggested in a study on the fluke. When glucose was made available to the fluke, serotonin produced an increase in glucose uptake (Mansour, 1967). Serotonin alone caused an increased utilization of glycogen. These effects are similar to those produced by serotonin in mammals (Mansour, 1967). Whether the action of serotonin in A. suum is the same as in the fluke or by some other mechanism has not been established. However, the worm may very well be exposed to serotonin since it dwells in the

gastrointestinal tract of a mammal and most of the serotonin of mammals is found in their gastrointestinal tracts (Harpur, 1973).

Anthelminthics

The World Health Organization has estimated that 25% of the world's population is infected with Ascaris lumbricoides (WHO, 1967). Few deaths occur from the infection, but the resulting morbidity is certainly of significant magnitude to constitute a major health problem. Currently, the principle medical effort is directed toward improvement in sanitation and mass chemotherapy (Desowitz, 1971). Since most infection by Ascaris is accompanied by infection with other parasites, a treatment using drug with a toxicity limited to Ascaris would be of little use. To effectively control helminths parasitization, a broad spectrum anthelmintic of low host toxicity is needed. Before such a drug can be produced, there must be a better understanding of the Ascaris biochemistry. There is much less knowledge concerning the biochemistry of helminths than of the biochemistry of vertebrates or bacteria (Saz, 1966). The study of the mode of action of anthelmintics can lead to a better understanding of the differences between the biochemical characteristics of the parasite and those of its host.

In general, the mode of action of most anthelmintics is to interfere with the energy yielding pathways or the neurochemical mechanism of the parasite (Saz, 1972). Piperazine

causes a flaccid paralysis of Ascaris due to a blocking action on the neuro-muscular junction. Apparently, the formation of acetylcholine is inhibited. The resulting paralysis allows for the worm's expulsion by the intestinal peristalsis of its host. The cyanide compounds, such as dithiazanine and pyrvinium act on the energy yielding pathway. It has been demonstrated that dathiazanine irreversibly blocks glycolysis in Trichuris vulpis (Desowitz, 1971). The uncouplers of oxidative phosphorylation of aerobic mitocheondria oligomycin and rotenone also inhibit the anaerobic phosphorylation that occurs in Ascaris mitochondria. Presumably this means that there is an electron transport associated with phosphorylation in Ascaris. The anticestoidal agents, chlorosalicylamide, desaspidin, SKF compound 90,625, and BW compound 61-435 inhibit the mitochondrial phosphorolation system of Ascaris in low concentrations. However, all these compounds are chemotherapeutically inaffective against the intact worm (Saz, 1971).

One compound that seems very promising as a broad spectrum anthelmintic is mebendazole. This compound inhibits the uptake of glucose, fructose, 3-0-methlyglucose, glycine, methiozine, proline and palmitic acid. The underlying biochemical change which mebendazole induces is not clear. Glucose phosphorylation is unaffected, even though glucose uptake is reduced. Phosphorylase a and a+b activity was unaffected even though glycogen consumption is increased. Mebendazol's mode of action may be that it interferes with the diffusion of

glucose across the intestinal membrane (Van den Bossche, 1972). Electron micrographs of the intestinal cells of mebendazole treated nematodes show marked deterioration of the cytoplasmic microtubules (Borger, 1975). Concurrently, there is a depletion of secretory granules at the brush border and an accumulation of secretory granules at the Golgi apparatus. Presumably, the microtubules are responsible for the movement of secretory granules from the Golgi apparatus where they are manufactured to the brush border where they are released. The secretory granules may contain material which facilitates the movement of glucose into the cell.

CHAPTER III

METHODS

Tissue

Adult female Ascaris suum are collected at the packing house and transported to the laboratory in saline (Harpur, 1963) that is maintained between 32 and 39 C. The intestine is removed from the worm, placed in a dish of warm modified Harpur's saline (Na_2SO_4, 45 mM; KCl, 25 mM, NaCl, 16 mM; NaH_2PO_4, 12.1 mM; $CaCl_2$, 6 mM; $MgSO_4$, 5 mM; Na_2HPO_4, 4.9 mM) and sliced open. The pH of modified saline is 6.8.

Chamber and Experimental Procedure

A modified Ussing chamber (Figure 2A) is used to mount the tissue for the in vitro experiments. A length of the resulting ribbon of the intestine is placed on a small strip of nylon mesh and positioned between two rectangular sheets of Lucite plastic with matching openings. This procedure is illustrated in Figure 3. The area of the opening is the choice of the investigator, and in these experiments they are either 1.6 cm^2 or 0.125 cm^2. The cartridge of Lucite-tissue-Lucite is inserted into the chamber, and the two resulting compartments are rendered water-tight by rubber "O" rings which press

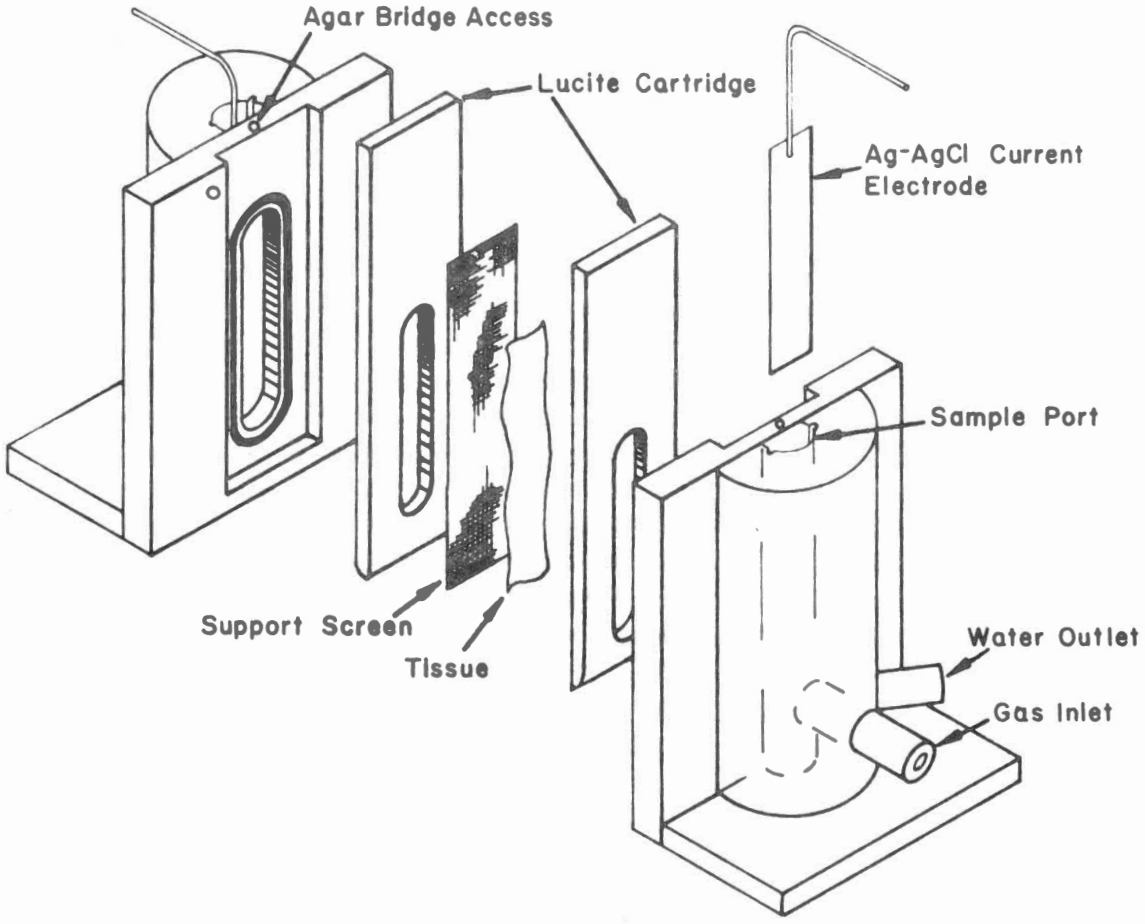

Figure 2. Tissue Incubation Chamber. A. Assembly diagram shows the position of the tissue membrane be- the two compartments of the chamber. Ports for the potential sensing agar bridges are located near the tissue while the current passing electrodes are held parallel to the tissue by grooves in the inner compartments. Warm water (37°C) enters one water jacket, and is passed to the water jacket on the opposite side through a small port, and then is circulated back to the water heater.

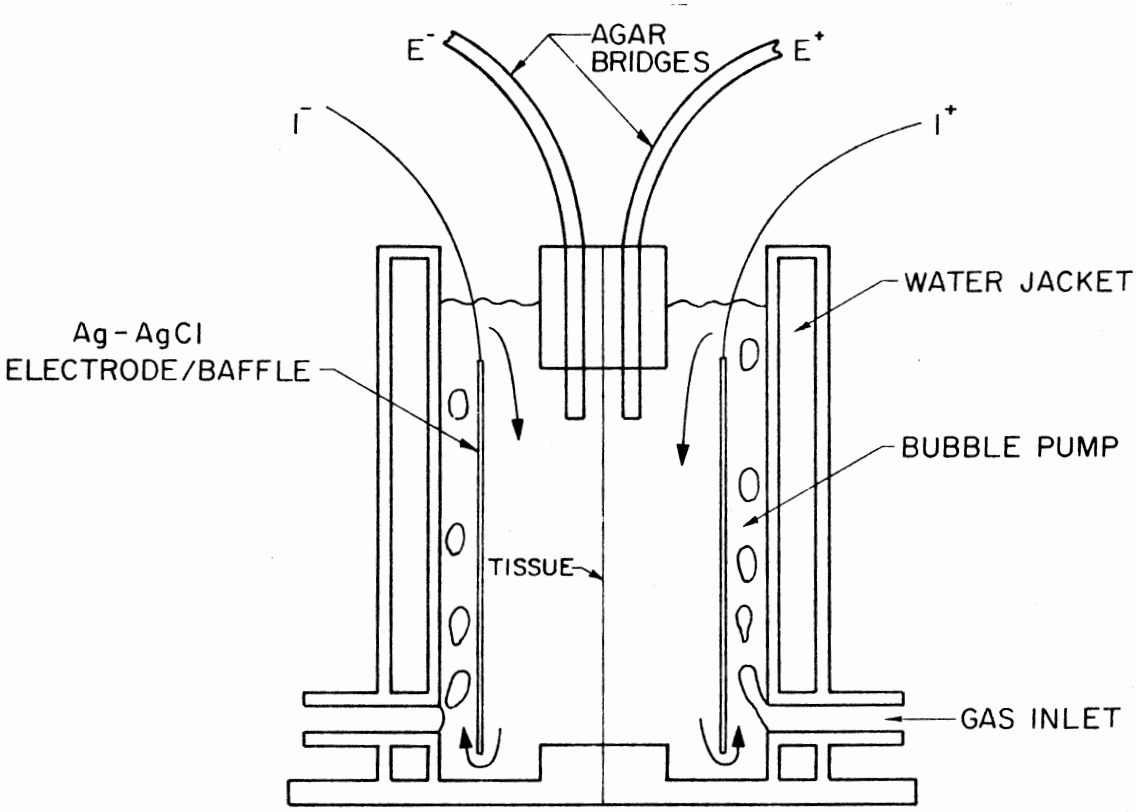

Figure 2. (con't.) Tissue Incubation Chamber. B. Shows the geometrical relation of the tissue and the electrodes. The incubation solution is aerated with N_2-CO_2 (95:5). This aeration causes the solution to be continuously circulated over the tissue.

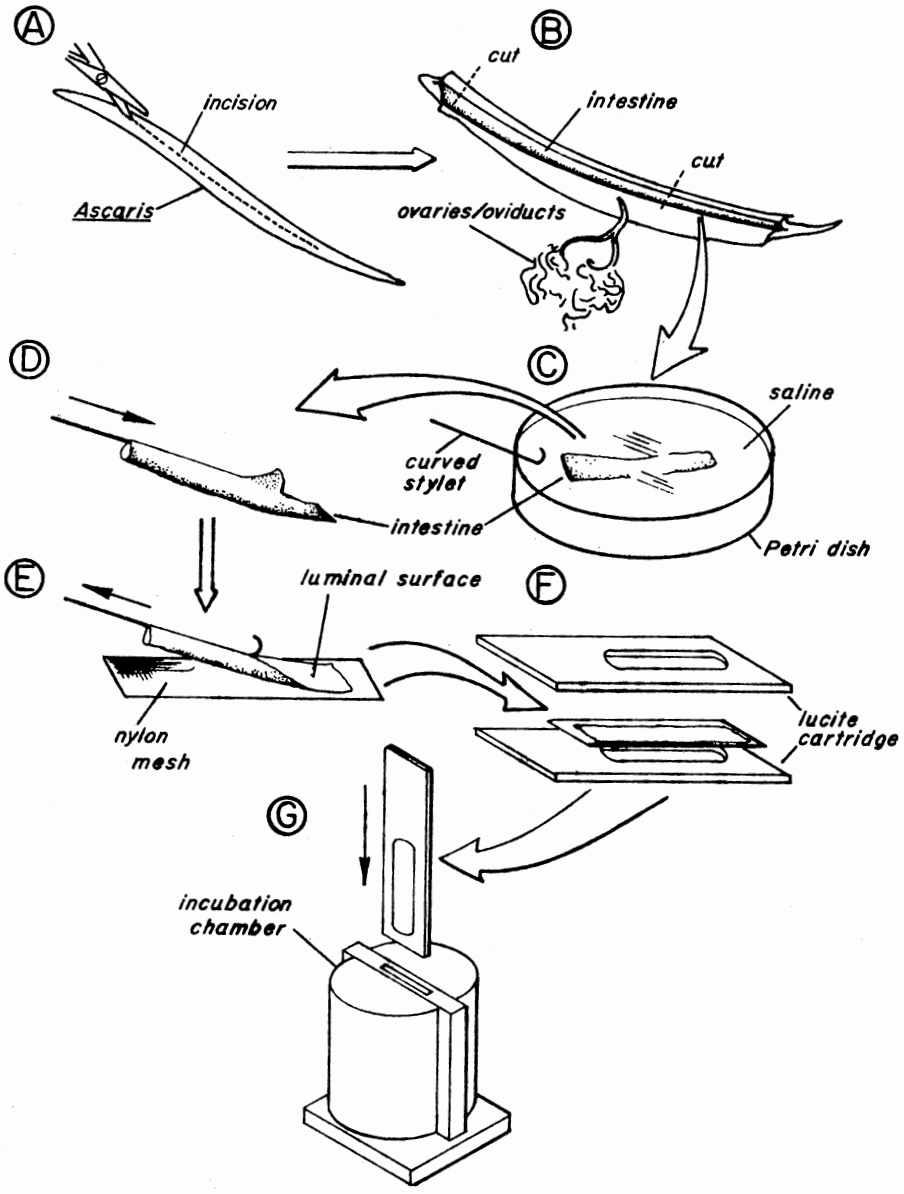

Figure 3. Steps in Preparing the Tissue. The intestine is excised from the worm by longitudinal incision and placed in a physiological salt solution. The tissue is sliced open using a curved stylet to form a ribbon. A nylon mesh is used to transfer the tissue ribbon from the saline to the Lucite cartridge and to support the tissue on the pseudocoelomic side. The tissue-Lucide assembly is sealed with petrolatum and inserted into the chamber.

against the Lucite sheets. A maximum volume of 3.5 ml of modified Harpur saline is place in each compartment. Circulation of the fluid in each compartment is accomplished by a gas-lift system as shown in Figure 2B. The gas is humidified 95% N_2 - 5% CO_2. Unless it is stated otherwise in the results, the temperature of the incubation fluid is 37 C. The baffles of the gas-lift system are rectangular sheets of silver, and they also serve as current clamping electrodes.

Electrical Measurements

Tips of agar- 3.0 M KCl bridges (1.2 mm I.D.) are placed within 1.5 mm of each surface of the longitudinal center of the membrane strip as shown in Figure 2 (A and B). The potential difference (PD) between the bridges is measured with a pair of matched calomel electrodes, which lead into the non-inverting inputs of a pair of high input impedence operational amplifiers. The system is essentially that described by Rothe, et al. (1969) and is an automatic voltage clamp device with compensation for solution resistance. A schematic of the circuit is presented in Appendix A. The summed outputs from the amplifiers are connected to one side of the membrane by a silver-silver chloride current electrode. The opposite of the membrane is grounded through another silver-silver chloride electrode to a current-to-voltage amplifier. The potentials were recorded on a Heath SR-255B strip chart appropriately calibrated with a built-in dummy membrane or with a Coleman model 110 pH meter that is used in the millivolt

mode. Corresponding currents (short circuit current) are recorded in the same manner.

Ion Transport

Procedures similar to those that are described by Schultz and Zelusky (1964) are used to determine the flux of Na^+, K^+, and Cl^- across the ascarid gut. Identical chambers and adjoining sections of intestine from the same animal are used to measure the luminal to pseudocoelomic and pseudocoelomic to luminal flux of ^{22}Na, ^{42}K and ^{36}Cl (New England Nuclear Corp., Boston, Mass.). Bidirectional flux of Na^+ is determined also with ^{22}Na and ^{24}Na using single ribbons of intestine.

Sugar Transport

Bidirectional flux of glucose across the intestine is investigated by using ^{14}C and ^{3}H labeled glucose. Concentrations of glucose on one side of the intestine are varied while the other is held constant and the flux rate in each direction is determined at various time intervals.

Extracellular Space

Extracellular space values are determined by following the procedures described by Esposito Csaky (1974) and employing ^{14}C-polyethylene glycol or ^{14}C-inulin (New England Nuclear).

Diffusion Potentials

The tissue's relative permeability to the major physiological ions Na^+, K^+, and Cl^- were determined by the formation of diffusion potentials across the membranes using the procedures that are described by Frezzell and Schultz (1972). In one experimental condition (shown in Figure 4) the concentration of NaCl in the solution perfusing the pseudocoelomic side was changed by substituting isosmotically mannitol for NaCl. The resulting potential that was created due to the Na^+ and Cl^- gradients from the luminal side to the pseudocoelomic side reflected the relative permeability of the tissue to these ions. Asymetrical electrode potentials were corrected prior to the experimental condition by using an electrode voltage offset compensation circuit incorporated in the electrometer circuit. The spontaneous transmembrane potential was reduced to near zero by conducting the measurements at 20-24°C.

In another experimental condition, the relative permeability of the tissue to Na^+ and K^+ was determined by substituting NaCl on the pseudocoelomic side with KCl.

In a few experiments, the solution perfusing the luminal side was altered by substitutions while the pseudocoelomic side was held constant. This was done to determine whether the observed potentials were influenced by compartmentalization in the tissue.

The relative permeabilities of the basement membrane for some ions were also determined. The basement membrane was

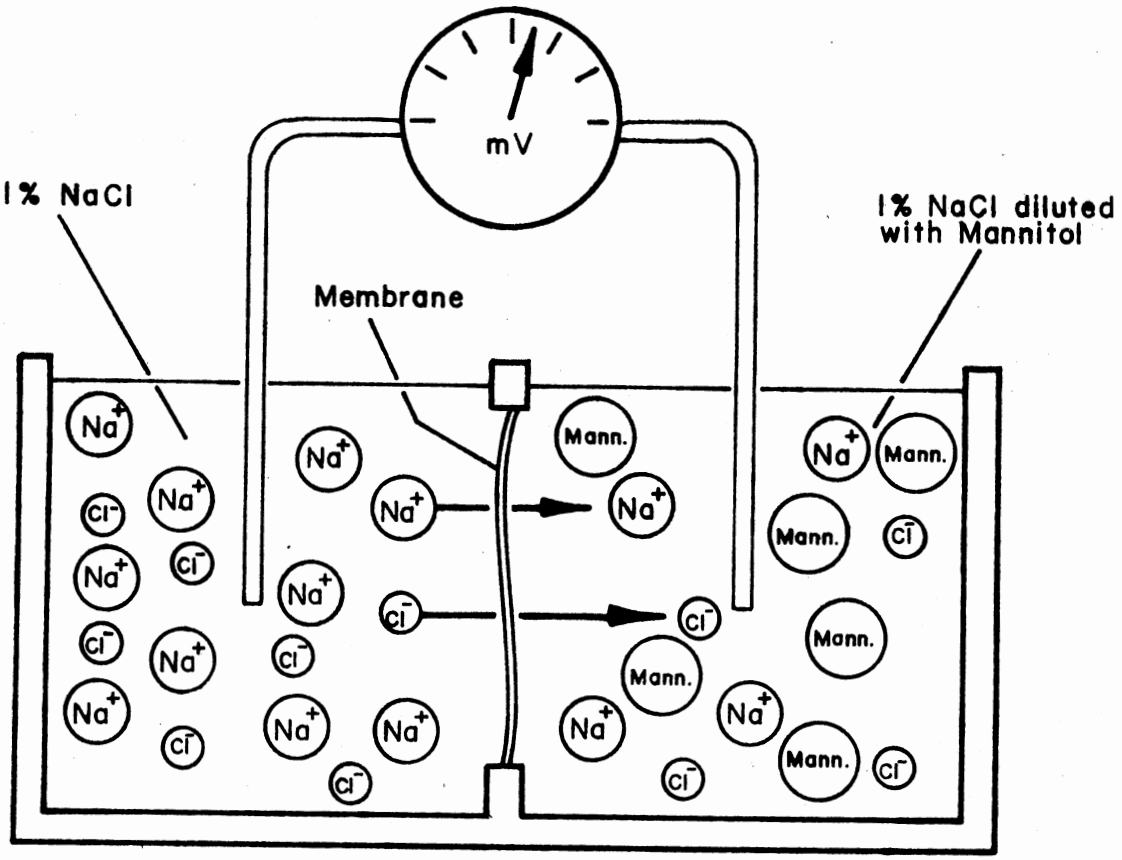

Figure 4. Diagram of Experimental Condition to Measure Diffusion Potentials. The concentration of NaCl in the solution perfusing the pseudocoelomic side is changed by substituting isosmotically mannitol for NaCl. Asymetrical electrode potentials were first determined by substituting Parafilm for the tissue membrane. Measurements were conducted at room temperature and under rapid perfusate circulation.

separated from the epithelial cells by sonication and mounted in the chamber as was the whole tissue. The same series of dilutions on the pseudocoelomic side was carried out as described above for the whole tissue.

Unidirectional Flux for Na^+

The above procedures were used to characterize the permselective characteristics of the whole tissue and presumably a functional part of it which may act as a barrier to the flow of materials through the shunt pathway, that is, the basement membrane. The other barrier of the shunt pathway is the apical junctional complex. Since there is no means by which a continuous sheet of tissue that is large enough to mount in a chamber can easily be prepared away from the basement membrane, a series of unidirectional flux measurements were carried out following the procedure described by Frezzell (1972). This procedure is based on a mathematical model which equates total unidirectional flux of ions into the tissue as the sum of two components, the influx across the mucosal cell membrane and the influx across the apical junctional complex. In this model, it is assumed that the influx across the apical junction is by diffusion and is influenced by the transmembrane potential. Consequently, any changes seen in the total influx under different voltage clamp conditions may be ascribed to the change associated with the diffusional compartment.

The tissue was mounted in the chamber as described above. The tissue was preincubated for 10 min with a balanced salt

solution containing 1 μCi ^{14}C-polyethylene glycol (PEG). Just prior to the voltage clamping, the DC resistance of the membrane was measured. The voltage clamping was enforced by an automatic voltage clamping circuit preset to the desired voltage. The essential experimental condition for voltage clamping is shown in Figure 5. ^{22}Na (10 μCi) was added to the luminal perfusate which was rapidly mixed by the bubble life pump. Since the perfusate volume was only 3.5 ml and the turnover volume was approximately 2-3 time that per sec, the mixing of the ^{22}Na throughout the perfusate would take about 3 seconds at the most. Following the addition of ^{22}Na, the tissue is incubated for 60 seconds, and the luminal perfusate was rapidly removed by a vacuum line into a sample bottle, and the tissue is removed and washed by briefly immersing it three times in ice cold mannitol (5.5%) solutions. Transfer of the tissue from the chambers to the first mannitol solution takes less than 3 seconds. The tissue is placed in 0.5 digesting fluid (Soluene, Packard Instrument Co.) for 3-4 hours and then into scintillation fluid and counted the next day with aliquots of the luminal and pseudocoelomic perfusates. Internal standards were used to determine quenching.

Coupled Transport

A series of measurements were made to determine the relationship between glucose and Na^+ absorption. After being mounted in the chamber, the gut epithelia were preincubated with iodoacetic acid (1 mM) for 10 min or until the trans-

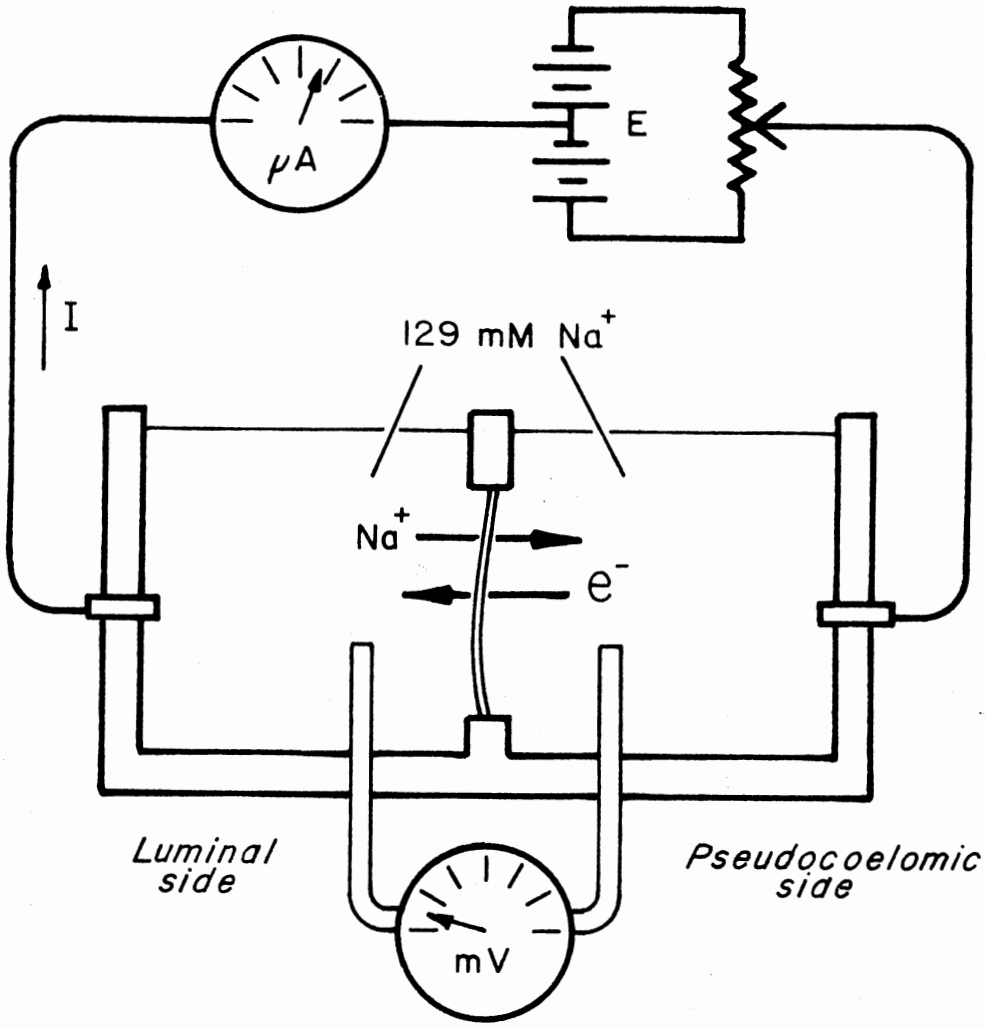

Figure 5. Diagram of the Experimental Condition to Measure the Undirectional Flux of Na^+. The tissue was preincubated on the luminal side for 10 min with a balanced salt solution containing 1 μCi PEG. Then 10 μCi ^{22}Na was added to the luminal perfusate. After one minute, the tissue was removed and counted for ^{14}C and ^{22}Na. The measurments were conducted at 20°C to reduce any spontaneous potentials. Electron flow is denoted by e^-.

membrane potential dropped to zero. The tissue was automatically clamped at zero mV, and the ^{14}C-glucose and ^{22}Na were added to the luminal perfusate simultaneously. After 10 min, the luminal solution was rapidly removed and the tissue rinsed three times in cold isotonic mannitol solution (5.5%). The tissue and luminal solutions were prepared for liquid scintillation counting using standard procedures. All three nuclides were counted on a Packard Tricarb Scintillation Counter using multichannel counting techniques that corecting for crossover and quenching.

Radioisotope Counting

Standard techniques for single label and double label counting of radioactivity are employed (Chase, 1965). Radioisotope counting is done with a Packard Tri-Carb Scintillation Counter and Scinti Verse (Fisher Scientific Co.) scintillation fluid. The tissue is digested for at least three hours in Soluene (Packard Instrument Co.) before the scintillation fluid is added. Quenching is corrected by internal standardization. In some instances, radioiosotope counting is done with a Packard Auot-Gamma Spectrometer. Specific activity is given in the caption for the appropriate Table or Figure.

CHAPTER IV

RESULTS

Electrical Characteristics

A potential difference (PD) develops spontaneously across the intestine and rapidly increases after the tissue is positioned in the chamber. A typical graph of such PD is shown in Figure 6. Considerable variation (10 to 30 mV) is observed between individual preparations but in our determinations the polarity is consistently pseudocoelomic negative with respect to the luminal side. Within 5-10 minutes, the PD reaches its greatest amplitude and then slowly falls. The PD is increased and prolonged by the addition of glucose to the solution bathing the pseudocoelomic side of the intestine as is shown in Figure 6. These results are the means of measurements with adjoining lengths of intestine that are placed in essentially identical chambers and incubated at the same time.

The short circuit current (SCC) that is required to zero the transmural potential follows a time course of change that is very similar to that of the PD. The results of the change in SCC of four separate intestinal preparations are shown in Figure 7. An occasional preparation maintains a SCC of 30 μA or more for 60-120 minutes.

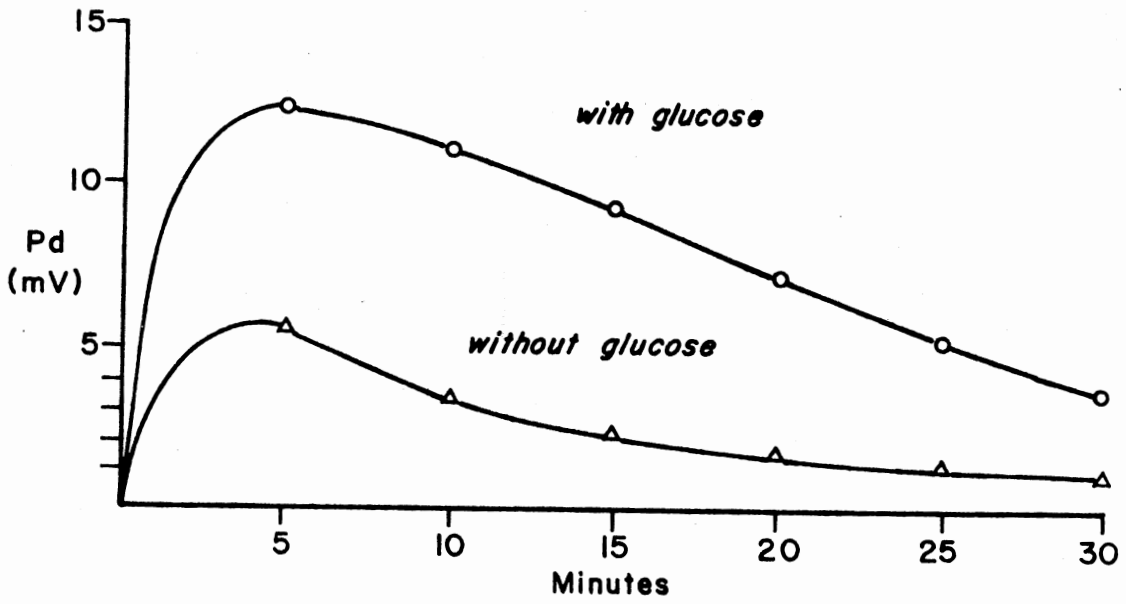

Figure 6. Spontaneous Difference Across the Intestine of Ascaris With and Without Glucose (10mM) added to the Pseudocoelomic Perfusate. Each point was determined from six measurements using paired tissue preparations from the same animal. One half of the tissue was treated with glucose. Paired t-test gave t=5.47** (dif 5). The polarity of the potential represented by the graph is a positive luminal surface relative to the pseudocoelomic surface.

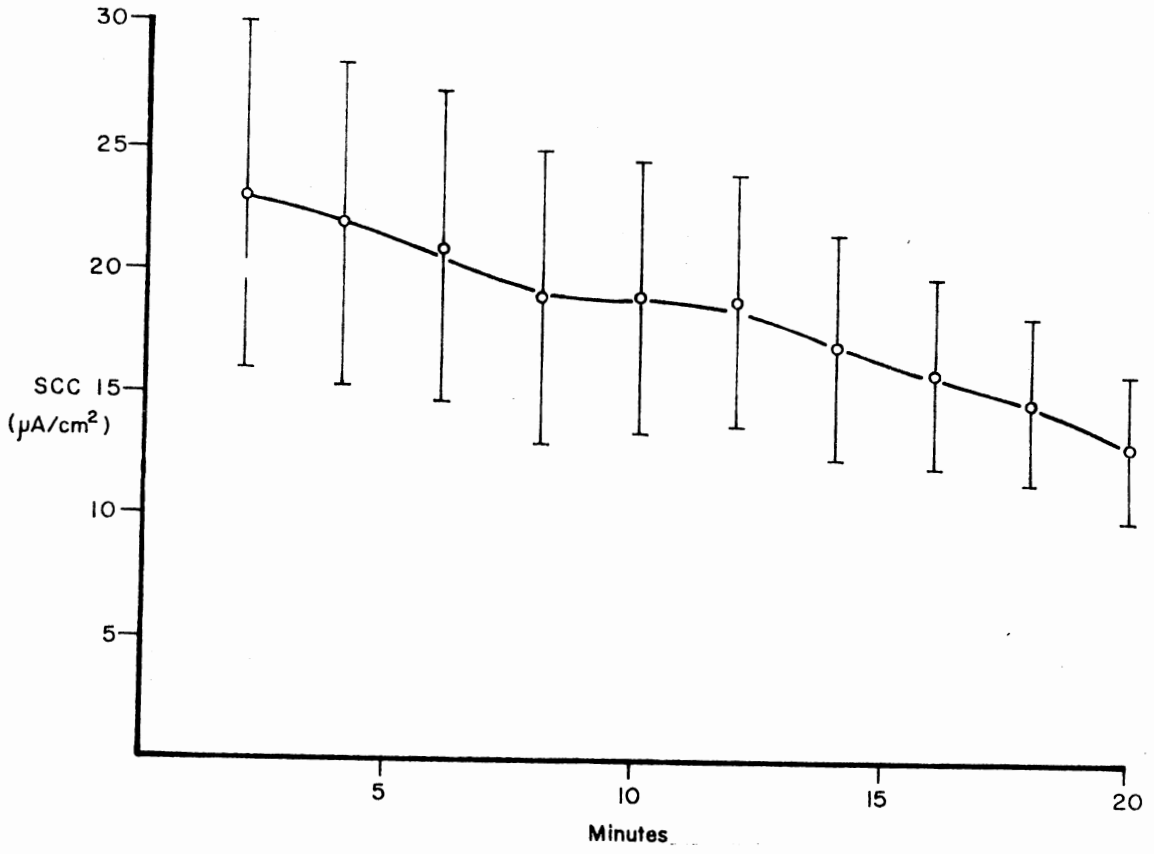

Figure 7. The Short Circuit Current (SCC) Required to Zero the Transmural Potential Across the Intestine of Ascaris. The vertical bars represent $\pm\overline{S.D.}$ The direction of positive current is towards the luminal surface.

Extracellular Space

For any calculation of the flux of material across an epithelial layer it is necessary to have a reasonable estimate of the extracellular space. Inverted and non-inverted sac preparations of the intestine were used to determine the extracellular volumes on the luminal and pseudocoelomic surfaces respectively. In addition, ribbons of the intestine were used to determine the total extracellular space. Results of these measurements are presented in Table III. The extracellular space on the pseudocoelomic side is approximately twice that of the luminal side. The luminal surface appears to reach equilibrium sooner than the pseudocoelomic side. The extracellular space value for ribbons of intestine is essentially the same as the sum of the value for the inverted and non-inverted sac preparations.

Ion Flux

Harpur (1965) reports that Na^+, K^+, and Cl^- are the predominate ions of the hemolymph of Ascaris. With this in mind, it seemed possible that differences in the rate for the forward and back flux of one or more of these ions could account for the observed transmural potential. Results of the determination of the forward and back flux of Na^+, K^+, Cl^- and the SCC are shown in Table IV. Obviously, the current-flux equivalent (CFE) calculated from the SCC is not accounted for by the net flux of any of the measured monovalent ions. The possibility that H^+ flux is responsible in part or whole for the trans-

TABLE III

EXTRACELLULAR SPACE MEASUREMENTS

	Sac Preparation			Ribbons
Time (min)	Inverted	Non-inverted	Inverted plus Non-inverted	
30	0.073±0.002	0.130±0.030	0.203	
60	0.100±0.014	0.141±0.019	0.241	
90	0.100±0.012	0.226±0.022	0.326	0.326±0.032

Extracellular space expressed as fraction of total tissue water. Preparations were incubated in saline containing 0.9 μCi of ^{14}C-inulin (2.5 mCi/mmole) per ml. Each value for the sac preparations represents the mean of 3 determinations ± S.E. The value for ribbons represents the mean of 6 determinations ± S.E. Temp 37°C. Gas phase 95% N_2-5% CO_2.

TABLE IV

FLUX MEASUREMENTS OF Na^+, K^+ AND Cl^- DETERMINED FROM THE BIDIRECTIONAL MOVEMENT OF THEIR RADIOACTIVE ISOTOPES

Ion	Influx μeq/H SD(n) L-P (1)	Outflux μeq/H SD(n) P-L (1)	Net μeq/H (L-P)-(P-L)	SCC μA SD(n)	SCC μeq/H (2)
Na^+	0.77±0.64(5)	0.75±0.52(5)	0.02 L-P	28.5±14(5)	1.00
K^+	1.10±0.70(5)	0.63±0.09(5)	0.47 L-P	21.8±9.7(5)	0.81
Cl^-	0.03±0.27(6)	0.06±0.04(6)	0.03 P-L	11.6±4.9(5)	0.43

(1) L-P: direction of flux from luminal side to the Pseudocoelomic side.

P-L: direction of flux from Pseudocoelomic side to the luminal side.

(2) μeq/Hr is the calculated current-flux equivalent for each of the measured short circuit currents (SCC). These SCC's represent a net positive ion flux across the intestine from P-L.

mural potential was tested by determining the change in pH of non-buffed saline bathing inverted and non-inverted sac preparations. A total of 4 preparations incubated for 60 minutes gave a mean pH change of 0.69 $\pm$ 0.18 (SD) for the inverted and 0.81 $\pm$ 0.27 (SD) for the non-inverted sacs. The initial pH was 6.34 and 10 mM glucose was present in the saline at the start of the incubation. The difference between means is not significant ($P < 0.5$, t-test) and suggests that H^+ production alone is not responsible for the transmural potential. The rather large outflux of Na^+, K^+ and Cl^- (Table IV) suggest that the tissue is permeable to these ions and that a diffusional component of their bidirectional flux could be substantial.

Effects of Sugars Upon the Short Circuit Current

A rapid change in the SCC follows the addition of glucose to the luminal solution as shown in Figure 8. When the glucose solution is replaced with basal salt solution (Figure 8, wash) the SCC returns towards its "base line" level. Increasing concentrations of glucose on the luminal side of the intestine results in a corresponding change in the SCC until the concentration reaches approximately 20 mM (Figure 9). Similar changes in the SCC are produced with fructose, 3-0-methyl glucose, mannose, and galactose; however, the changes seen with mannose and galactose are much smaller than that seen with the other sugars. The relation between concentration of these

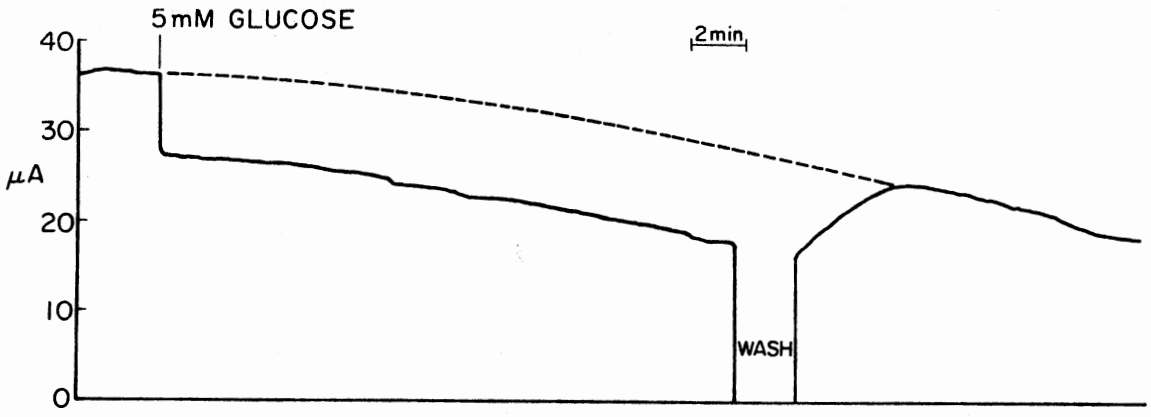

Figure 8. Effect of Glucose on the SCC when Added to the Luminal Perfusate. The graph represents a typical response shown by the intestine when glucose is added to the luminal perfusate to give a final concentration of 5 mM. When the luminal solution is replaced with salt solution containing no glucose (wash), the SCC returns to the pre-glucose level (corrected for decay). Glucose was added to the perfusate on the pseudocoelomic side to give a 10 mM solution.

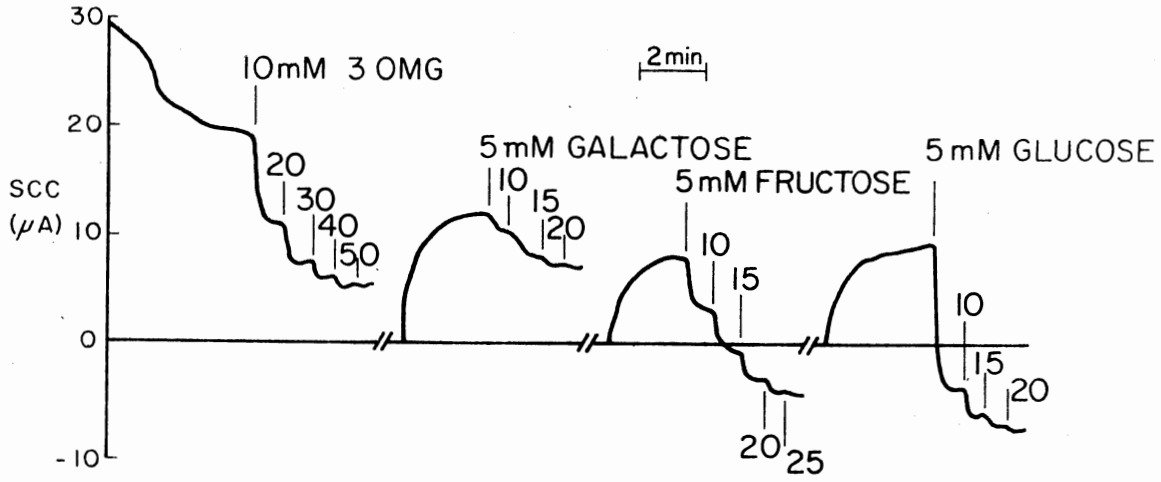

Figure 9. Effects of Various Sugars on the SCC when added to the Luminal Solution. The graph represents a typical response in the SCC of a single tissue preparation. The perfusate was exchanged before a new sugar was added.

sugars in the luminal solution and the change in the SCC is presented in Figure 10. The change in the SCC is a saturable function of the sugar concentration. This relation resembles typical Michaelis-Menten kinetics.

Effects of Temperature Change on the Potential

Paired tissue preparations from the same animal were used to assess the effects of low temperature on the spontaneous transmural potential. The results are presented in Figure 11. The temperature of the experimental tissues was lowered from 37°C to 15°C over a 6 minute period. Because of the procedure used to cool the perfusate, the temperature decreased very rapidly at first then tapered off after the first minute. Concurrent with the temperature change, the spontaneous potential decreased to near zero. Below 20°C, the potential established a new base line. When glucose was added to the luminal perfusate, there was a rapid decrease in the potential of the control intestines, but there was no change in the experimental tissues. The effects of the low temperature was reversible. When the temperature was again increased to 37°C, the spontaneous potential would increase to near the original values. Furthermore, the tissue would still respond to the addition of glucose to the luminal solution as reflected by a rapid decrease in the potential.

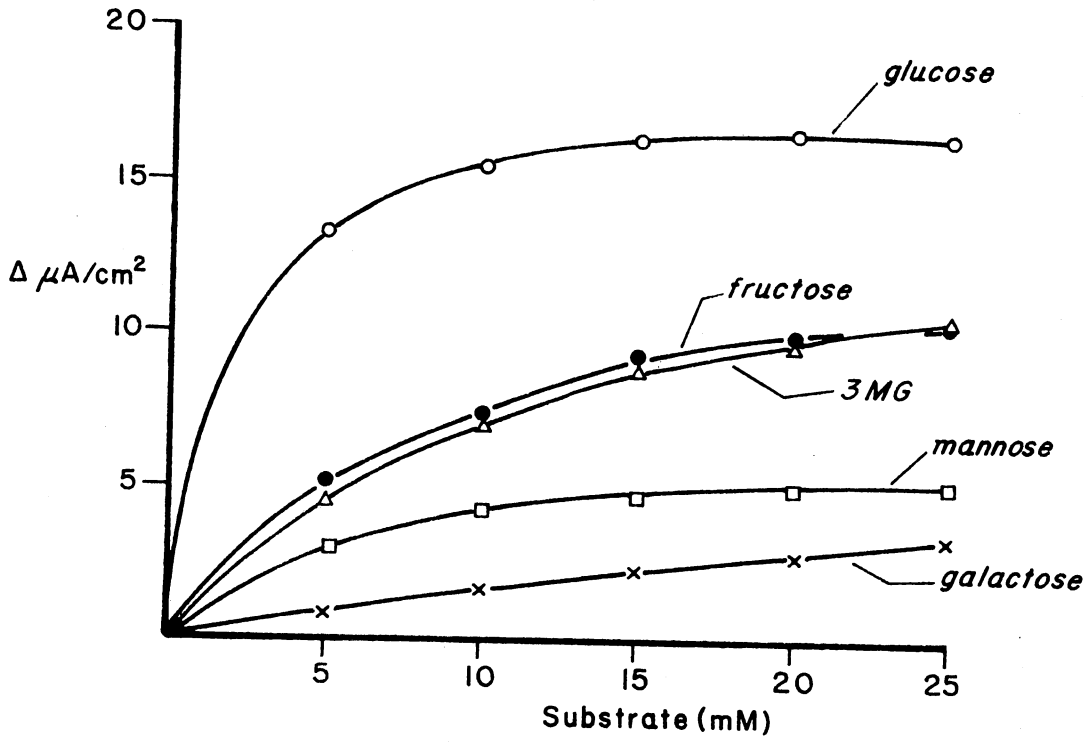

Figure 10. Effects of Various Sugars at Different Concentrations have on the SCC. Each point represents the mean from 15 measurements for glucose; 4 measurements for fructose, 3-0-methyglucose (3MG), and mannose; and 3 measurements for galactose.

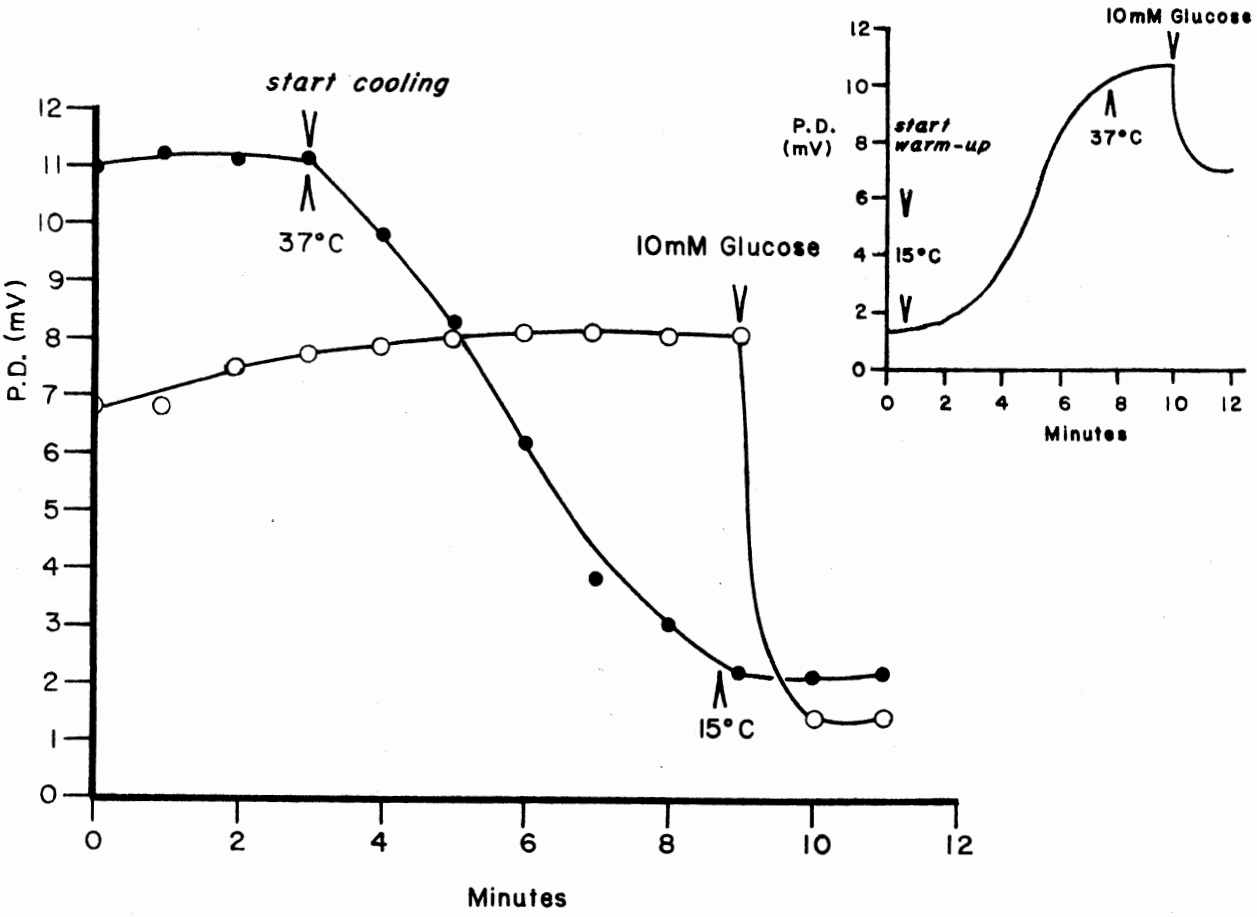

Figure 11. Effects of Temperature Change on the Transmural Potential of Ascaris Intestine. The points on the curves represent the means from 5 measurements using paired tissue preparations from the same animal. The open circle represents the control and the closed circles represent the experimental. Baselines were established for 3 minutes at 37°C. Afterwards, the experimental tissues were cooled from 37°C to 15°C. When the low temperature was reached, glucose (10 mM) was added to the luminal solution of both the control and experimental tissues. The inset graph shows the effect of re-warming a single gut preparation to 37°C after it had been cooled down to 15°C. Glucose was added to the luminal solution after the temperature reached 37°C.

Diffusion Potential

Two membrane preparations (ribbons of intestine and basement membrane) were used to measure the potentials generated by the diffusion of ions due to concentration gradients across the membrane. The measurements were conducted at room temperature to reduce the spontaneous potential across the tissue to near zero. Both preparations showed distinct difference in their permselective characteristic.

Ribbon of Intestine

When the NaCl in the luminal perfusate was substituted with mannitol using the ribbon of tissue, the pseudocoelomic side became positive relative to the luminal side. This potential increased as the gradient across the membrane was increased as seen in Figure 12. Little or no change was seen in the potential when the Na^+ concentration was reduced below 10 mM. When the NaCl in the pseudocoelomic perfusate was substituted for mannitol, the luminal side became positive relative to the pseudocoelomic side. However, the time necessary for the potential to reach a steady state was twice as long (i.e., 2-3 min for changes on the luminal side, and 5-6 min for changes on the pseudocoelomic side).

There was considerable variance in the diffusion potentials between samples of worms collected on various days and between fresh worms and worms older than 8 hours. As the tests were conducted over several days, it was observed that the diffusion potential decreased in magnitude to zero for

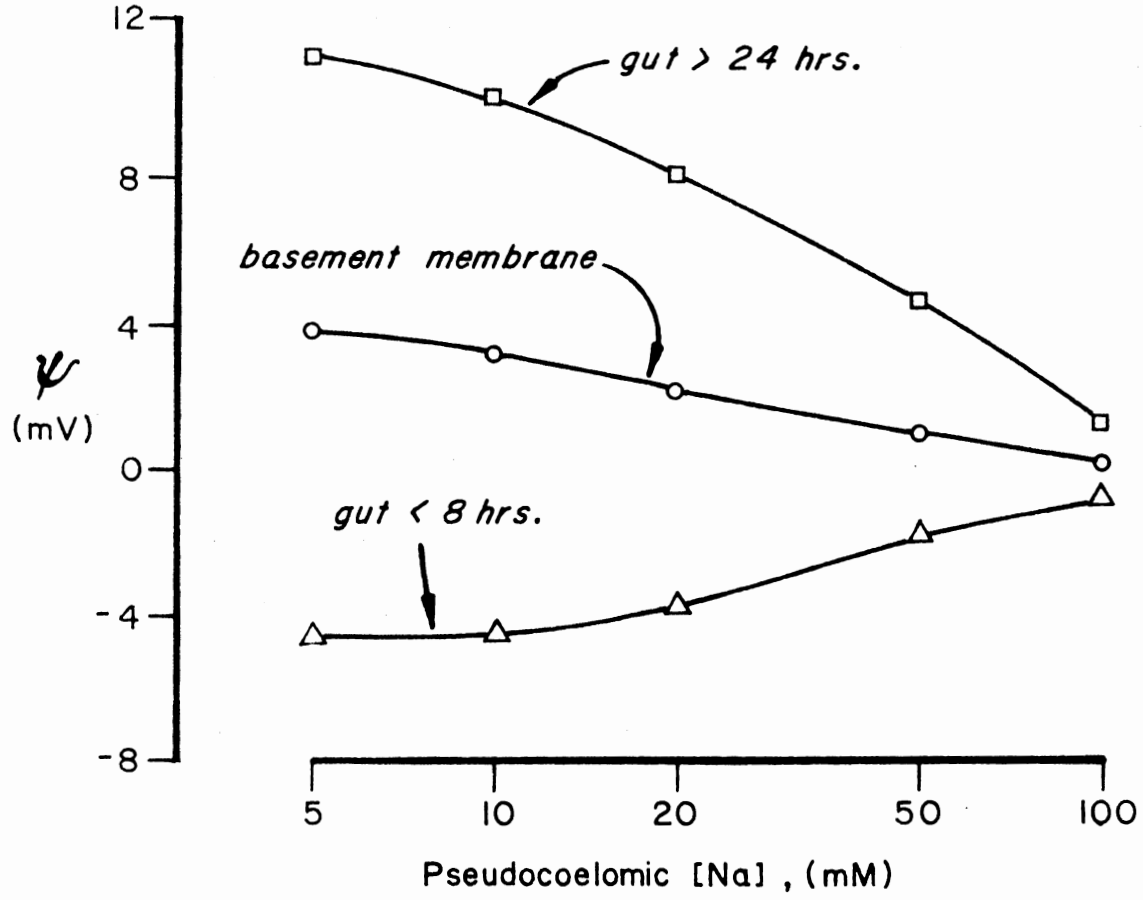

Figure 12. Diffusion Potentials Across the Whole Gut and Basement Membrane. The NaCl in the luminal perusate was substituted isosmotically with mannitol. There was no diffusion potential when KCl was used in place of mannitol. Each point represents the mean from 9 measurements made from whole guts less than 8 hours old, 4 measurements for whole guts more than 8 hours old, and 5 measurements for the basement membrane.

some day old worms, and in older worms, the potential was reversed. That is, NaCl was substituted with mannitol in the luminal perfusate the pseudocoelomic side became positive relative to the luminal side; however, as the worms became older, the positive potential diminshed to the point at which the luminal side became relative to the pseudocoelomic surface-a reversal of the potential.

There was no diffusion potential generated when substituting NaCl with KCl.

Basement Membrane

The diffusion potential across the basement membrane was opposite in polarity and nearly equal in magnitude than the whole tissue preparation when using the mannitol series dilution. The potential was positive on the luminal side and was approximately 1 mV less in its absolute value at any given dilution than the absolute value for the whole tissue. The potential tended to increase as the NaCl became more diluted. There was no diffusion potential when KCl was used to substitute for NaCl.

Permeability Coefficients

From the observed diffusion potentials and the known concentrations of the various solutes, the relative permeability coefficients for K^+ and Cl^- to Na^+ were calculated using the Constant Field Equation. Table V summarizes the results of these calculations. The significant difference seen between

TABLE V

RELATIVE PREMEABILITY COEFFICIENTS*
FOR Na, Cl AND K

	Ions		
Barrier	P_{Na}	P_{Cl}	P_{K}
Gut	1.0	0.8	1.0
Basement Membrane	1.0	1.2	1.0

*Permeability coefficients were determined by the numeric solution of the Goldman-Hodgkin-Katz constant field equation.

the whole gut preparation and that of the basement membrane is that the basement membranes showed a greater permeability to Cl^- than did the whole gut preparation. There was no difference between the permeabilities of the membrane for Na^+ and K^+.

Unidirectional Flux

The results of the unidirectional flux measurements for Na^+ are presented in Figure 13. Following the procedures described by Frezzell and Schultz (1972), the diffusion component ($_oJ_d$) was calculated from the slope of the curve. It was found to be 2.5 moles/cm^2/H. This is equivalent to the partial ionic conductance of Na^+ (i.e., G_{Na^+}). The solute ionic conductance of the tissue, the reciprocal of the specific tissue resistance, was measured to be 3.2 mmhos. The apical junction's permeability coefficient for Na^+ was calculated to be 0.019 by dividing $_oJ_d$ by [Na^+] in the luminal perfusate.

Adequacy of this procedure to measure the true unidirectional flux of Na^+ was judged from the linear uptake of Na^+ by the tissue with time and the rate of appearance of $^{22}Na^+$ into the pseudocoelomic perfusate.

Coupled Transport

Several attempts were made to correlate the rate of glucose absorption with the rate of Na^+ absorption and the change in SCC. In all these, the rate of glucose absorption was faster than the absorption of Na^+. It became apparent

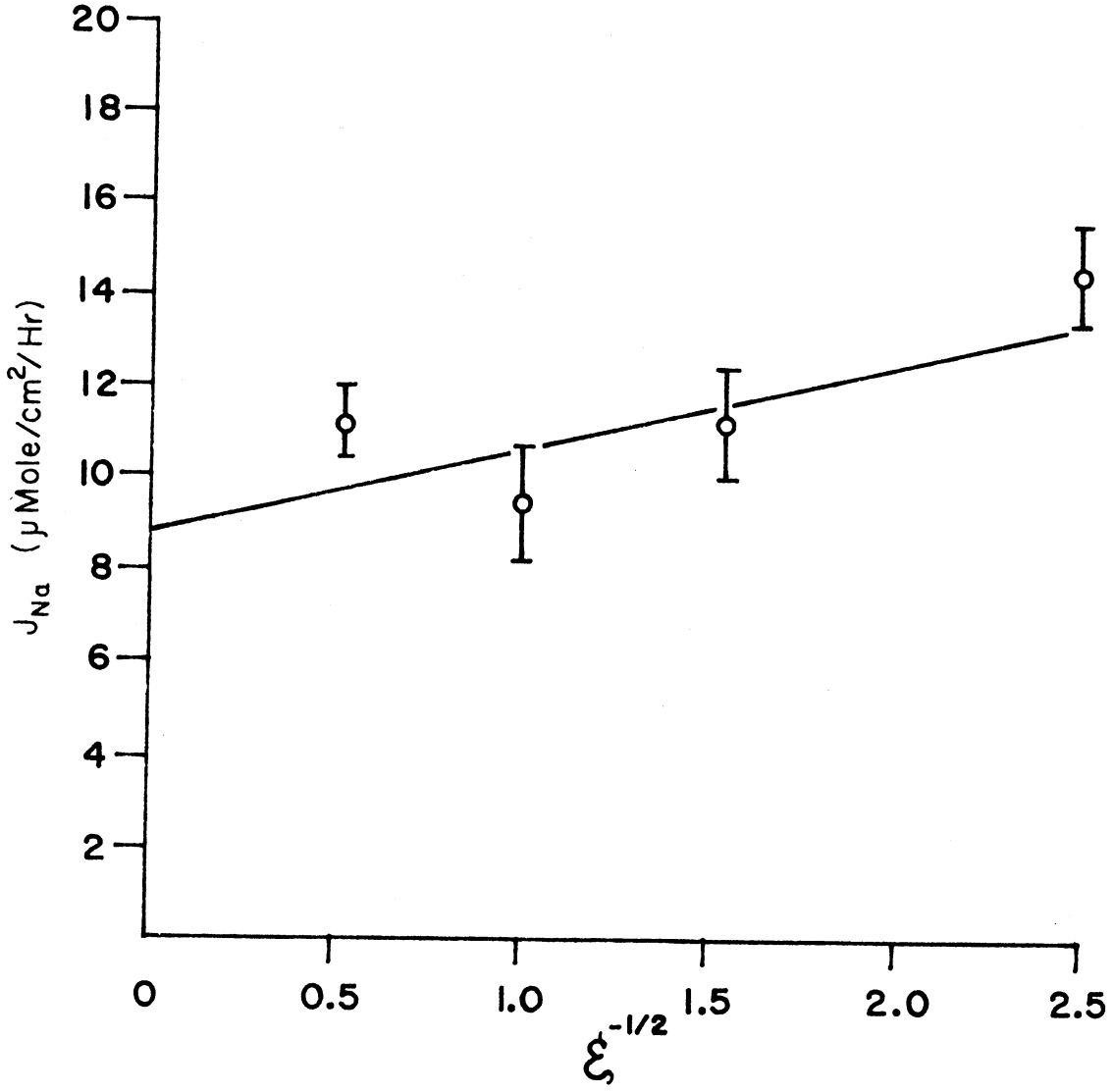

Figure 13. Unidirectional Flux of Na^+ into the Gut Epithelium from Luminal Solution when Clamped at Different Voltages. The graph represents the linear regression as expressed by : $J_{Na} = J_M^{Na} + {}_oJ_d \ \xi^{-\frac{1}{2}}$, where the total Na^+ influx (J_{Na}) is the sum of the influx into the cell (J_M^{Na}) and the ionic conductance ($_oJ_d$) which is influenced by the electrical activity (ξ) across the membrane. Each point represents the mean from 4 measurements.

after some work on the "shunt" pathway was completed that re-extrusion of Na^+ after absorption would confound the measurement of Na^+ uptake. It also was observed that the PD of tissues treated with the metabolic inhibitors iodacetic acid or sodium fluoride decreased to near zero but continued to demonstrate a rapid change with the addition of glucose to the luminal perfusate. It was presumed that the observed changes reflected the existence of a Na^+ gradient while the pump mechanism which maintained the gradient was effectively inhibited. Accordingly, these observations suggested that the re-extrusion of Na^+ from the tissue could be reduced by the use of IAA and that glucose and Na^+ uptake could still be measured over a 10 min period. Table VI shows the results of measurements on the uptake of Na^+ and glucose from the luminal perfusate by intestinal strips preincubated with IAA. Paired t-test between the equivalent current calculated from the observed Na^+ uptake and the measured change in the SCC showed no significant difference. Paired t-test between glucose uptake and Na^+ uptake was not significant. From this analysis it is concluded that the rate of glucose uptake by ribbon of intestine is equal to the rate of Na^+ uptake which is reflected in the observed change in SCC.

TABLE VI

UNIDIRECTIONAL UPTAKE OF $^{22}Na^{+}$
^{14}C-GLUCOSE INTO THE
EPITHELIAL CELLS[1]

Glucose Uptake	Na^{+} Uptake		Δ SCC
μmoles/min	μeq/min	Eq I[2]	(μA)
0.0005 ±0.0009(9)	0.006 ±0.0028(9)	9.8	7.1

Paired t-test (H_o = 0; d.f., 16) indicated no significant difference between Na^{+}, glucose and Δ SCC groups.

[1]Preincubated with 1.0 mM iodacetic acid for 10 min.

[2]Eq I is the equivalent current in μA calculated from the measured uptake of Na^{+}.

CHAPTER V

DISCUSSION

The main conclusion drawn from the data is that the gut epithelium of Ascaris actively transports some ion or ions. The transport of these ions generates a potential, and the net rate of transport can be quantitated by the SCC. The relationship between potential and ion transport is influenced by the anatomical characteristics of the membrane. There is a coupling between sugar absorption and ion absorption which reflects the existance of an ion pump mechanism. The sum of these conclusions characterize this membrane as an absorptive surface that is similar to many other membranes; however, this membrane is unique in the polarity of its potential, its anatomical features, and its anerobic metabolism.

The transmural potential of Ascaris intestine appears to be the result of diffusion potentials of ions within the extracellular spaces (shunt pathway). The primary barriers of this pathway are the apical junctional complexs and the basement membrane. Sodium is the major ion conducted in the shunt pathway. In addition, Na^+ is coupled to the absorption of glucose into the epithelial cells of the intestine. This coupled absorption suggests that a preexisting Na^+ gradient furnishes the necessary energy. This intracellular gradient

is establihsed probably by a Na^+ - K^+ pump typical of other tissues. Such a pump mechanism could generate diffusion potentials across the intestine by creating an ion gradient within the shunt pathway.

The existence of a transmembrane potential could only be demonstrated after a suitable chamber was designed. A Ussing chamber was modified to permit the handling of the membrane. The membrane is less than 100μ thick and is fragile. Once mounted in the chamber, the membrane would generate a potential in which the pseudocoelomic side was negative relative to the luminal side. The membrane was shown to be sensitive to lowering of the temperature. When the temperature dropped below 30 C the potential would decrease. At 20 C there was no potential. This evidence suggests that the PD is dependent on metabolic energy which is necessary for the active transport of ions. Further evidence for an energy dependency is the response shown by the tissue to the addition of glucose to the pseudocoelomic perfusate. With this addition, the tissue was able to maintain the potential at a greater amplitude and for a longer period. This observation suggests that glucose is utilized by the membrane as an energy substrate to run the ion pump mechanism. Furthermore, we have observed that the PD and SCC would rapidly decrease in the presence of either NaF or IAA in the incubation media (Beames, et al, 1976). Since these inhibitors are known to inhibit enzymes of the Embden-Meyerhof pathway, the data indicates that ion transport is dependent on the anaerobic metabolism of the cells of the tissue.

The data from the bidirectional flux measurements of Na^+, K^+ and Cl^- along with the observation on pH changes do not indicate any particular ion responsible for the observed SCC, however, the data does imply that there exists a large diffusional component in the movement of these ions across the membrane as represented by the relatively large outflux measurements when compared to the influx measurements. This large diffusional component reflects the existence of an extracellular channel which would act as a shunt for the passive movement of ions. Measurements of the extracellular space indeed indicated that the space on the pseudocoelomic side is larger than the space on the luminal side. The luminal surface would appear at first to have a larger extracellular space than the pseudocoelomic side due to its numerous mirovilli. However, the extracellular space measured for the pseudocoelomic side is larger than the luminal space. This is probably due to the free movement of inulin into the lateral intercellular spaces which are in contact with the pseudocoelomic surface through the basement membrane. These intercellular spaces could act as a shunt pathway for the diffusion of ions across the membrane and, consequently, account for the large backflux of the observed ions.

The data does not directly indicate how the potential across the gut epithelium is generated. However, within the limits of the existing data, a conceptual model of the membrane is used to explain the underlying mechanism generating the observed potential. Such a model is based primarily on

the anatomical features of the gut epithelium. The outstanding features of this membrane are its tall columnar cells, large apical junctional complex, and thick basement membrane. The role of the two structures the apical junctions and basement membrane as barriers was determined by diffusion potentials. The whole tissue preparation acted as a cation permeable membrane, while the basement membrane acted as a anion permeable membrane. Since the whole tissue preparation included the basement membrane, and apical junctions in series, the observed cation permeability must contain the anionic permeability component of the basement membrane and a much greater cation permeability component of the apical junction. The theoretical values for the permeability coefficients of membranes in series is discussed by others (Schultz and Frizzel, 1976).

Further insight into the permselective characteristic of the apical junctional complex was obtained by measuring the unidirectional flux of Na^+ into the tissue from the luminal (mucosal) side. The partial ion conductance of Na^+ (G_{Na^+}) was measured as the rate of Na^+ diffusing into the tissue or, in other words, the partial conductance of Na^+ ($_oJ_d$). This value was found to equal 80% of the total ion conductance of the tissue. Thus, Na^+ must be the predominant ion conducted into the shunt pathway. This observation reaffirms that the apical junction is permselective for cations. Presumably, the permeability of the apical junction to a cation is the same in either direction; therefore, a cation would move from the shunt path-

way to the luminal side faster than an anion.

The permselective characteristics of the two anatomical barriers of the shunt pathway are consistent with an ideal model for the segregation of ions found within the shunt. Cations would move away from anions at the apical junctions while anions would move away from cations at the basement membrane. The result of this charge segregation would be an orientation of polarity such that the luminal surface would be positive and the pseudocoelomic side would be negative. A diagramtical representation of the electrical model is presented in Figure 14. A membrane which possesses a shunt pathway system has been characterized as a membrane with a relatively low specific resistance or a "leaky" membrane. In fact, the gut epithelium of Ascaris has a high specific resistance characteristic of "tight" membranes such as the toad bladder (Ussing, 1974). This "tightness" of the gut is apparently due to the basement membrane. The resistance of the basement membrane detached away from the epithelial cells is some 10-20% of that of the whole tissue. The actual resistance of the basement membrane when attached to the epithelium is probably greater due to the adhesion of the cells over most of its surface. The sum of these observations suggest that the whole membrane system acts as a "leaky" membrane attached to a "tight" membrane: the sum of these resistances is that of a "tight" membrane.

One of the most consistent responses which the tissue gave has been a change in the PD with the addition of glucose

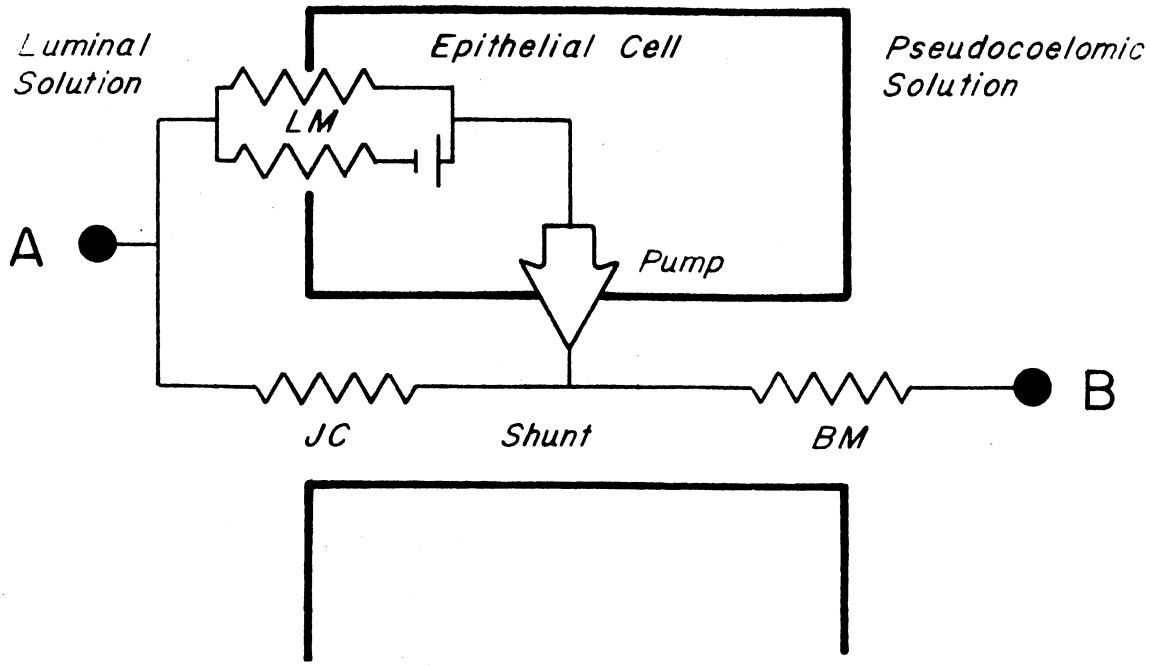

Figure 14. Proposed Electrical Circuit Model. The circuit depicts the movement of a cation from the luminal solution across the cell membrane (LM) along its electrical gradient. Subsequently, the cation is actively pumped into the "shunt" pathway. Anions would follow passively. Within the "shunt", the ions would distribute themselves according to the permselective characteristics of the junctional complex (JC) and the basement membrane (BM). The movement of these ions through these barriers would generate potentials that are detected at points A and B.

to the luminal perfusate. This change is always a rapid decrease in the negativity of the potential on the pseudocoelomic side. After a few minutes, the potential slowly becomes more negative again. The rapid change was interpreted as the cotransport of Na^+ with glucose into the cell from the luminal solution. This absorption of the ion initially results in the movement of positive charges towards the pseudocoelomic side. The extrusion of the ion into the lateral extracellular spaces or "shunt" results in the observed "rebound" of the PD. After the addition of a metabolic poison to the incubation media, the tissue continued to respond to the addition of glucose with a rapid decrease in the potential; however, there was no "rebound". Presumably, the active extrusion of Na^+ from the cell forms a Na^+ gradient across the mucosal membrane which drives the Na^+ - glucose cotransport mechanism. This gradient is apparently able to exist for some time after the tissue is poisoned and the PD drops to zero. After poisoning, the "rebound" no longer is observed because the Na^+ is not moved into the shunt pathway to form the diffusion potentials across the apical junctions and basement membrane.

There are obvious limitations to the interpretations given above. The cotransport of Na^+ and glucose is only prima facie evidence for the existance of an active transport mechanism for Na^+. Harpur (1973) observed that Ouabain had no effect on Na^+ transport across Ascaris gut. Schanbacher (1974) was unable to measure any inhibition in the absorption of glucose with guts preincubated with ouabain. If there is

a Na^+ pump in Ascaris gut epithelium its mechanism must be substantially different from those that have been described in other tissues. Alternately, Harpur (1973) suggests that the pumping sites may be situated within the lateral cell membranes, and ouabain is unable to reach these sites through the barriers of the shunt pathway.

The existence of a positive luminal potential could represent the active transport of some ion not investigate. Or the PD could be due to one of the ions measured but the experimental procedure was not sensitive enough to detect any difference in the bidirectional flux measurements. The decay of the potential may represent a general dysfunction of the tissue which would obviate any possible ion flux measurements. Tissue deterioration would have prevented a "steady state" from being reached. Furthermore, the response observed with the addition of glucose to the luminal perfusate could be due to an osmotic effect to which the membrane is sensitive. All these possibilibities together or independently could exist and would be difficult to confirm or deny with the present set of techniques. Not withstanding these possibilities, the model which has been described is consistent with the data and what is knwon about other tissues.

In agreement with our observation, Harpur (1973) measured a potential across the intestine of Ascaris. His results indicated a small positive potential on the luminal side of the intestine. Since his results were similar to those observed across the gall bladder (Barry, 1970), Harpur concluded

that the transport mechanisms involved were probably the same. Our results indicate the same polarity as reported by Harpur; however, the magnitude of the PD is much greater. If the comparison between Ascaris gut and the gall bladder is valid, there would have to be ion pumps along the lateral cell membranes which could establish an ion gradient within the shunt pathway, resulting in a diffusion potential across the apical junctional complex. Such a pump mechanism has not been demonstrated.

The intestine of Ascaris may be able to transport several ions. Hobson (1952) observed that Na^+ and K^+ concentration in the hemolymph were variable while Ca^{++} and Mg^{++} concentrations were relatively constant. Furthermore, it was observed that changes in Cl^- concentration was prevented when glucose was included in the incubation media (Harpur, 1965). Besides these ions, organic ions may be actively transported. A variety of volatile fatty acids are produced by Ascaris. These include succinate, tiglate, 2-methyl valerate, n-valerate, 2-methylbutyrate, propionate and acetate (Harpur, 1969). Even though these acids are produced in large amounts by the worm, the pH of the hemolymph is only slightly acidic. Harpur (1974) suggests that the worm is able to regulate these acids by its intestine. The sum of these observations suggest that the intestine is involved in the absorption of inorganic ions and the secretion of organic ion. These two functions may be interrelated. Our inability to correlate the SCC to any one of the ions investigated may be related to com-

plexity of the absorption and secretion processes. However, it must be kept in mind that this is a parasitic nematode with relatively limited metabolic capabilities. It has a fermentative metabolism and uses most of it absorbed nutrients in the production of eggs (Fairbairn, 1967). The metabolic economy of the proposed model would allow the diffusion of both small organic ions and inorganic ions along the electrical gradient established across the intestine by a single active process. A model presented in Figure 15 shows the relationship between coupled transport and the active transport of Na^+.

Addition of glucose to the incubation media does allow the tissue to maintain the PD for a longer period. This observation is related to the fact that Ascaris is an obligatory carbohydrate fermenter (Read, 1961; Beames, 1970). If there is a drastic reduction in carbohydrate substrate, there is a rapid depletion in glycogen stores in the intestine of the worm (Borger, 1974; Greichus, 1966, Schanbacher, 1973). Presumably, the addition of glucose to the bathing media helps to reduce the depletion rate of endogenous carbohydrates which supply the energy necessary for active ion transport.

Besides electrolytes, non-electrolytes can move across the intestine of Ascaris. Glucose has been shown to be moved across the intestine against a concentration gradient (Sanhuzea, 1968; Castro, 1969). Beames (1971) has shown an analogue of glucose, 3-0-methyl glucose, is transported into the gut cells when glucose is supplied in the incubation

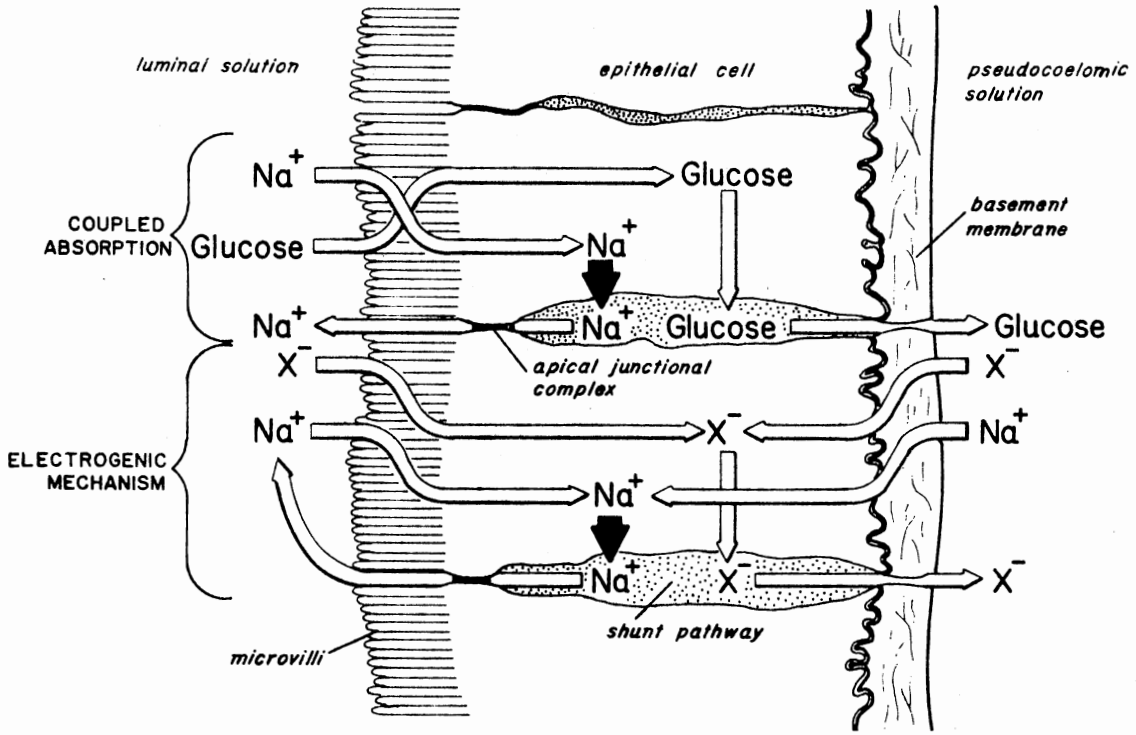

Figure 15. Proposed Model Related to the Anatomical Features of the Gut Epithelium of Ascaris. The pump mechanism (shown as a solid arrow) moves Na^+ out of the epithelial cells into the shunt pathway. Anions (probably Cl^-) follow after Na^+. The permselective characteristics promote the segregation of charges from one another such that there would be a positive luminal potential. The coupled transport mechanism is dependent on the electro-chemical gradient established by the same active pump as the electrogenic mechanism.

media. Three-0-methyl glucose is not metabolized by the tissue. In addition, 3-0-methyl glucose absorption is partially dependent on the concentration of Na^+ in the incubation media (Schanbacher, 1973). These observations give support to the observed correlation between Na^+ absorption and glucose absorption made in this report.

It is generally believed that there are similarities of biological processes between various parasitic helminths (Saz, 1972). In a recent review, a number of parasitic helminths were listed which have been shown to display a Na^+ - glucose coupling (Pappas and Read, 1975). In these species, the influx of glucose showed first order dependence on Na^+ concentration in the medium. The underlying reason for the Na^+ non-electrolyte coupled absorption seen in these animals and Ascaris may be due to the dependency of glucose absorption upon pre-existing Na^+ gradients formed by an active Na^+- K^+ pump mechanism (Crane, 1969). However, the existence of such a pump mechanism has not been demonstrated in any parasitic helminth including Ascaris.

The cotransport of Na^+ and glucose has been observed in other animals. Some of these animals are the rabbit, rat, hamster, dog, chicken and human (Schultz, 1970). The cotransport of Na^+ and glucose appear as a change in the transmural potential across the animals intestine in which the serosal surface becomes electrically more positive. This observation is correlated with the simultaneous transport of glucose and Na^+ across the mucosal cell membrane -- a net movement of pos-

itive charges towards the serosal side. The driving force for this cotransport process is generally described as the asymmetrical distribution of Na^+ across the mucosal membrane (Rose and Schultz, 1971; Munck and Schultz, 1969; Lee, 1972). It is proposed that the low intracellular activity of Na^+ in turn is maintained by ion pumps located on the lateral cell membranes (Lee, 1972). These pumps move Na^+ into the lateral intercellular spaces or shunt pathway. It is through these spaces that sodium moves according to its electrochemical gradients and the permselectivity of the barriers found within it. Since the tight junction of vertebrate gut epithelia found at the apical region is generally believed to be the principle barrier of the shunt pathway the net movement of Na^+ is in the direction of least resistance or, in other words, towards the serosal surface. Unlike the vertebrates Ascaris has a very thick (3-4 μ) basement membrane attached to the gut at its pseudocoelomic pole. The basement membrane is equivalent to a second barrier in the shunt pathway. Consequently, the distribution of charges within the shunt is not only determined by the permselective characteristic of the apical junctional complex found in Ascaris but also the permselectivity of the basement membrane. It is these barriers which cause the observed transmural potential to be opposite in polarity to that observed in most vertebrate intestines.

CHAPTER VI

SUMMARY

The transmural electrical potential of isolated ribbons of the intestine from Ascaris suum were studied in terms of factors which effect the potential and its relationship to similar phenomena observed in other animal tissues. An attempt was made to identify the ion or ions responsible for the short circuit current. The membrane was characterized further by determining the extracellular space in contact with the luminal and pseudocoelomic surfaces. The effects of selected monosaccharides were compared in terms of the observed change they caused in the short circuit current when they were perfused over the luminal surface of the intestine. The results of these procedures are consistent with what is generally understood about the absorptive function of the intestine of Ascaris and absorptive surfaces in general. The gut of Ascaris generates a potential in which the pseodocoelomic side is negative relative to the luminal side. This potential is due to the active transport of a yet unidentified ion or ions. The anatomical features of the intestine suggest that a "shunt pathway" could exist which would influence the distribution of actively transported ions and result in the observed polarity of the potential. The response shown by the short

circuit current to the addition of surgars to the luminal surface indicates a coupling between ion and sugar absorption.

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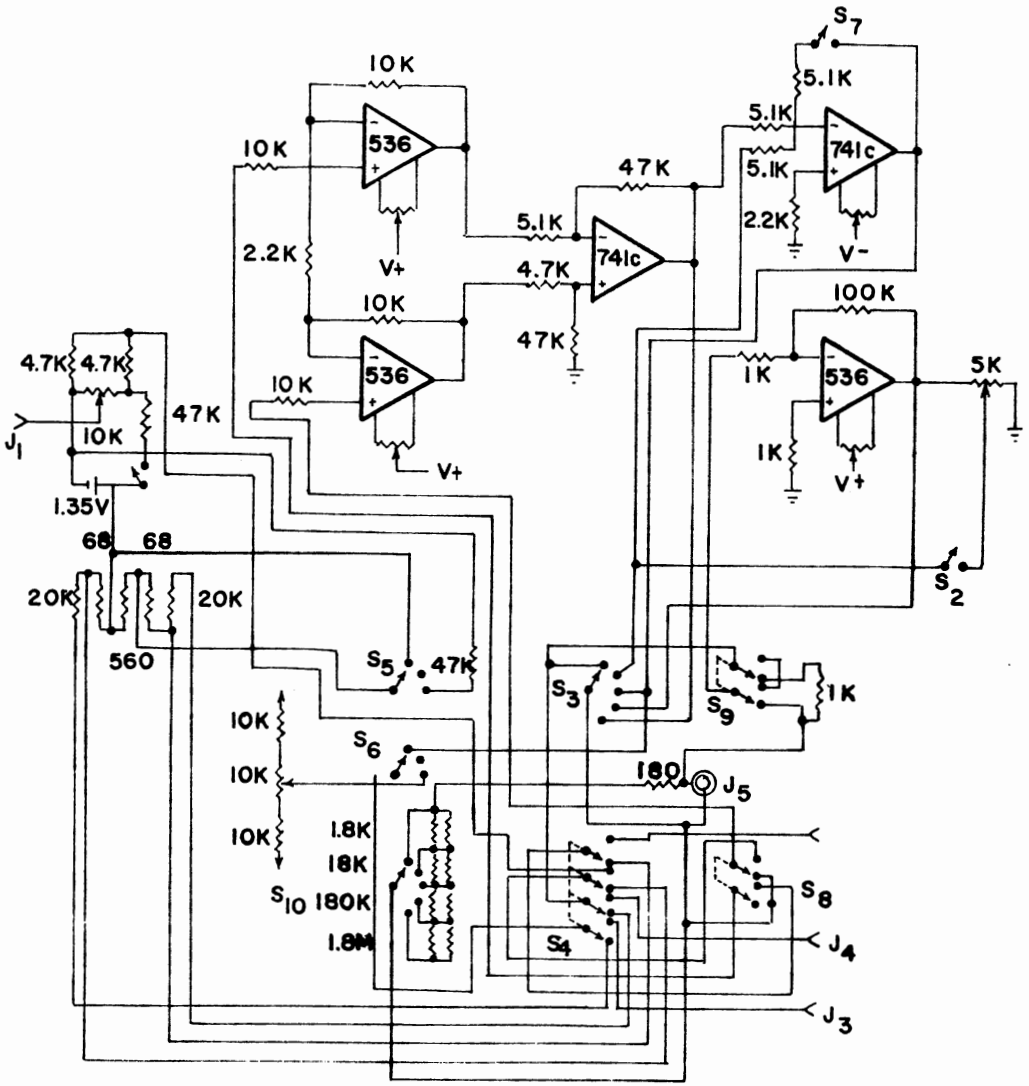
APPENDIX

DIAGRAM OF THE ELECTRICAL CIRCUIT FOR THE AUTOMATIC SHORT-CIRCUITING APPARATUS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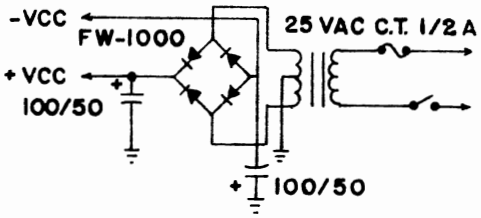
10K
2.2K
V+
10K
536
10K
V+
4.7K
4.7K
47K
10K
J1
1.35V
68
68
20K
20K
560
S5
47K
10K
10K
10K
S6
S10
1.8K
18K
180K
1.8M
S4
5.1K
741c
47K
4.7K
47K
S7
5.1K
5.1K
5.1K
741c
2.2K
V-
100K
1K
536
1K
V+
5K
S2
S3
S9
1K
180
J5
S8
J4
J3


-VCC
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25 VAC C.T. 1/2 A
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100/50

VITA[2]

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