STUDIES OF THE MECHANISM AND *IN VITRO* REQUIREMENTS FOR THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE INTESTINE OF *ASCARIS SUUM*

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

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CHAPTER I

INTRODUCTION

Foreword

The continued prevalence and world-wide distribution of ascarid infections in man and his domesticated animals has stimulated much concern from a public health standpoint as well as presented a constant economic threat to the swine industry. Ascaris lumbricoides Linnaeus, 1758 is the large nematode parasite of man, and a closely related species, Ascaris suum Goeze, 1782, infects swine. These two species have similar life cycles and morphological characteristics but are host-species specific under natural conditions.

The role of A. suum in human infections has been the subject of much controversy. However, there is no doubt this parasite can infect man. This point has been proven under controlled experimental conditions by a number of accidental laboratory infections where the only species involved was A. suum (Koino, 1922; Jaskoski, 1961).

A. suum has been used as the "experimental model" for studying the biochemistry and physiology of nematode parasites for many years. The reasons most investigators select this particular parasite for scientific studies are: 1) adult A. suum are readily available from infected swine at local slaughterhouses; 2) their large size facilitates isolation of specific tissues for experimental studies in vitro; and 3) adults can be maintained in the laboratory in a buffered-saline solution under

anaerobic condition for long periods of time. Many questions concerning the life cycle, epidemiology, pathology, and physiology of nematode parasites have been answered through continued research in the physiology and biochemistry of A. suum.

Historical Remarks

Historically, human ascarids were among the first parasites to be recognized and recorded. Hoopli (1959) cites several references to ascarid infections recorded in ancient texts from Mesopotamia, Greece, Rome and China. The first recorded knowledge of human ascarids was found in Eber's Papyrus, written between 1553-1550 B.C. Early Greeks were aware of human ascaris and Aristotle's Historia animaluim described both this worm, the pinworm (Enterobius vermicularis) and the tapeworms (Taenia saginata and T. solium). The famous Greek physician Hippocrates (460-377 B.C.) wrote of his knowledge of and experience with human ascarids (Levine, 1968).

Ascaris lumbricoides undoubtedly has been one of man's most faithful and constant companions. Chandler (1958) believes that early man's burden of ascarids most likely came through his domestication of wild pigs infected with the closely related species, A. suum. Early man's habits and close association with swine made possible the development of a special strain or species that became particularly well adapted for residence in his own intestine. This intestinal parasite has successfully clung to mankind through the stone, copper and iron ages, but continued improvements in sanitary plumbing and personal hygiene threatens to separate this partnership.

Infected children are the greatest reservoir for ascarids and if

they can be "yard-broken", the spreading of the infection to adults and other children can be drastically reduced. Improvements in sanitary management and chemotherapeutic programs contributed a great deal to the control of ascarids in swine. However, these programs are limited severely due to a lack of parasite-specific treatments that are economically feasible and do not harm the host. In spite of these improvements, wherever soil pollution persists and wherever there is warmth and moisture, ascarid infections are common in both man and swine.

Structural Characteristics

Mature adult worms of both species are quite similar in structure. They are large, heavy-bodied, unsegmented worms with a simple non-bulbar esophagus. They have a thick cuticle that covers the external surface of the body composed of nine separate layers with three distinct regions (outer cortex, matrix layer, and inner fibre layers) (Bird and Deutsch, 1957; Chitwood and Chitwood, 1950). A combination of chemical composition and X-ray diffraction studies on the outer cortical layer suggest that it is formed mainly of keratin containing some collagen (Lee, 1965). The digestive tract is a long, straight, tube-like structure that runs the entire length of the worm. Lee (1965) divides the alimentary canal of Ascaris into three distinct regions. First, the most anterior portion of the canal which includes the lips, mouth, buccal cavity and pharynx (esophagus) is called the stomadaeum. Located more posteriorly is the intestine which is flattened dorso-ventrally and is lined with a single layer of microvillated columnar epithelial cells. The intestine can be subdivided into three regions: the anterior region, mid-region, and posterior region. These regions differ from one another in the size and

shape of the lumen, the height and contents of the epithelial cells, and in their secretory and absorptive functions (Carpenter, 1952; Lee, 1961; Harpur, 1966; Schanbacher and Beames, 1973). More details of the intestinal structure and function are presented in Chapter II. The pseudocoelomic surface of the intestine is covered with an unusually thick basement membrane as compared with such structures in vertebrate tissues. The luminal surface of each cell is covered with numerous microvilli which differ in height and density from one region of the intestine to another (Kessel, et al., 1961; Shefield, 1964). The most posterior region of the alimentary canal is the proctodaeum. This region consists of the rectum and anus in females and the cloaca in males. Both the stomadeaum and proctodeaum are lined with a cuticular-like layer which is continuous with the cuticle covering the outer surface of the worm.

Mature female Ascaris commonly measure from 20-35 cm in length and from 0.3-0.6 cm in width. Male worms are generally smaller than females. They measure from 15-31 cm in length to 0.2-0.4 cm in diameter. Both males and females are spindle shaped, and cream white to pink in color. The most anterior end is blunt due to the infolding appearance of three finely denticulated lips that contain a pair of papillae on each side. The posterior end is more pointed at the tip, and males are readily distinguished from females by their ventrally curved tail. In the female, the genital pore is located ventrally at the junction of the anterior and middle thirds of the body. Male worms produce amoeboid-like sperm from a single testis and females produce eggs from a bilateral opesthodelphic reproductive system. Following copulation the sperm are stored in the seminal receptacles of the female and fertilization occurs as the eggs

are released from the ovaries. Fertilized ova develop two distinct envelopes. The primary envelope consists of three distinct layers: the fertilization membrane (0.5 μ); the chitenous layer (3.0 μ thick); and the vitelline membrane. The latter develops from the deposition of ascarosides from within the eggs as they progress down the uterus (Fairbairn, 1957). The secondary envelope consists of a sticky albuminous coat secreted by the uterus which tightly adheres to the outer surface of the ova and aids in protecting the embryonating eggs from adverse environmental factors.

Life Cycle

Although Ascaris is one of man's longest known parasites, details of its life cycle were learned only after the turn of the century. In 1916 Stewart recognized that A. lumbricoides larvae migrate to the lungs. In 1917 Ransom and Foster showed the life cycle to be direct and that the main route of migration is via the hepatic portal system.

Mature female ascarids normally inhabit the upper small intestine where they feed on the predigested foodstuffs provided by its vertebrate host. Fertile females lay unsegmented eggs which leave the digestive tract with the feces. In 1925, Cram estimated by egg count that each female A. lumbricoides can produce 200,000 eggs per day, and can lay a total of 26-27 million eggs in her lifetime. The fecundity of the swine ascarid, A. suum, is apparently much greater. Kelly and Smith (1956) experimentally infected pigs with A. suum and estimated that each female deposited between 1.0-1.6 million eggs per day. Later, Olsen, et al. (1958) determined that each female in naturally infected pigs produced nearly two million eggs per day. Their studies also indicated that

females stored a sufficient quantity of sperm to fertilize eggs for at least sixteen weeks after the last male was removed from the host. The tremendous egg laying capacity of A. suum is further realized by considering Fairbairn's (1957) calculations which show that a mature female can lay her own weight in eggs and accompanying secretions in 10 days. These eggs store an abundance of complex nutrients for aerobic development of the embryos and the females must rely upon the absorption and subsequent assimilation of host nutrients to maintain viable egg production and growth.

Fertile eggs from Ascaris are undifferentiated when they reach the ground. Their aerobic development is triggered by various stimuli. For complete development to infective-stage larvae, they require a temperature lower than that of the host's body, and a small amount of moisture and oxygen (Levine, 1968). Developing ova are very susceptible to extreme temperatures. They gradually degenerate at temperatures above 55°C and cease to develop at temperatures below 3°C. The optimum temperature for development lies between 21-30°C (Brown, 1927, 1928; Caldwell and Caldwell, 1928). Complete desiccation of ova is lethal to the embryonation process, but in most soil ova remain viable for years (Otto, 1929). If ambient conditions remain favorable, viable ova will develop active embryos within 10-14 days. However, the embryos do not reach the infective second stage larval form until they have gone through one molt which requires a total of 21 days (Rogers, 1958).

The continuance of the life cycle is assured when the appropriate host ingests infective eggs. The embryos emerge in the intestine as second stage larvae (Rogers, 1958). Optimum conditions for hatching in vitro requires at least 4 stimuli: 1) a temperature near that of the

body; 2) a concentration of CO_2 at about 5 volumes per liter; 3) a pH near 7.0; and 4) non-specific reducing conditions such as those produced by cysteine, glutathione, sodium bisulfite or sulfur dioxide (Fairbairn, 1961). The larvae penetrate the intestinal wall and migrate through the tissues. The route of migration was first worked out by Ransom and Foster (1917) and has since been confirmed by others (Olsen and Kelley, 1960). The larvae enter the mesenteric veins and pass via the hepatic portal system to the liver. A few may pass via the lacteals to the mesenteric lymph nodes, by-pass the liver and go directly to the lungs via the right heart. The larvae develop in the liver, molting to the third stage larvae 4-5 days after infection. Then they leave the liver and are carried by the blood stream through the heart into the lungs. Here, the larvae develop further, and molt to the fourth stage after 5-6 days. They burrow out of the alveoli into the bronchioles and bronchi, and finally are swept up the trachea. The peak of this fourth stage larvae migration occurs about 12 days post infection. The larvae are carried up to the pharynx, swallowed and reach the small intestine where they go through a fourth molt, and become adults. It is at this point they begin an anaerobic existence. The prepatent period is about 49-62 days (Schwartz, 1959), and very few adults live more than a year (Kelley and Olsen, 1958). Ascaris are facultative anaerobes as can be seen from the previous discussion on their life cycle. Genetic adaptations have undoubtedly occurred in the process of the evolutionary development of these nematodes which facilitates this type existence (Fairbairn, 1970).

Epidemiology of Ascariasis

In 1947 Stoll estimated that over 644.4 million people throughout the world were infected by A. lumbricoides; on the average one person out of every four were infected. This estimate was a composite of the incidence in various geographical locations. His survey showed that in North America the incidence of human ascariasis ranged from 12% in urban to 62% in rural areas. In Mexico where ascariasis is endemic, the incidence ranged from 45-71%. In Europe the incidence was low in large cities, ranging from 2-4%, but 52% in rural communities. In the U.S.S.R. the incidence was 70-80%, in Africa 30-95%, in Asia 80-90%, and in the Pacific Islands infection rates ranged from 2-5% in urban centers to 50% in rural areas. With an increase in the world population and subsequently a greater density of people in any given area, there are probably more total cases today than ever before despite a conserted effort to control these parasites.

In the United States, Ascaris infections are more common in the southern than northern states, rural communities maintaining a higher incidence of infection than urbanized areas. Furthermore, the greatest number of cases are reported most frequently from the mountainous rural areas of the southeastern states and from southern Louisiana (Chandler, 1958). Ascariasis is so rare in New Hampshire that Bullock (1961) found only two children infected with ascarids.

Ascarid infections are not as common in adults as in children. This is thought to be due in part to the adult's acquired immunity developed during childhood infections and in part to an increased frequency of exposure as a child from unsanitary eating and playing habits (Levine, 1968). In those parts of the world where "night-soil" is routinely used

to fertilize crops and unwashed raw vegetables are eaten, the incidence of ascariasis is high. From an epidemiological standpoint the contributing factors to the persistence and dissemination of these parasites in crowded quarters are: 1) dense shaded areas; 2) an abundance of moisture; and 3) infected children who defecate in these habitats (Chandler, 1958).

Pathogenesis of Ascariasis

From a pathological standpoint the symptoms and lesions caused by A. lumbricoides depends upon the stage and location of the infection. If a sufficient number of larvae are present, they may cause pneumonia. This occurs most frequently in young children suffering from malnutrition or other conditions causing lowered resistance to infections. Clinical signs of this condition include an abundance of eosinophils in the patient's sputum, persistent coughing and sometimes the presence of larvae in the heavy respiratory exudate. Loeffer's syndrome may occur in both children and adults as a result of sensitization. This is a pneumonic condition with eosinophilic infiltration of the lungs (Levine, 1968). There is a slight initial fever, the sputum is yellowish, and a leucocytosis and eosinophilia with relatively few lymphocytes is noticeable. Young pigs frequently show respiratory symptoms known as "thumps". A similar symptom has been observed in humans with heavy pulmonic infections of Ascaris (Chandler, 1958).

Some serious problems may develop when the larvae migrate to various other organs. If the larvae migrate into the lymph nodes, spleen, liver, kidney, or brain, they may induce an inflammatory reaction. The tissue responds to its foreign visitor by forming small inflammatory nodules

which eventually become necrotic, the larvae are destroyed and encapsulated. Depending on their number and location, these organ's functions may be seriously impaired. This is especially true in the nervous system (Sprent, 1949; Levine, 1968).

Sometimes migrating larva do not produce encapsulated lesions, but do produce minute local damage. Such a condition is known as visceral larva migrans. This is seen especially in young children between the ages of 16-36 months of age that insist on eating dirt. The condition results from prolonged continuous migration of animal nematodes in human tissues other than skin as first described by Beaver, et al. (1952) in three children near New Orleans. They indicate that most cases in the United States are a direct result of either the larvae of (1) the dog ascarid Toxocara canis, (2) A. suum in areas where swine are raised, or (3) the horse ascarid Parascaris equorum. The disease is characterized by hypereosinophilia, hyperglobulinemia, hepatomegaly and general poor health. Migrating larvae will also cause pneumonia, central nervous system involvement, or endophthalmitis and retinal granuloma (Dent, et al., 1956). The incidence of nematode larval migrans is probably much higher than one realizes since it is often misdiagnosed, and therefore, not reported in the literature. Levine (1965) has reviewed several case reports and shows this to be the case.

After reaching maturity in the intestine, adult ascarids may or may not disrupt the tranquility of the intestine. This is directly related to the parasite load. A few adults in the intestine may cause no serious symptoms, but heavy infections readily compete for nutrients and may promote abdominal pain and discomfort from irritation of the mucosal lining. These conditions are often associated with nausea, vomiting,

diarrhea or constipation, low-grade fever, loss of weight, or in some cases, convulsions (Chandler, 1958). A large mass of worms may completely obstruct the intestine, sometimes causing a volvulus or intussusception (Arean and Crandall, 1971). This condition may be fatal unless it is diagnosed quickly and the worms removed surgically.

The list of dangerous complications of Ascaris infections is greatly enlarged by the fact that the worms have a "wanderlust" and tend to explore ducts and cavities (Chandler, 1958). They may completely occlude the bile and pancreatic ducts and enter the gall bladder or even go into the liver. Ascaris sometimes pass through the intestinal wall and cause fatal peritonitis or may even come through the umbilicus or groin. Similar pathological conditions exist in swine infections and this accounts for a considerable loss each year (Arean and Crandall, 1971).

Economic Impact of Ascariasis on the Swine Industry

Ascaris suum is by far the most economically important parasite of swine and it causes a substantial loss of pigs raised under unsanitary conditions. According to the USDA (1965) swine ascariasis causes an annual loss of $34,812,000 in the United States alone, of which $683,000 is due to deaths and $34,129,000 to morbidity. However, in terms of monitary losses this figure is very conservative.

Many studies have been conducted on the economic effects of parasitism in swine and the above figures do not take into account factors that indirectly affect the losses due to parasitism. The death loss of infected animals, and the condemnation of carcasses and edible parts at slaughter only make up a small portion of the total economic loss. Slow

growth and poor weight gains are much more important economic factors. Spindler (1947, 1951) estimated that the average loss due to condemnation of edible portions of swine carcasses was 50 cents per 100 pounds body weight. The loss on an average slaughter hog weighing 250 pounds was $1.25. Since about 60 million hogs were slaughtered under federal inspection in 1948, Spindler (1951) estimated the loss due to condemnation was $75 million in that year alone.

Such estimates for the monetary losses can be brought up to date if the current higher prices of market animals are considered. Furthermore, if one takes into account the high cost of the extra grain that is fed to parasitized hogs to bring them to a marketable weight, and the extra labor required to finish parasitized hogs for slaughter, the financial losses become even greater. Taking all these factors into consideration, the most current and conservative estimate of losses to the swine industry in the United States during 1962 exceeded $200 million (Levine, 1968). Considering the current prices of pork and grain which have almost tripled since 1962 and the higher cost of labor, today the total monetary losses to the swine industry caused by Ascaris must be some 2-3 times greater than it was in 1962.

Rationale and Research Objectives of the Study

Rationale

Adult ascarids rely almost exclusively upon the catabolism of carbohydrates to meet their demands for metabolic energy. The intestine serves as the principal route for the absorption of nutrients by nematodes (Fairbairn, 1957; von Brand, 1966). Although the intermediary metabolism of adult Ascaris has been the subject of numerous studies, comparatively

few details concerning the mechanism and requirements of the movement of carbohydrates across the nematode intestine are known. Additional experiments *in vitro* on the movement of carbohydrates across the intestine of *Ascaris* will provide valuable information to help explain "unique" differences in the process of nutrient absorption. Such differences as the inability to absorb galactose (Sanheuza, et al., 1968; Beames, 1971) and a requirement for both carbon dioxide and glucose to facilitate the movement of hexoses (Beames, 1971) presently suggest that the sugar transport system has specificity and certain metabolic requirements unlike those described for the mammalian intestine. Exogenous glucose in the system significantly enhances the rate of movement of 3-O-MG. Endogenous glycogen and trehalose are present in the intestinal epithelium and hemolymph (Fairbairn and Passey, 1957). In these tissues the total carbohydrate is approximately 1% of the wet weight. Beames (1971) suggests that under conditions *in vitro* it is possible that the small endogenous supply of carbohydrate is exhausted very rapidly and this could explain the glucose requirement he observed. Alternatively, the epithelial cells may not be able to mobilize the endogenous carbohydrate reserves to facilitate the movement of sugars. Additional information on the role of endogenous carbohydrate metabolism and other requirements of 3-O-MG movement will help to clarify specific details of the sugar transport process. Such measurements may also provide the basis for a rational approach in developing parasite specific chemotherapeutic agents for irratification of nematode parasitic diseases in both man and his domesticated animals.

Research Objectives

The objectives of this study are: (1) to further characterize the requirements for moving 3-0-MG across the intestine; (2) to determine the role of exogenous sugars in facilitating the movement of 3-0-MG from the luminal to the pseudocoelomic solution of sac preparations; and (3) to determine if various metabolic inhibitors, membrane blockers, or omission of certain cations effect the movement of 3-0-MG across the intestine of *Ascaris* as has been shown in other systems.

CHAPTER II

LITERATURE REVIEW

Introduction

The vertebrate small intestine is well adapted for the digestion and absorption of carbohydrates and it provides an abundant supply of predigested nutrients to intestinal parasites. The nematode's intestine is undoubtedly the principle route for the absorption of these nutrients. However, such differences as (1) the inability to absorb D-galactose, and (2) an *in vitro* requirement for CO_2 and glucose for moving hexoses across the intestine of *Ascaris* (Beames, 1971), strongly suggest that the specificity for sugar transport and its metabolic requirements are unlike the mammalian sugar transport system (Crane, 1960). Therefore, the current concept of the mechanism and requirements for the intestinal absorption of sugars in the mammalian intestine will be reviewed to serve as a reference for comparing similarities and differences between the two systems.

Movement of Materials Across Cell Membranes

Before progressing further, it is necessary to define several mechanisms by which substances such as sugars move across cell membranes. Hence, a brief description of the distinguishing features of the mechanisms essential for moving sugars across cellular membranes is presented.

Passive Diffusion

When a substance crosses a selectively permeable membrane by random molecular motion it is called passive diffusion. The driving force responsible for the net movement of passively diffused materials is the movement of dissolved substances from an area of higher concentration to an area of lower concentration. At equilibrium the net movement of diffusable substances stops. Therefore, a favorable concentration gradient must exist across the membrane. In passive diffusion the transported solute does not interact with any molecular species or carrier in the membrane. This mechanism does not exhibit Michaelis-Menton saturation kinetics and the rate of diffusion is a linear function of the solute concentration. Kaback (1970) suggests that passive diffusion mechanisms may be modified by solvent drag in which the penetrating substance is swept through aqueous pores in the cell membrane by (1) bulk water flow, (2) membrane charge that either attracts or repels an ionizable substance, and (3) the degree of hydrophobicity of the diffusion barrier--the cell membrane. Since monosaccharides are (1) unionized in solution, (2) relatively insoluble in lipids having a low solubility coefficient, and (3) too large to pass through membrane pores, it is doubtful that these compounds move across the cell membrane by passive diffusion.

Facilitated Diffusion

The second type of transport mechaism is facilitated diffusion. In this process the transported sugar is presumed to combine specifically and reversibly with a carrier component in the membrane (Rosenberg and Wilbrandt, 1955). It is generally thought the carrier or carrier-substrate complex is free to oscillate between the inner and outer

surfaces of the membrane, releasing and binding molecules on either side (Ussing, 1952). The distance covered by the mobile carrier or carrier-substrate complex is relatively short. It is thought that thermal energy and/or molecular deformation resulting from the binding and release of a substrate can account for the small amount of molecular motion required to move sugars across the otherwise limiting membrane (Giese, 1968). A net movement of substrate occurs only in response to a concentration difference. With low concentrations equilibrium is attained more rapidly than can be accounted for by simple diffusion processes. Facilitated diffusion differs from simple diffusion in that the rate of sugar movement becomes limited with increasing substrate concentrations, and the carrier system elicits Michaelis-Menten type saturation kinetics. For example, facilitated diffusion rather than simple diffusion accounts for the rapid uptake of sugars in erythrocytes (Rosenberg and Wilbrandt, 1957). Many other tissues show similar kinetic parameters and the intestinal epithelium is no exception. The entry of sugar into the mucosal epithelium occurs by a facilitated diffusion process (Widdas, 1952).

The process of facilitated diffusion does not require the expenditure of metabolic energy since the presence of the potent metabolic inhibitor, iodoacetate, does not alter the rate of movement of fructose, mannose or the pentoses (Venzar and Sullmann, 1936; Riklis and Quastel, 1958). The transport system shows specificity and is located on the luminal surface. The addition of the competitive inhibitor phlorizin to the luminal solution markedly decreases the rate of intestinal absorption of sugars (Alvarado and Crane, 1962).

Active Transport

The distinguishing feature of actively transported solutes is that accumulation or movement occurs against an opposing concentration gradient. Rosenberg (1954) has used and restricted the term active transport for those instances in which metabolic energy is required. Furthermore, the clearest experimental evidence to support an active transport process is the net movement of molecules against a concentration difference. The classic model for describing intestinal active transport of sugars was proposed by Crane (1962). He postulated that the penetrating sugar combined with a carrier in the brush border membrane and that the carrier or carrier-substrate complex is then subjected to molecular modification in the membrane. The expenditure of metabolic energy is essential to this process since either the lack of O_2, the presence of an oxidative uncoupler 2,4-dinitrophenol (DNP) or the addition of a glycolytic inhibitor iodoacetate (Cori, 1925; Verzar and McDougall, 1936) markedly decrease the rates of intestinal absorption of glucose or galactose.

Modifications of the carrier occur in such a way that it has a higher affinity for the substrate on the luminal surface of the membrane. Molecular changes in the carrier on the inner surface of the membrane lowers the affinity of the carrier for the substrate and unidirectional flux of sugar is established across the intestine (Crane, 1968).

Na^+-Coupled Transport

Recognition of the involvement of sodium ions (Na^+) with the movement of actively transported sugars has resulted in modifications in the mechanism of intestinal active transport of sugars. Riklis and Quastel (1958) first recognized that Na^+ must be present in the luminal solution

for active absorption of glucose by the guinea pig small intestine. Later this observation was confirmed by Bihler and Crane (1962) for the accumulation of the nonmetabolizeable sugar 6-deoxy-glucose in strips of hamster intestine and they concluded that the primary interaction of Na^+ was localized to the brush border membrane. Csaky (1963) later demonstrated that the addition of Na^+ to a Na^+-free media bathing the luminal surface of the intestine activates the sugar transport system. Furthermore, the addition of the cardiac glycoside ouabain to the serosal side of the intestine drastically reduces the active absorption of sugars (Csaky and Hara, 1965), has shown that a Na^+-K^+ pump is coupled with active sugar transport. The above observations led several investigators (Schultz and Zalusky, 1964; Schultz and Curran, 1969; Crane, 1962, 1968) to propose the Na^+-coupled sugar transport mechanism which has the essential features of (1) an energy-dependent, ouabain sensitive, Na^+-carrier mechanism on or near the serosal membrane whose rate of transport is a function of the intracellular Na^+ concentration, and (2) a Na^+-coupled, phlorizin-sensitive sugar transport mechanism on or near the brush border of the cell whose rate of transport is a function of both the Na^+ and the sugar concentrations.

Conclusions

The conclusion from all of these studies is that some sugars (e.g., fructose, mannose, and some pentoses) move from the lumen of the intestine into the blood by a facilitated diffusion process while others (e.g., glucose and galactose) move through the plasma membranes and across the intestine by a Na^+-coupled energy dependent active transport process. As Widdas (1952) suggested active absorption of hexoses may be

a two step process. First, sugars bind to the membrane carrier by an energy-independent Na^+-coupled process which has a high affinity for actively transported sugars. Secondly, the sugar is released from the Na^+-carrier-sugar complex by an energy dependent ATPase system allowing the sugar to be concentrated in the cell. The system is tightly coupled to the Na^+-K^+ pump which maintains the low intracellular Na^+ levels and decreases the affinity of the carrier for sugar. This complex process assures a unidirectional flux of actively transported sugars from the luminal to the serosal side against an opposing concentration gradient which requires the expenditure of metabolic energy.

The Vertebrate Intestine

Early Studies on the Absorption of Sugars

In 1939, Barany and Sperber observed the continued disappearance of glucose from an intestinal loop of the rabbit small intestine when the concentration of glucose in the loop fell below the level in the blood. However, their experiments suffered to some extent from the lack of proof that glucose, at low concentrations, disappeared from the luminal solution as a result of absorption into the blood stream rather than by metabolism in the epithelial cells or by breakdown by intestinal microbes. Later, Campbell and Davson (1948) conclusively demonstrated that 3-0-methyl-D-glucose, a compound which is not apparently metabolized (Campbell and Young, 1952) was absorbed against a concentration gradient from an intestinal loop in the cat. Modifications in experimental technique allowed Atkinson, et al. (1957) to study the absorption of glucose by placing ^{14}C-glucose in a jejunal loop of the dog and collect blood from a vein draining the loop. They found that the venous blood

contained a higher concentration of labeled glucose than the luminal solution.

Earlier studies by Cori (1925) on the rates of absorption of naturally occurring sugars led to the separation of sugars into two classes on the basis of their relative rates of absorption and the effects of metabolic poisons. Cori (1925) found that the relative rates of absorption of various sugars in the rat intestine was galactose > glucose > mannose > fructose > pentoses and this observation has since been confirmed not only in the rat (Verzar and McDougall, 1936) but in many other species (Crane, 1960). In addition the rates of absorption of glucose and galactose were found to be maximal at relatively low luminal concentrations while the rates of absorption of mannose, fructose and the pentoses, ribose and xylose, only increased proportionally with increases in their concentration (Cori, 1926). Furthermore, the finding that the addition of iodoacetate to the luminal solution drastically reduced the rates of absorption of only glucose and galactose, and had little effect on the absorption of other sugars, led investigators to conclude that of the naturally occurring sugars, only glucose and galactose are actively absorbed against a concentration difference (Crane, 1960).

Location of the Sugar Absorption Process

The intestinal epithelial cells of vertebrates are functionally polarized since the net movement of sugar is from the lumen to the blood. Morphologically the mucosa is well adapted for this absorptive function since the luminal margins of its epithelial cells have developed a brush border of numerous tightly packed microvilli. The absorptive epithelial cells are 22 to 25 μ long and microvilli measure from 1.0 to 1.4 μ in

length to about 0.08 μ wide. Although there are no obvious morphological differences in the absorptive cells along the length of the small intestine, the density of villi supporting these cells changes from 10 to 40/mm^2 (Bloom and Fawcett, 1968). Deane (1966) stated that the villi are taller and more numerous in the jejunum than in the ileum. In the distal part they are short and scattered, finally disappearing on the surface of the ileocecal valve. Thus, differences in the absorptive surface area occur along the length of the vertebrate small intestine. Fisher and Parsons (1950) showed that the gradient of mucosal surface area per unit length of intestine increased from the ileum to the duodenum of the rat.

Earlier work by Magee and Reid (1931) indicated that the rate of absorption of sugars is not uniformly distributed along the length of the small intestine of the rat, since the rate of glucose absorption in the ileum is less than the absorption by either the duodenum or the jejunum. Later, Fisher and Parsons (1950) confirmed this observation and further demonstrated the presence of a linear gradient for the absorption of glucose from the lumen. They showed that the rate of glucose absorption rose sharply as the mean distance from the ileocecal valve is increased. Overall, there was a 4-fold increase in the rate of glucose absorption in the duodenum as compared to the ileum. With galactose, a sugar which is not utilized as rapidly as glucose and for which there are not large intracellular stores, Fisher and Parsons (1953) found maximal absorption in the mid-portion of the rat small intestine. The results of experiments in vitro by Crane and Mandelstam (1960) showed that the active absorption of glucose, galactose or the nonutilizable 1, 5-anhydro-D-glucitol occurs more rapidly across the upper jejunum than in other segments of the hamster intestine. Thus it is apparent that a functional

gradient for the absorption of sugars exists along the length of the vertebrate intestine which is related to functional alterations in the luminal surface morphology.

Specificity of Sugar Absorption

The structural features of some sugar molecules enhance their absorption and some "mechanism" in the mucosa, most likely a complex molecular arrangement of a carrier molecule at the mucosal interface, specifically interreacts with certain of the sugars and not others to facilitate transfer from the mucosal to serosal compartment. Crane (1960) studied the absorption of 49 sugars and related compounds from a structural standpoint and found that only fourteen of these compounds could be accumulated against a concentration gradient on the serosal side of everted sac preparations of the rat or hamster intestine. On the basis of the common structural features of the actively absorbed sugars, Crane (1960) proposed that the minimum structural requirements of the sugar are (1) the presence of a D-pyranose ring structure, and (2) a hydroxyl group attached to carbon-2 (C-2). Furthermore, Crane (1960) indicates that those sugars which are not actively absorbed possess either a particularly large or bulky group (e.g., 3-O-butyl-D-glucose) or an ionized (e.g., gold-thioglucose) substituent on some part of its structure or they completely lack one of the essential structural features (e.g., 2-deoxy-D-glucose, fructose, pentoses).

More recently, the structural features of actively transported hexoses of the vertebrate intestine have been modified. For example, Barnett, et al. (1968) extended his earlier work to show that absorption of hexose involves hydrogen bonding at the carbon-1 position. They

demonstrated that L-glucose has a much lower affinity for the mechanism than D-glucose and is absorbed at a much reduced rate. This also appears to be the case with D-xylose; a sugar which lacks a 6th carbon but is homomorphic to D-glucose in the first five carbons (Alvarado, 1966). It now appears that a decrease in active transport occurs when replacements or deletions are made in positions 1, 2, 3, 4, and 6 of the sugar molecule, suggesting that contact at five points is necessary between the sugar and the membrane component (Whittam and Wheeler, 1970). Glucose is considered the ideal sugar, and substitution or deletion on any part of the structure results in a decreased affinity for the carrier system.

Kinetics of Sugar Absorption

The fact that facilitated and active transport of sugars follows Michaelis-Menton saturation kinetics is well established in the vertebrate small intestine and indicates that a carrier mediated system is involved in moving the substrate across the membrane. Of the naturally occurring sugars, only glucose and galactose show saturation kinetics at low concentrations. Like most enzyme mediated reactions a graphical expression or plot of the rate of sugar absorption versus increasing substrate concentrations results in a characteristic hyperbolic curve that approaches a maximum rate at high substrate concentrations. This characteristic response is typical of a Michaelis-Menton saturation curve for an enzyme mediated reaction (Christensen and Palmer, 1967). The entry of substrate into the cell at very low substrate concentrations is proportional to its concentration and follows first-order reaction kinetics. However, as the substrate concentration is increased, the entry becomes zero-order at high substrate concentrations. Hence, the reactive sites

of the carrier system are saturated. By definition the Michaelis constant for transporting solutes, K_t, is the concentration of substrate that gives one-half the maximum velocity or rate of substrate entry, and is a kinetic parameter used to estimate the affinity of a carrier system for the substrate. The other kinetic parameter, V_{max}, represents the limiting rate of entry or transport of the substrate by the carrier mediated system.

In intestinal transport studies the value of the K_t apparently depends on the experimental technique or preparation for determining this parameter. For example, Fisher and Parsons (1949) perfused the isolated intestine of the rat with various concentrations of glucose on the mucosal side and maintained a constant concentration of 28 mM glucose on the serosal side. They observed that the change in rate at which the glucose moved from the mucosal to the serosal side followed Michaelis-Menton saturation kinetics with a K_t for glucose transport of 8-9 mM. In similar experiments with galactose as the substrate on the mucosal side a lower affinity for the carrier system was determined since the K_t for galactose transport increased to 35 mM. Later Riklis and Quastel (1958) using similar preparations of the guinea pig small intestine with sugar present only in the mucosal solution found an apparent K_t value of 7 mM for glucose transport to the serosal solution. Using another preparation, Crane and Wilson (1958) studied the movement of both glucose and galactose across everted sac preparations of the hamster intestine with varying, but equal, concentrations on both sides and found the K_t values for the accumulation of these sugars in the mucosal epithelial tissue were 1.5 and 2.2 mM, respectively.

Studies on the competitive inhibition of sugar transport indicate

that the carrier system for moving sugars across the vertebrate small intestine has a relatively higher degree of inhibition with certain sugars, and that the magnitude of inhibition can be predicted from their transport constants, K_t. Cori (1926) first reported mutual inhibition between glucose and galactose transport across the rat small intestine, in vivo, and this observation has since been confirmed, in vitro, with everted preparations of the hamster small intestine (Crane, 1960). Since glucose has a higher affinity (K_t = 9 mM) for the transport mechanism than does galactose (K_t = 35 mM), the absorption of glucose should depress galactose absorption relatively more than galactose absorption depresses the transport of glucose. Riklis, et al. (1958) demonstrated this point by placing 14 mM galactose on the mucosal side of a 10 cm length of intestine and adding an equal concentration of glucose. The addition of glucose under these conditions reduced the absorption of galactose by some 80%. Further, it was shown that the addition of an equal concentration of galactose to a similar preparation absorbing 14 mM glucose only reduces the rate of glucose transport 20-30%. In these experiments, the control rate of glucose absorption alone was 37 μmoles per hour. Csaky (1958) reported mutual inhibition of a competitive type between glucose and 3-O-methyl-D-glucose when the substrate-inhibitor concentrations were 28 mM or greater. With equal molar concentrations, glucose absorption was inhibited some 40-50% by the addition of 3-O-methyl-D-glucose. Finally, Crane (1960) found that the pairs, glucose and galactose, glucose and 1, 5-anhydro-D-glucitol, glucose and 6-deoxy-D-glucose, and 1, 5-anhydro-D-glucitol and 6-deoxy-D-glucose mutually inhibit one another during in vitro accumulation with rings of everted hamster intestine, and that the degree of inhibition was approximately that expected

from their respective K_t values.

Relation of Absorption to Cell Metabolism

When transport of sugars has been measured in cells incubated under favorable and unfavorable metabolic conditions, the results show that energy-yielding reactions are needed for active transport processes. The fact that active absorption is an energy-requiring process is made clear by the properties of absorption against a concentration gradient and the capacity to concentrate glucose in the tissue some 100 times the luminal concentration (Wilson and Crane, 1958).

Several experiments in vitro have shown that the active sugar absorption in the vertebrate intestine is dependent upon aerobic energy metabolism since the replacement of air or O_2 in the incubation system with nitrogen (Crane and Mandelstom, 1960; Darlington and Quastel, 1953; Wilson and Vincent, 1955), or the addition of the uncoupler of oxidative phosphorylation, dinitrophenol, to the incubation media (Crane and Mandelstom, 1960; Darlington and Quastel, 1953) drastically reduces the absorption of glucose, galactose and 3-0-methyl-D-glucose. Furthermore, active absorption is dependent upon glycolysis or glycolytic energy production since the addition of the well known glycolytic inhibitor, iodoacetate, at low (1×10^{-6} M) concentrations inhibits the accumulation of both glucose and galactose in the rat (Riklis and Quastel, 1958) and the hamster (Crane, 1960) intestine.

In the course of being actively absorbed, glucose does not entirely depend upon its own metabolism for metabolic energy to drive its own transport. The observation that large amounts of free glucose are absorbed into the blood was demonstrated by Kiyasu, et al. (1957) in the

rat and by Atkinson, et al. (1957) in the dog intestine using the isolated loop preparation. Both groups of investigators found that U-^{14}C-glucose placed in isolated loops of intestine appeared in the blood collected from a vein draining the isolated segment. In the case of the rat intestine it was shown that 97% of the absorbed radioactivity from the loop was recovered in the blood in the form of glucose (82-92%), lactate (4-16%) and alanine (1-5%). In the isolated intestinal loop of the dog, Atkinson, et al. (1957) recovered 70-80% of the radioactivity in the blood as glucose and 7-17% as lactic acid. Only small amounts (< 3%) of radioactive CO_2, alanine and pyruvic acid were recovered. Furthermore, glucose is not resynthesized from lactate in the gut tissue in significant amounts since Hawkins and Wills (1957) found less than 1% of the labeled blood glucose was randomly labeled when absorbing glucose-1-^{14}C. Finally, absorbed glucose does not go through the tissue pool of glucose-6-phosphate since the specific activity of the glucose-6-phosphate pool previously labeled with galactose-1-^{14}C is diluted less than 10% by absorbed glucose in the tissue of everted sacs of the hamster intestine (Landau and Wilson, 1959).

Whittam and Wheeler (1970) have reviewed the relationship between the Na^+-K^+ pump and active transport processes, and indicate that the activity of the Na^+-K^+ pump plays an important part in regulating Na^+-coupled active transport processes by maintaining a low intracellular Na^+ concentration and/or altering cellular metabolism. This concept suggests that such coupled systems have a basic economy and simplicity in that the active pumping of Na^+ out of the cell provides the driving force either directly or indirectly necessary for the active transport of K^+, and glucose into the cell. This observation is supported best by specific

inhibition of the ATPase dependent Na^+-K^+ pump in erythrocytes with the cardiac glycoside, ouabain, reducing lactate production by lowering the intracellular K^+ concentration and increasing the intracellular Na^+ concentration sufficiently to prevent disassociation of the Na^+-sugar-carrier complex on the inner surface of the membrane. Hoffman and Parker (1967) have shown that the production of metabolic energy (ATP) is regulated by the flux of cations and the activity of the Na^+-K^+ pump. This close relationship influences the availability of intracellular ATP and thus helps to regulate the entry of Na^+ coupled substrates such as glucose into the cell.

In respiring tissue the role the Na^+-K^+ pump plays is complicated by oxidative energy production and O_2 consumption in mitochondria, which is far removed from the cellular membrane. Landon (1967) shows that the addition of isolated cellular membrane fragments of kidney cortex stimulates the respiration of kidney mitochondria in vitro which indicates an interaction between mitochondrial ATP production and ATPase activity of the membrane-bound Na^+-K^+ pump exists. Most of the ATP production in kidney cells is used to transport Na^+ (Lehninger, 1970). He states that if the active transport of Na^+ in kideny cortex preparations is inhibited by ouabain, the rate of respiration is depressed some 80%. The Na^+-K^+ pump in intestinal tissue is thought to operate similar to kidney tissue (Crane, 1968). Csaky and Hara (1965) shows that the addition of 1×10^{-5} M ouabain to the serosal side of the frog intestine gradually reduces 3-O-MG transport from the mucosal to the serosal solution. Crane (1968) attributes this reduction in movement to an inability to accumulate the 3-O-MG within the epithelial cell as a result of a high intracellular concentration of Na^+.

Metabolic Poisons

"Poisons" of specific metabolic pathways can indicate whether or not the energy of those pathways provide an obligatory drive to a specific cellular process. Iodoacetamide blocks glycolytic metabolism by inactivating glyceraldehyde-3-phosphate dehydrogenase and inhibiting the utilization of triose phosphates. Although the inhibitory effect of this metabolic poison is nonspecific, it irreversibly oxidizes sulfhydryl groups which are necessary for glyceraldehyde-3-phosphate dehydrogenase activity. Velick (1955) indicates that a concentration of 3.3×10^{-3} M iodoacetamide inhibits a purified yeast preparation of glyceraldehyde-3-phosphate dehydrogenase some 51 to 100%. Phiefer (1960) studied the effect of iodoacetamide upon the uptake of glucose in the tapeworm Hymenolopis deminuta. He shows that the uptake of glucose in these parasites is reduced some 53-71% with iodoacetamide in the incubation system. Sodium fluoride is another glycolytic inhibitor. It blocks utilization of 2-phospho-D-glycerate at the enolase step. This potent inhibitor also blocks those kinase reactions that require both phosphate and magnesium ion for enzymatic activity at their reactive site. Bucher (1965) indicates that a Mg^{+2}-fluorophosphate inhibitor complex is formed in the presence of both fluoride and phosphate ions which competitively inhibits enolase activity. The metabolic inhibitor 2,4-dinitrophenol (DNP) is a well known uncoupling agent of oxidative phosphorylation in mammalian mitochondria (Slater, 1967) and does not effect substrate phosphorylation (Lebninger, 1970). It also inhibits the incorporation of inorganic phosphate and increases the utilization of malate in isolated muscle mitochondria from Ascaris. Saz (1972) and Van de Bossche (1972) both show that concentrations of DNP of 5×10^{-5} to 1×10^{-4} M inhibit

the incorporation of inorganic phosphate in mitochondrial preparations some 33 to 67%.

Studies With Ascaris and Other Parasites

Anaerobic Carbohydrate Metabolism of Ascaris

In considering the energy dependent movement of sugars by Ascaris, the nature of this nematode's intermediary metabolism and its relationship to the movement of sugars across its intestine becomes important. Adult Ascaris live in an essentially anaerobic environment in the vertebrate small intestine and must rely upon the anaerobic catabolism of carbohydrates for energy to carry out many of their physiological processes (Fairbairn, 1970). Some biochemical adaptations occur in the terminal steps of the glycolytic pathway. These changes aid in balancing the shuttle of cytoplasmic pyridine nucleotides by replacing the inadequate pyruvate kinase step (Bueding and Saz, 1968). This sequence of steps ultimately provides the substrate for mitochondrial oxidation. Bueding and Saz (1968) have shown the presence of a very active cytoplasmic enzyme, phosphoenolpyruvate (PEP) carboxykinase, which fixes CO_2 to phosphoenolpyruvate to form oxaloacetic acid; the reaction that replaces the pyruvate kinase step. Oxaloacetic acid is converted to malic acid by a cytoplasmic malic dehydrogenase (Saz and Hubbard, 1957). This step regenerates the oxidized NAD utilized by the glyceraldehyde-3-phosphate dehydrogenase reaction for triose phosphate oxidation in the glycolytic pathway. Malic acid is thus made available to the mitochondrial fumarase (Saz and Lescure, 1969) to form fumarate. Fumarate is reduced to succinate and various volatile fatty acids (Saz and Bueding, 1966). During anaerobic reduction of fumarate to succinate in the

mitochondria, electrons are transferred from a reduced pyridine nucleotide to fumarate by a flavoprotein-succinic dehydrogenase shuttle system generating an ATP from ADP and inorganic phosphate (Kmetic and Bueding, 1961; Seidman and Entner, 1961). This mechanism along with the glycolytic pathway in the cytoplasm provides energy in the form of three ATP equivalents for various physiological activities. Since these organisms fix CO_2, removing CO_2 from the tissue should effectively block PEP carboxykinase activity, and inhibit such physiological activities as the energy dependent movement of hexose across the intestine; a result verified by Beames (1971) using midgut sac preparations incubated under various conditions *in vitro*.

In addition to CO_2, Beames (1971) showed that the movement of sugars across the midgut of *Ascaris* required an exogenous energy source such as glucose. Adding glucose to the incubation system significantly enhanced the rate of movement of 3-0-MG and fructose in a 95% N_2-5% CO_2 gas atmosphere. Removing CO_2 and incubating in 99% N_2, drastically reduced the effect of exogenous glucose on the movement of 3-0-MG. Furthermore, Beames (1971) showed that the movement of 3-0-MG from the luminal to the pseudoceolomic solution against a concentration gradient occurred only when glucose was present as an energy source. These observations are consistent with what is known of the worm's carbohydrate metabolism and suggest that the movement of sugars *in vitro* across the intestine of *Ascaris* requires metabolic energy as it does in vertebrates.

Structural and Functional Aspects of the Intestine

Although some of the structural characteristics of the intestine of Ascaris have been mentioned previously, it is important to point out the relationship between the structural and functional aspects of the intestine.

In general, the appearance of the epithelial cell of the intestine of Ascaris is quite similar to the mucosal cells lining the vertebrate small intestine. However, in contrast to the complex multi-layered structure of the vertebrate intestine, the intestine of Ascaris suum is lined with a single layer of tall columnar epithelial cells. The intestine runs the entire length of the worm beginning at the junction of the intestine-esophageal valve and terminating in the posterior end at the rectum.

One of the most complete structural-functional studies of ascarid intestine is Carpenter's survey of its hydrolytic enzymes correlated with its morphological variations throughout its length (dissertation, 1952). Her observations suggest that differences between the absorptive and secretory functions along the length of the intestine may exist. For example, she observed that the epithelial cells in the anterior region were 200 μ tall and they contained an abundance of densely stained secretory granules concentrated in the apical end. Furthermore, she noted that some cells in this region were essentially void of cytoplasmic secretory granules, and there were numerous secretory blebs of granular material and protoplasmic projections associated with or extending through the cellular membrane into the lumen. In some instances the secretory bleb was "pinched off" or ruptured in the vicinity of the

microvillar region.

In the mid-region at the level of the uterine pore, the average height of the epithelial cells increased to 300 μ, and the microvillar layer remained 16-18 μ thick. There was only a slight decrease in both the staining density of secretory granules and the number of secretory blebs in the epithelial cells from this region. In female worms at the level of the reproductive organs striking differences were observed in the morphological appearance of the intestinal epithelium. In this upper posterior region Carpenter (1952) determined that the epithelial cells were considerably shorter, measuring an average length of 100 μ. In this region the intestine appeared more folded than in the anterior region. Also, the secretory granules and blebs decreased in intensity and were less apparent. The height of the microvillar layer at this level remained unchanged. However, the perimeter of the upper posterior region was approximately twice that of the anterior region. In the lower posterior end of the intestine just anterior to the rectum and caudal to the reproductive tissue the intestine assumed a dorsoventrally flattened appearance and the perimeter of the gut in this region was some three times greater than the anterior region. The height of the epithelial cells in the lower posterior region was 35-50 μ. Histochemically the density of secretory granules and the number of blebs was described as "slight" in this region. Also, the appearance of the microvillar layer maintained a constant height of 16-18 μ but appeared much coarser in the lower posterior region.

Carpenter (1952) also measured the relative activity of various hydrolytic digestive enzymes from cellular homogenates of the anterior, mid and posterior regions of the intestine. From this series of

measurements she determined that all the enzymes studied (e.g., dipeptidases, proteinase, maltase, lipase, and amylase) exhibited their greatest hydrolytic activity in the most anterior region of the intestine. These measurements agree with the histochemical appearance and intensity of secretory activity in the epithelial cells along the length of the intestine. The differences in histological appearance, histochemical staining characteristics, and the relative difference in activities of the hydrolytic enzymes, indicate that the function of the anterior region of the gut is primarily secretory while the posterior region plays a more absorptive role. Using histochemical methods, Lee (1962) studied the distribution of esterase enzymes in frozen sections from various regions of whole Ascaris suum. He showed that the cells of the intestine gave a strong positive reaction for esterase along its entire length. The esterase appeared to be in the form of small granules which were concentrated in the basal two-thirds of the cell. In some cells there were large masses of esterase activity described as merocrine secretions. These secretions are liberated from the cell through the plasma cap and microvillar layer into the lumen of the intestine. Lee (1962) indicated that although esterase activity is distributed throughout the whole intestine, the cells showing merocrine secretions are most numerous in the anterior part of the intestine. He suggested that the anterior region is more actively involved in secretion of enzymes to carry out extracellular digestion than other regions. These observations are similar to Carpenter's (1952) findings.

Studies with the electron microscope have revealed several interesting details concerning the fine structure of the epithelial cells of the intestine of Ascaris. First, Kessel, et al. (1961) described the apical

bacillary layer to be composed of minute cylindrical projections called microvilli. This region is currently referred to as the brush border. The microvilli are fingerlike projections of the plasma membrane measuring 6-7 μ long and 0.08-0.1 μ in diameter. The area just beneath the brush border is the terminal web which shows electron dense material corresponding to the terminal bars or tight junctions associated with most epithelium. At a much higher magnification and with better resolution Sheffield (1964) showed that the microvilli contain numerous tubular filaments that extend their rootlets into the terminal web of the cell. Furthermore, surrounding the outer surface of each microvilli is a fine network of extracellular material commonly referred to as the fuzzy coat or glycocalyx. There was no evidence to support the presence of cilia, since basal bodies and the "9 plus 2" arrangement of fibers could not be demonstrated by Sheffield (1952). The presence of secretory blebs surrounded by a plasma membrane were also observed by both investigators.

The function of the microvilli is to increase the surface area of the intestinal epithelium. Assuming that (1) the cell is flat on the luminal surface, (2) the microvilli cylindrical, (3) the spacing between the microvilli constant, and (4) the cell surface a perfect square, Kessel, et al. (1961) estimated that the surface area was increased by a factor of 75. Assuming that the cell's surface is circular, the surface area is increased by a factor of 90. Hence, these values represent the limits for increasing the cell's surface area and would directly increase their absorptive or secretory capacity. Both Kessel, et al. (1961) and Sheffield (1964) frequently demonstrated that branching of the microvilli would increase the absorptive surface area of the epithelial cells even more.

In contrast to the relative heights of the epithelial cells measured by Carpenter (1952) with the light microscope, measurements with the electron microscope by Kessel, et al. (1961) and Sheffield (1964) indicate that the epithelial cells in the anterior region of the intestine are considerably shorter than she (Carpenter, 1952) reported. Their measurements indicate that the dimensions of the epithelial cell, excluding the brush border, are approximately 10 μ wide and 50 μ high. The epithelial cells are attached to a basal lamella having two layers--a thick inner layer of approximately 6 μ and a thinner outer layer of approximately 0.2 μ. The total length of the epithelial cell including the brush border and basal lamella is approximately 63 μ. The basal region of these cells is further characterized by numerous infoldings of the plasma membrane and is similar to the basal structure of the epithelial cells in the mammalian kidney described by Pease (1955).

The cytoplasm contains large rows of glycogen granules that are closely associated with the rough endoplasmic reticulum. The nucleus is located in the basal region of the columnar epithelial cell. Mitochondria are distributed throughout the cytosol but are more abundant in the apical end of the cell just below the terminal web. They are few in number below the nucleus. Mitochondrial morphology is not constant in all cells. Structural variation within a cell and between cells may reflect differences in the physiological activity of the cells at the time of fixation (Sheffield, 1964). The epithelial cells are distinctly separated by their closely associated plasma membranes that are frequently interdigitated along the apical and basal margins. These structures are thought to interlock portions of two adjoining cells and undoubtedly contribute to the tight binding of epithelial cells. Desmosomes located

along the laterial margins of the cell were also observed (Sheffield, 1964).

Studies on the Absorption and Utilization of Sugars

Cuticular Versus Intestinal Absorption. Adult Ascaris are continuously bathed in a liquid diet in the upper small intestine rich in predigested nutrients. However, the cuticle does not function in moving low molecular nutrients such as glucose. Mueller (1929) showed that perfused cylinders of the body wall did not absorb glucose. Cavier and Savel (1952) later confirmed this observation by showing that the body wall of living parasites did not absorb glucose. Studies in vitro showed that the cuticle of Ascaris is permeable to water, some ions, and to certain hydrophilic chemicals (Fairbairn, 1960). There is little doubt that the intestine rather than the cuticle serves as the principle route for the absorption of nutrients (Fairbairn, 1957, 1960; Lee, 1965; von Brand, 1966). Castro and Fairbairn (1969) studied the role of intestinal versus cuticular absorption of glucose in adult Ascaris suum and their results indicated that glucose is absorbed freely from the lumen of the intestine against a concentration gradient but provided no support for any significant uptake of this compound via the cuticle.

Extracellular Digestion of Dissacharides. Since microvilli increase the surface area of the gut, they play an important role in extracellular or membrane digestion of disaccharides and the absorption of free sugars. Gentner, et al. (1972) shows that the disaccharidases, maltase, sucrase, palatinase, and trehalase, are tightly bound to and highly active in isolated brush border preparations from the intestine of Ascaris.

Lactase and cellobiase activity was not found in either cell homogenates or isolated brush border preparations of the intestine. Since the specific activity of the former four enzymes was from 5-10 times more in the brush border fraction than in the cell homogenates, these authors conclude that the disaccharidases are bound to the brush border and are involved in membrane digestion. The advantage of this system is that the hydrolyzed substrate, mainly glucose, can be readily absorbed at the membrane surface where it is formed. Cavier and Savel (1952) report that Ascaris can synthesize glycogen in vitro if either glucose, fructose, sorbose, sucrose or maltose is added to a mineral incubation medium. They also show that added galactose or lactose does not stimulate glycogen synthesis; an observation that could be explained by the absence of lactase activity in intestinal tissue as showed by Gentner, et al. (1972).

Studies on the Mechanism and Requirements for Movement of Materials Across the Intestine. Harpur (1969) showed that the volatile organic acids derived as end products of carbohydrate metabolism are excreted into the lumen of the anterior region and selectively reabsorbed by the mid and posterior regions of the gut before leaving the worm by the feces. This study was the first to provide evidence supporting the hypothesis that the function of the anterior region of the gut is secretory, while the function of the mid-posterior regions is absorptive.

Sanhueza, et al. (1968) studied the movement of several sugars across sac preparations of the mid-gut of Ascaris. They reported that glucose and fructose are rapidly abosrbed from the luminal solution and appear in the pseudocoelomic fluid. The rate of absorption of glucose is controlled partially by a process that is sensitivie to phlorizin and

dependent upon the presence of Na^+ in the lumen. With the conditions they employed *in vitro*, little or no absorption of galactose or 3-O-MG was observed. Their results indicated that specific processes for the glucose transport system exist on the luminal surface of the epithelial cells but that glucose and other hexoses are not moved across the intestine against a concentration gradient. Later, Castro and Fairbairn (1969) showed that isolated ribbons of the intestine rapidly absorbed glucose when the final tissue concentration was some three times that in the luminal solution following one hour incubation. Under these conditions, the ribbons of intestine did not accumulate 3-O-MG against a concentration gradient.

Beames (1971) determined the effect of various gases (95% N_2-5% CO_2, 95% O_2-5% CO_2, 95% air-5% CO_2, and 99+% N_2) and the presence of glucose upon the movement of 3-O-MG, galactose and fructose across sac preparations of ascarid intestine *in vitro*. He showed that 3-O-MG moves from the luminal to the pseudocoelomic fluid (at a rate of 1.29 μmoles/cm^2/hour) when glucose was added as a substrate and the gas atmosphere was 95% N_2-5% CO_2. Furthermore, his data indicated that the movement of 3-O-MG is drastically reduced (0.28-0.56 μmoles/cm^2/hour) when glucose is omitted or when one of the gases other than 95% N_2-5% CO_2 is present in the incubation system. Fructose also moved from the luminal to the pseudocoelomic fluid and its rate of movement was more than doubled by the addition of glucose to the pseudocoelomic fluid. Unlike 3-O-MG and fructose, the movement of galactose was insignificant under similar incubation conditions. It was also shown that the system for moving 3-O-MG across the intestine of *Ascaris* is directional since only 0.18 μmoles moved/cm^2/hour from the pseudocoelomic fluid to the luminal

solution containing glucose. From these studies Beames (1971) concluded that the movement of hexoses across the intestine of Ascaris requires an exogenous energy source such as glucose and the presence of CO_2 in the incubation system. These observations are consistent with the metabolic requirements of Ascaris and he suggested that the small endogenous supply of carbohydrate is rapidly exhausted in vitro. If this is the case, it could explain the glucose requirement he observed. Another alternative suggested by Beames (1971) to explain the exogenous glucose requirement was that the epithelial cells may not be able to mobilize the endogenous glycogen for physiological processes such as the movement of sugars across the intestine. Sanhueza, et al. (1968) indicated that the specificity in the transport of sugars by the intestine of Ascaris is different from the mammalian intestine (Crane, 1960). Furthermore, the specificity of the sugar transport system in Ascaris is different than the sugar absorption mechanism described in the cestode Hymenolepis diminuta. Read (1961) showed that galactose and glucose competitively inhibit each other and that both sugars are actively taken up by this tapeworm. They concluded that glucose and galactose share a common absorption site, and both hexoses are taken up by an active transport process.

CHAPTER III

MATERIALS AND METHODS

Materials

Chemicals

All chemicals used in this study were of the highest purity available. Radioactive 3-0-methyl-^{14}C-D-glucose (^{14}C-3-0-MG) with a specific activity of 5-10 mCuries/mMole was purchased from New England Nuclear Corporation, Boston, Massachusetts, and stored in the refrigerator at 4 C until the seal was broken. Before preparing the working stock solution of ^{14}C-3-0-MG the ethanol solution was evaporated from the ampule with extra dry nitrogen in a water bath maintained at 40 C. The radioactive material was reconstituted in either buffered saline or distilled water so that 1.0 ml of the stock solution contained 10 μCuries of radioactivity. The stock solution was stored at -15 C and allowed to thaw just prior to use.

The chemical and radiochemical purity of ^{14}C-3-0-MG was checked prior to use by the thin-layer chromatography procedure described by Beames (1971). Unlabeled 3-0-MG was purchased from Sigma Chemical Company, St. Louis, Missouri. The chemical purity of unlabeled 3-0-MG was checked by a combination of GLC separation of the trimethylsilyl derivatives (Sweeley, et al., 1963) and the TLC separation procedure described by Beames (1971). The Sigma 3-0-MG was free of any hexose

contaminants.

The metabolic inhibitors ouabain (octahydrate form), iodoacetamide and sodium fluoride were also obtained through Sigma Chemical Company, St. Louis, Missouri. Phlorizin was purchased from Calbiochem Company, Los Angeles, California. The 2,4-dinitrophenol used in this study was a product of the J. C. Baker Chemical Company, Phillipsburg, New Jersey.

Glucose oxidase, sold under the trade name Fermco-Test SFG, was purchased as a commercial clinical preparation from Fermco Laboratories, Chicago, Illinois, and used for the colorimetric determination of glucose as described by the accompanying pamphlet. As noted by the manufacturer this source of glucose oxidase was essentially free of the disaccharidases, maltase and trehalase.

All solutions used for tissue incubation studies were prepared from either refrigerated stock solutions or dry crystalline powders on the day of the experiment. Preparation of solutions in this manner minimized bacterial growth and the formation of breakdown products. Reagents used for colorimetric assays were stored in the refrigerator at 4 C and discarded after their recommended shelf-life had expired. Anthrone reagent was prepared according to the procedure described by Carroll, et al. (1956) and discarded after one month.

Methods

Collection of Animals

Adult female Ascaris suum were collected from the small intestine of infected swine at a local packing house (Wilson and Company, Oklahoma City, Oklahoma). Female worms greater than 20 cm long were selected and rinsed in warm tap water (< 40 C). The rinsed worms were immediately

placed in a warm buffered saline solution (Harper, 1963), maintained between 35 and 39 C, and transported to the laboratory. In one instance, a group of worms was collected and transported to the laboratory in an ice-cold buffered saline solution to determine the total carbohydrate content in the intestine. During collection of these animals special care was exercised not to wash the worms in tap water above 40 C, and to avoid collecting worms in the drain trap. The worms in the latter case were often exposed to hot running tap water for an unknown period of time. Worms exposed to temperatures > 40 C usually contained soft intestines which were difficult to handle and considered undesirable for experimental purposes.

Upon arrival at the laboratory the worm container was placed in an incubation oven and maintained in buffered saline solution at 37 C. The worms were used within 2-4 hours.

General Procedure for Preparing and Incubating Intestinal Sac Preparations

Individual female worms ranging from 27 to 36 cm in length were selected from the holding solution, blotted dry on paper toweling and opened longitudinally on the dorsal side with scissors to expose the intestine. At this point, worms were rejected for experimental purposes if (1) the intestine was mottled black or dark brown, or (2) both horns of the uterus were not fully developed and extended with eggs. After careful inspection, the intestine was cut free at the anterior and posterior ends and separated from the surrounding reproductive tissue. The reproductive tissue was discarded and the intestine repositioned in the open body cavity previously moistened with buffered saline. Sac

preparations were prepared according to the procedure described by Beames (1971).

The lumen of the intestine was filled with the appropriate buffered saline solution containing a known concentration of 3-O-MG (15,960 dpm/μmole) and was briefly inspected for leaks by blotting on Whatman No. 1 filter paper. The preparation of intestinal sacs required an average of 4-5 minutes from the time the worms were slit open until the sacs were placed in shell vials (35 x 19 mm). The vials contained 1.0 ml of the desired incubation media and were incubated under the conditions described by Beames (1971) unless otherwise indicated. The incubation media basically consisted of a buffered saline solution (final pH 7.1-7.4) with or without 0.04 M glucose. In some experiments metabolic inhibitors or other sugars were added to the incubation media. Specific details for each experiment are described in the appropriate table or figure presented in the Results.

At the end of each experiment the sacs were removed from the incubation vials. Ten μl samples of the incubation media were spread on planchets, dried and the radioactivity measured using a Nuclear Chicago gas-flow planchet counter. Appropriate volumes of the standard radioactive working solution were counted along with each set of samples to correct for efficiency changes. The planchet counter maintained an average counting efficiency for ^{14}C material of 30 $\pm$ 2%.

The luminal solution was drained from the sac preparation and the surface area determined by measuring the length and width of the segment to the nearest 0.5 mm. In those experiments where the tissue weight was determined, the excess water was drained on hard cracked ice and the tissue weighed to the nearest 0.1 mg. The dry weight was determined after

drying the tissue in an oven at 100 C to constant weight.

Procedure for Preparing Sac From the Anterior, Mid and Posterior Regions of the Intestine

A series of experiments were designed to determine if various regions of the intestine of Ascaris had the capacity to move 3-0-MG from the luminal to the pseudocoelomic solution at relatively different rates. For this study it was necessary to remove the entire length of intestine from the worm free of damage and make all three sac preparations from one intestine. Sac preparations from equal thirds of the intestine, representing the anterior, mid and posterior regions, respectively, were prepared in the following manner. The intact isolated intestine was positioned along a straight line divided into equal thirds on a glass plate moistened with buffered saline. Silk sutures (No. 00) were placed at appropriate points along the intestine. The entire lumen was flushed with a 0.04 M ^{14}C-3-0-MG solution then the most anterior suture was tied and the intestine filled. The remaining sutures were tied securely and the various regions were separated by cutting the tissue between adjacent sutures. The sac preparations from each region were individually rinsed, blotted dry and placed in individual incubation vials. The sacs were incubated under the conditions described by Beames (1971). At this point, if any one of the prepared sacs leaked the entire group was discarded and another group prepared. In this manner it was possible to make statistical comparisons of the rate of movement of 3-0-MG among the various regions as treatment groups and each intestine represented a complete replicate. Immediately following the experiments the surface area was measured and the radioactivity of the incubation media determined as

described previously.

Incubation of Intestinal Tissue and Analysis of Total Carbohydrate

Sections of the posterior two-thirds of the intestine ranging from 5-8 cm in length were removed and immediately placed in ice-cold buffered saline solution (Harpur, 1963). The isolated sections were then slit open by gently pulling the tissue over the edge of corneal eye scissors. The ribbons of the intestine were rinsed in three changes of ice-cold buffered saline solution. Three ribbons of tissue were placed into a 25 ml Erlenmeyer flask containing 5.0 ml of buffered saline (pH 7.4) with or without 0.04 M glucose. All flasks were gassed for 2 minutes by bubbling a 95% N_2-5% CO_2 gas mixture through the incubation media, sealed with serum vial rubber stoppers and incubated at 37 C in a metabolic shaker for either 0, 5, 10, 20, 40, or 80 minutes. At the end of the incubation periods the tissue was removed, rinsed in three changes of ice-cold buffered saline, drained on crushed ice and placed in a tared, ice-cold test tube containing 5.0 ml of 5% tricholoroacetic acid (TCA, w/v). The wet weight of the tissue was determined gravimetrically. The tissue was homogenized in a prechilled 10 ml Potter-Elvehjem tissue grinder in the 5.0 ml of 5% TCA solution. The homogenate was centrifuged for 10 minutes at 400 x g at room temperature and the supernatant was decanted and saved. The precipitate was resuspended in 2.0 ml of 5% TCA by stirring the suspension with a Vortex mixer and centrifuged as mentioned previously. The supernates were combined and adjusted to a known volume. Aliquots of the supernate were removed and the TCA soluble carbohydrate was determined by the anthrone method described by Carroll, et

al. (1956). The reducing sugars were determined from aliquots of the supernatant by the Nelson's alkaline copper reagent test as described by Nelson (1944). Glycogen was precipitated from aliquots of the supernate with 5 volumes of 95% ethanol, and the glycogen determined colorimetrically by the anthrone method described by Carroll, et al. (1956).

Extraction of Radioactive 3-O-Methyl-D-Glucose From Intestinal Tissue

The effect of unlabeled sugars on the absorption of 0.01 M ^{14}C-3-O-MG from the luminal solution was determined by measuring the radioactivity extracted from the tissue. At the end of the incubation period sac preparations were removed from the incubation vials, the luminal solution drained, and the surface area determined as described previously. The silk suture was removed, the intestine was placed in ice-cold buffered saline, sliced, drained on crushed ice and placed in a tared, ice-cold test tube containing 2.0 ml of 70% ethanol. The wet weight of the tissue was determined gravimetrically. The tissue was extracted for 24 hours, a 0.5 ml sample of the ethanol extract was dried and the radioactivity determined in a Nuclear Chicago gas-flow counter. The concentration of 3-O-MG in the tissue was calculated from the following formula:

$$X = \frac{\text{total cpm} \div \text{specific activity}}{\text{mg tissue wet wt} \times K}$$

where X is the concentration of 3-O-MG in moles/liter, the specific activity of the working solution is expressed as the cpm/μmole of 3-O-MG, and K is the constant 0.877 for percent tissue water used to determine the μl of water in the tissue sample.

Statistical Design and Analysis

Experiments were arranged in a completely randomized block design consisting of 3 to 10 treatment groups with a total of 4 to 11 replicates for each treatment. All data were subjected to statistical analysis by either the Students t test or analysis of variance, and when found to be significant, differences in treatment means were detected by Duncan's new multiple-range test (Steel and Torrie, 1960). Linear regression lines were computed with an Olivetti Underwood Programma 101 computer.

CHAPTER IV

RESULTS

Movement of 3-0-Methyl-D-Glucose Across Various Regions of the Intestine

Results of the movement of 3-0-MG across the anterior, mid and posterior regions of the intestine of Ascaris are presented in Table I. The rate of movement of 3-0-MG across the various regions of the gut are expressed on the basis of surface area and tissue dry weight. From casual inspection of data it is obvious that the rate of movement of 3-0-MG across the intestine progressively increases from the anterior to the posterior region. Differences between means were tested for significance at the 0.01 and 0.05 probability levels by analysis of variance and Duncan's new multiple range test. On the basis of surface area the mean values for the mid and posterior regions are significantly greater ($P < 0.01$) than the mean for the anterior region. The posterior region shows a slightly higher mean rate of movement than does the mid region. However, the difference between the means is not statistically significant ($P > 0.05$). When the data are expressed on the basis of mg tissue dry weight, the mean rates of movement of 3-0-MG for the mid and posterior regions are significantly greater ($P < 0.01$) than the mean for the anterior region. Further, the mean for the posterior region is significantly greater ($P < 0.01$) than the mean of the mid region.

TABLE I

THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE ANTERIOR, MID AND POSTERIOR REGIONS OF THE INTESTINE[a]

Criteria	Region of Intestine		
	Anterior	Mid	Posterior
µmoles/cm^2/hr	0.48 ± 0.06[b]	0.79 ± 0.06	0.93 ± 0.09**
µmoles/mg dry wt/hr	0.14 ± 0.02	0.32 ± 0.04**	0.44 ± 0.03**

[a]The luminal solution was buffered saline (pH 7.4) with 0.04 M 3-O-methyl-^{14}C-D-glucose (15,960 dpm/µmole). The incubation media was 1.0 ml of buffered saline (pH 7.4) containing 0.04 M glucose. Incubation time was 60 min; gas phase 95% N_2-5% CO_2; temperature 37 C.

[b]Each value represents the mean of eleven observations ± standard error.

**P < 0.01.

The Effect of Incubation Time on the TCA Soluble Carbohydrate of Intestinal Tissue Incubated With or Without Exogenous Glucose

Results of the determinations of the TCA soluble carbohydrate of ribbons of intestine incubated for various periods of time with or without exogenous glucose are presented in Figure 1. When intestinal tissue is incubated without glucose there is a rapid depletion of the TCA soluble carbohydrate. The endogenous carbohydrate of tissue incubated for 5 minutes without glucose is some 62% of the zero time control value of 3.10 mg of TCA soluble carbohydrate/g wet weight of tissue. After 10 minutes of incubation the endogenous carbohydrate of the tissue is further reduced to 32 percent, and after 80 minutes incubation it is only 19 percent of the zero time control value. In contrast, the total carbohydrate content of tissue incubated for 5 minutes with 0.05 M glucose is some 2 times greater than the zero time control value of 3.7 mg total carbohydrate/g wet weight of tissue. This rapid increase in the tissue carbohydrate is more or less maintained throughout the 80 minute incubation when glucose is present in the incubation medium.

The Effect of Incubation Time on the Glycogen and Reducing Sugar of Intestinal Tissue Incubated With Glucose

The change in the glycogen and total reducing sugar of intestinal tissue incubated in 0.04 M glucose for various periods of time is shown in Table II. TCA soluble carbohydrate of the tissue is due to an initial rapid uptake or 2-fold increase in the reducing sugar within the first 5-10 minutes of incubation. This increase is maintained throughout 80

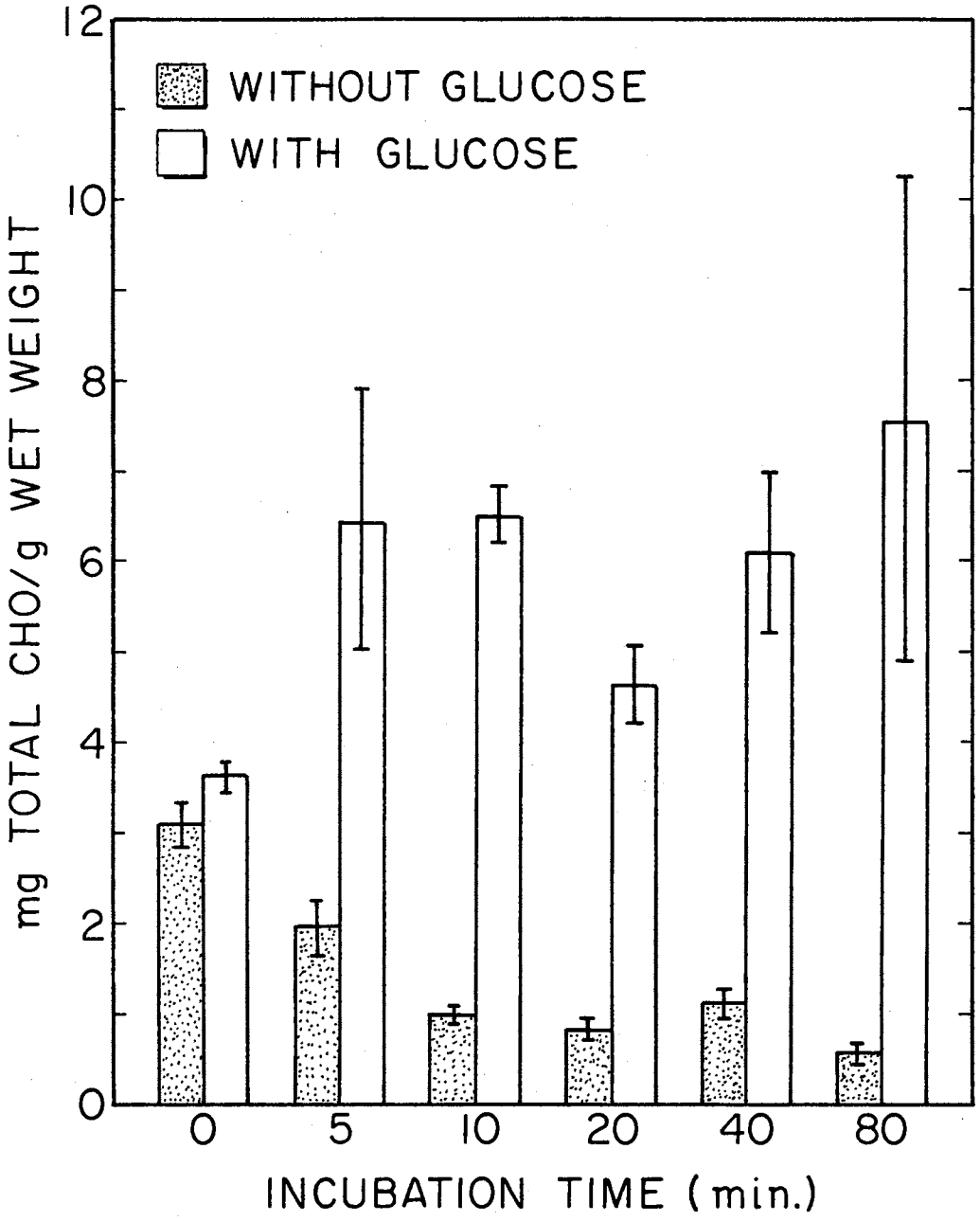

Figure 1. The Effect of Incubation Time on the TCA Soluble Carbohydrate of Intestinal Tissue Incubated With or Without Exogenous Glucose. All values are expressed as glucose.

TABLE II

THE EFFECT OF INCUBATION TIME ON THE GLYCOGEN AND REDUCING SUGAR OF INTESTINAL TISSUE INCUBATED IN GLUCOSE

Incubation Time (minutes)	mg/g wet weight				
	TCA Soluble CHO	Glycogen	% Increase in Glycogen	Reducing Sugar	% Increase in Reducing Sugar
0	2.84[a]	0.62	---	1.97	---
5	5.54	0.81	31	4.22	215
10	6.22	1.71	175	4.30	220
20	5.14	0.44	0	5.12	280
40	5.61	1.61	160	5.00	270
80	7.05	3.26	426	3.71	177

[a]Each value represents the mean of two observations at each time period. All values are expressed as glucose. Tissue strips from the posterior two-thirds of the intestine were incubated in a 0.04 M glucose buffered-saline solution (pH 7.4) at 37 C in a 95% N_2-5% CO_2 gas atmosphere for the desired time period. All measurements were determined on intestinal tissue from two of the experiments reported in Figure 1.

minutes of incubation. Further, the glycogen remains fairly constant for the first 5 minutes and then rapidly increases between 10 and 80 minutes. At the end of 80 minutes of incubation a 4-fold increase in glycogen was measured.

The total carbohydrate of intestinal tissue from 4 female worms collected and maintained on ice was determined upon arrival at the laboratory. The mean TCA soluble carbohydrate $\pm$ SE was 3.14 $\pm$ 0.2 mg per g wet weight of tissue.

The Influence of Preincubation Upon the Movement of 3-0-Methyl-D-Glucose Across the Intestine

Since the endogenous carbohydrate of the intestine is maintained, and in fact increases when the tissue is incubated with glucose, a series of experiments were carried out to determine the influence of the endogenous carbohydrate level upon the movement of 3-0-MG. The results of this series of experiments are presented in Figure 2. The rate of movement of 3-0-MG across the intestine is essentially linear when sac preparations are preincubated for 10 minutes and incubated with glucose. At the end of 80 minutes incubation 2.3 µmoles of 3-0-MG/cm^2 were moved across the intestine. When sac preparations are preincubated with 0.04 M glucose for 10 minutes and then incubated without glucose the rate of movement of 3-0-MG is linear and equal to the rate of the control with glucose during the first 10 minutes of incubation. After 10 minutes, the rate of 3-0-MG movement begins to decline and between 20-80 minutes of incubation it becomes equal to the rate of movement shown for the control without glucose.

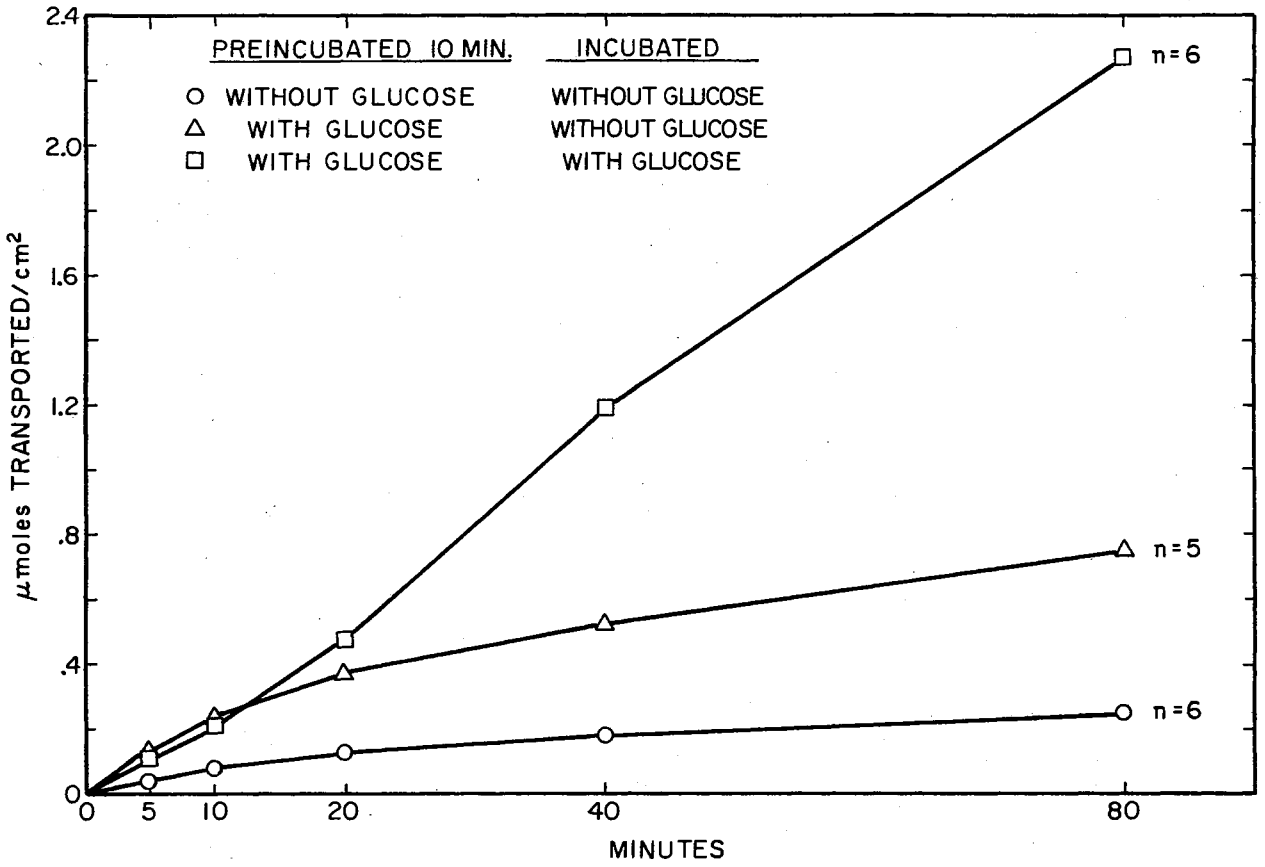

Figure 2. The Influence of Preincubation Upon the Movement of 3-O-Methyl-D-Glucose Across the Intestine. The luminal solution was buffered saline (pH 7.2) with 0.04 M ^{14}C-3-O-MG (32,050 dpm/μmole). The preincubation and incubation media was 1.0 ml of buffered saline (pH 7.2) with or without 0.04 M glucose as indicated. Sacs were preincubated and incubated at 37 C with a gas phase of 95% N_2-5% CO_2. During the 80 min incubation a 10 μl aliquot of the incubation media was taken at the end of each time period.

The Influence of Exogenous Glycogen, Trehalose and Maltose Upon the Movement of 3-O-Methyl-D-Glucose Across the Intestine

Experiments were carried out to determine if the movement of 3-O-MG across the intestine could be facilitated when either glycogen, trehalose or matose was added to the incubation media. For comparison, measurements were carried out with sac preparations incubated with or without glucose in the incubation media. These results are presented in Table III. When either 0.4% glycogen, or 0.04 M trehalose or maltose was added to the incubation media the movement of 3-O-MG is only slightly higher than the control without sugar. However, statistical analysis of these data using the Students t test indicates that the difference between the means is not significant ($P > 0.05$). Increasing the concentration of glycogen to 0.8% in the incubation media does not increase the rate of movement of 3-O-MG across the intestine. On the other hand, the mean rate of 3-O-MG movement across sac preparations incubated in buffered saline with 0.04 M glucose added is significantly higher ($P < 0.05$) than the control rate without glucose.

The Effect of pH Upon the Movement of 3-O-Methyl-D-Glucose Across the Intestine

Experiments were carried out to determine the effect of the pH of the luminal solution upon the rate of movement of 3-O-MG across intestinal sac preparations incubated in the presence or absence of 0.04 M glucose. The pH of the luminal solution was determined electrometrically

TABLE III

THE EFFECT OF VARIOUS SUGARS UPON THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE INTESTINE[a]

Additions to Incubation Media	n	moles 3-O-Methyl-D-Glucose/cm^2/hr (± S.E.)
Glucose (40 mM)	6	1.36 ± 0.28*
Trehalose (40 mM)	12	0.42 ± 0.10
Glycogen (0.4%)	6	0.52 ± 0.10
Glycogen (0.8%)	6	0.44 ± 0.15
Maltose (40 mM)	7	0.55 ± 0.07
None	8	0.29 ± 0.10

[a]The luminal solution was buffered saline (pH 7.1) with 0.04 M 3-O-methyl-^{14}C-D-glucose (15,960 dpm/μmole). The incubation media was 1.0 ml of buffered saline (pH 7.1) with the desired sugar added as indicated. Sacs were prepared from the posterior region and incubated for 1 hour at 37 C in a 95% N_2-5% CO_2 gas atmosphere.

*$P < 0.05$.

before and after incubation. The pH of the luminal solution after incubation did not change more than ± 0.1 of a pH unit from the initial pH. The effect of the pH of the luminal solution upon the rate of 3-0-MG movement across the intestine is presented in Figure 3. The upper curve represents the effect of pH on the net rate of movement of 3-0-MG when glucose is present, and the lower line shows the effect of pH on the movement of 3-0-MG when glucose is omitted from the incubation system. The results indicate that when glucose is present the net movement of 3-0-MG sharply increases from pH 5.5, to an optimum rate of 4.3 moles 3-0-MG/cm^2/hr at pH 6.5, and that the net movement rapidly declines as the pH of the luminal solution increases further. There is little movement of 3-0-MG when glucose is omitted from the incubation system. However, the movement that does occur shows the same pH optimum as the system with glucose. The luminal fluid in the posterior two-thirds of the intestine was collected separately from several adult worms and the pH of the gut contents determined electrometrically (see Table IX in Appendix). The average pH of the gut contents is 6.75 ± 0.08 and ranges from a low of 6.10 to a high of 7.28.

The Effect of Temperature Upon the Movement of 3-0-Methyl-D-Glucose Across the Intestine

Results of the effect of temperature upon the movement of 3-0-MG across the intestine of Ascaris are presented in Table IV. From the determinations at 25 through 45 C it is obvious that the movement of 3-0-MG increases with increasing temperature when exogenous glucose is present in the system. There is no increase in the movement of 3-0-MG with increasing temperature when glucose is omitted from the system.

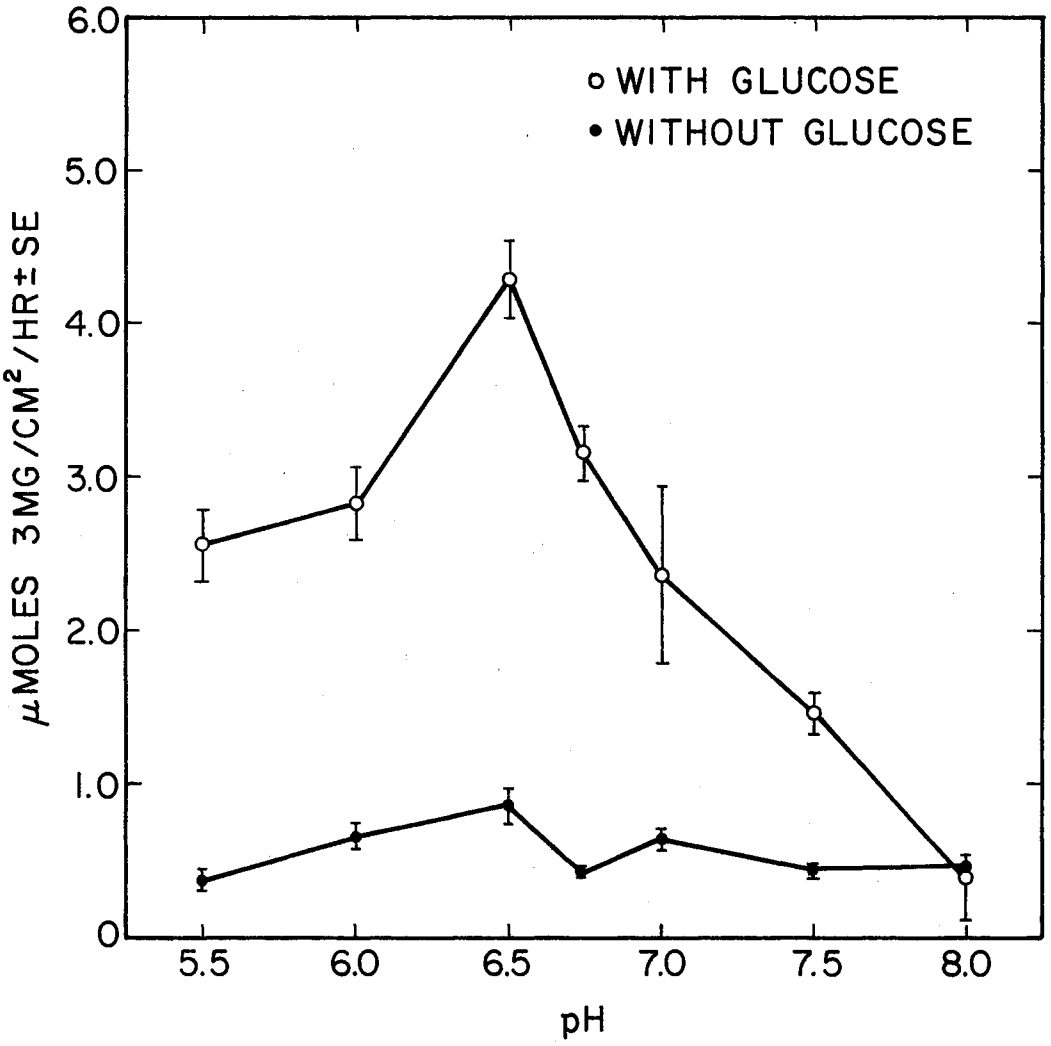

Figure 3. The Effect of the Luminal pH Upon the Movement of 3-O-Methyl-D-Glucose Across the Intestine. The luminal solution was 0.15 M phosphate buffer adjusted to the desired pH as indicated. Incuabtion media was buffered saline (pH 7.1) with or without 0.04 M glucose. Each value represents the mean of four measurements ± SE.

At 20 C there is little movement of 3-0-MG with or without the addition of glucose. At 60 C the sac preparations become opaque and hardened shortly after they are placed in the incubation media. Relatively large amounts of 3-0-MG move across the intestine at this high temperature, but there is little difference in the rate of movement with or without exogenous glucose in the system.

From 25 through 45 C the increase in the movement of 3-0-MG is a logarithmic function as is shown in Figure 4. The Q_{10} values for the increase in movement of 3-0-MG with each 5 C rise in temperature from 25 through 45 C range from 2.85 to 3.17 (Table IV). When the values in Figure 3 are expressed in an Arrhenius plot of the ln flux versus 1/T, a straight line results (Figure 5) with a slope of -10033. The energy of activation calculated from this slope is 19938 calories/mole of 3-0-MG that moves across the intestine.

Saturation Kinetic Studies

When glucose is present in the incubation system, increases in the substrate (3-0-MG) concentration result in a linear rise in the movement of 3-0-MG across the intestine. The system does not appear to saturate. However, if the rates of movement are corrected for the "diffusion" component, as determined by incubating sac preparations in the absence of glucose, a net rate of movement of 3-0-MG representing the glucose-dependent 3-0-MG transport system is obtained for each substrate concentration as shown in Figure 6. The data indicate that the system for moving 3-0-MG across the intestine can be saturated.

Analysis of the data in Figure 6 by the Hofstee method permits the graphic expression of the results shown in Figure 7. The line drawn

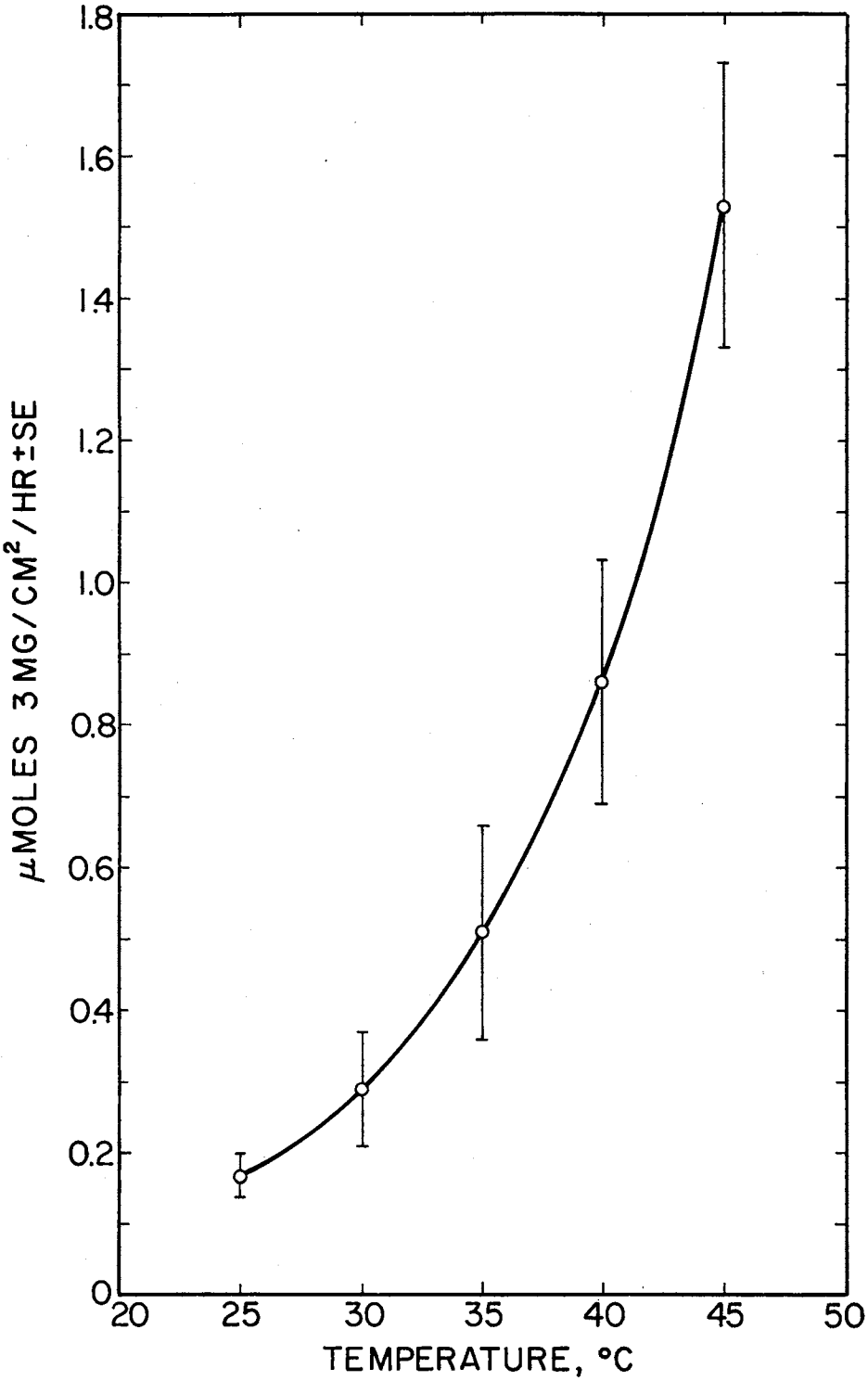

Figure 4. The Influence of Temperature Upon the Movement of 3-O-Methyl-D-Glucose Across the Intestine

TABLE IV

THE EFFECT OF INCUBATION TEMPERATURE UPON THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE INTESTINE[a]

Incubation Temperature °C	μmoles 3-O-MG/cm^2/hr±SE			Temperature Range	Q_{10}
	With Glucose	Without Glucose	Difference		
20	0.04 ± .01 (9)	0.02 ± .01 (4)	0.02 ± .01		
25	0.28 ± .03 (7)	0.11 ± .02 (4)	0.17 ± .03		
30	0.34 ± .08 (9)	0.05 ± .01 (4)	0.29 ± .08	25-30	2.91
35	0.65 ± .15 (10)	0.14 ± .03 (5)	0.51 ± .15	30-35	3.16
40	1.06 ± .17 (8)	0.20 ± .04 (5)	0.86 ± .17	35-40	2.85
45	1.64 ± .20 (10)	0.11 ± .03 (5)	1.53 ± .20	40-45	3.17
60	1.40 ± .02 (10)	1.26 ± .04 (4)	0.14 ± .02		

[a]Intestinal sacs were prepared from the posterior region of the intestine. The luminal solution was buffered saline (pH 7.1) with 0.04 M 3-O-methyl-^{14}C-D-glucose (15,960 dpm/ mole). Incubation media was 1.0 ml of buffered saline (pH 7.1) with or without 0.04 M glucose for 1 hour at the desired temperature. The gas phase was 95% N_2-5% CO_2. The number of determinations is in ( ).

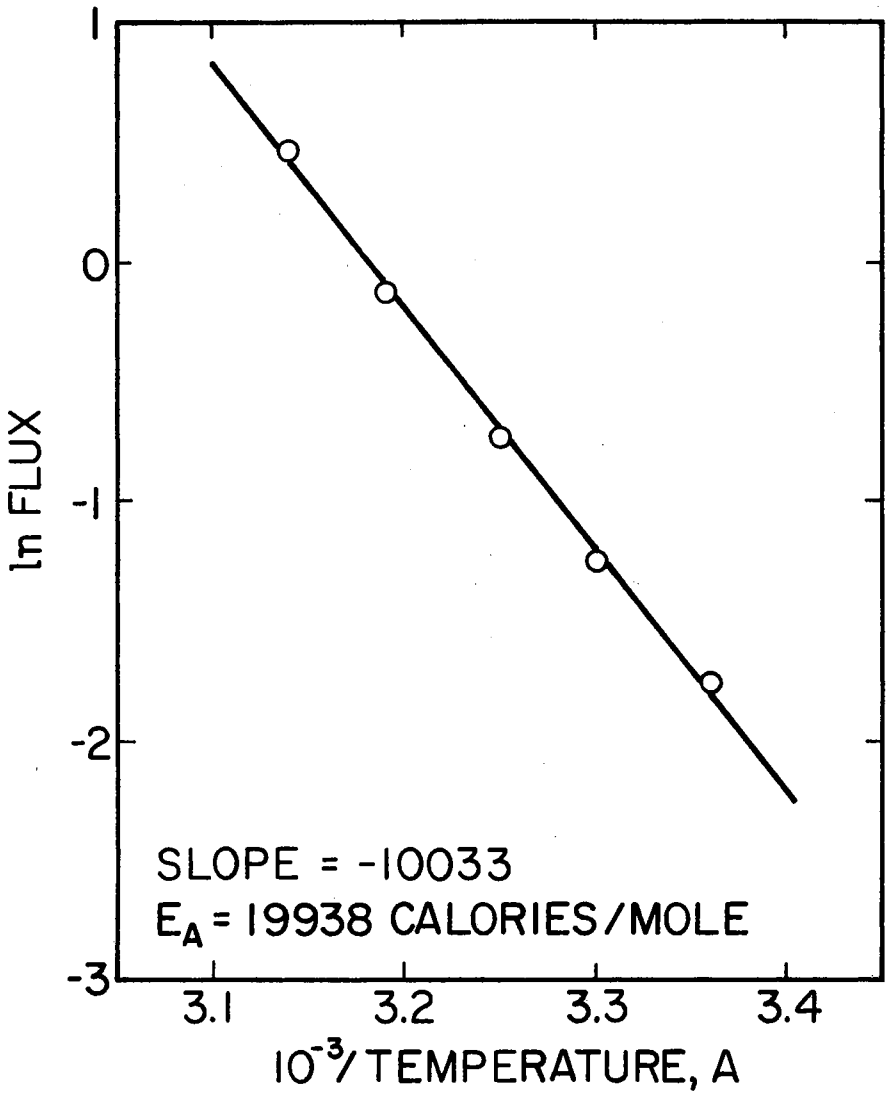

Figure 5. Arrhenius Plot of the ln Flux Versus the Reciprocal of the Absolute Temperature

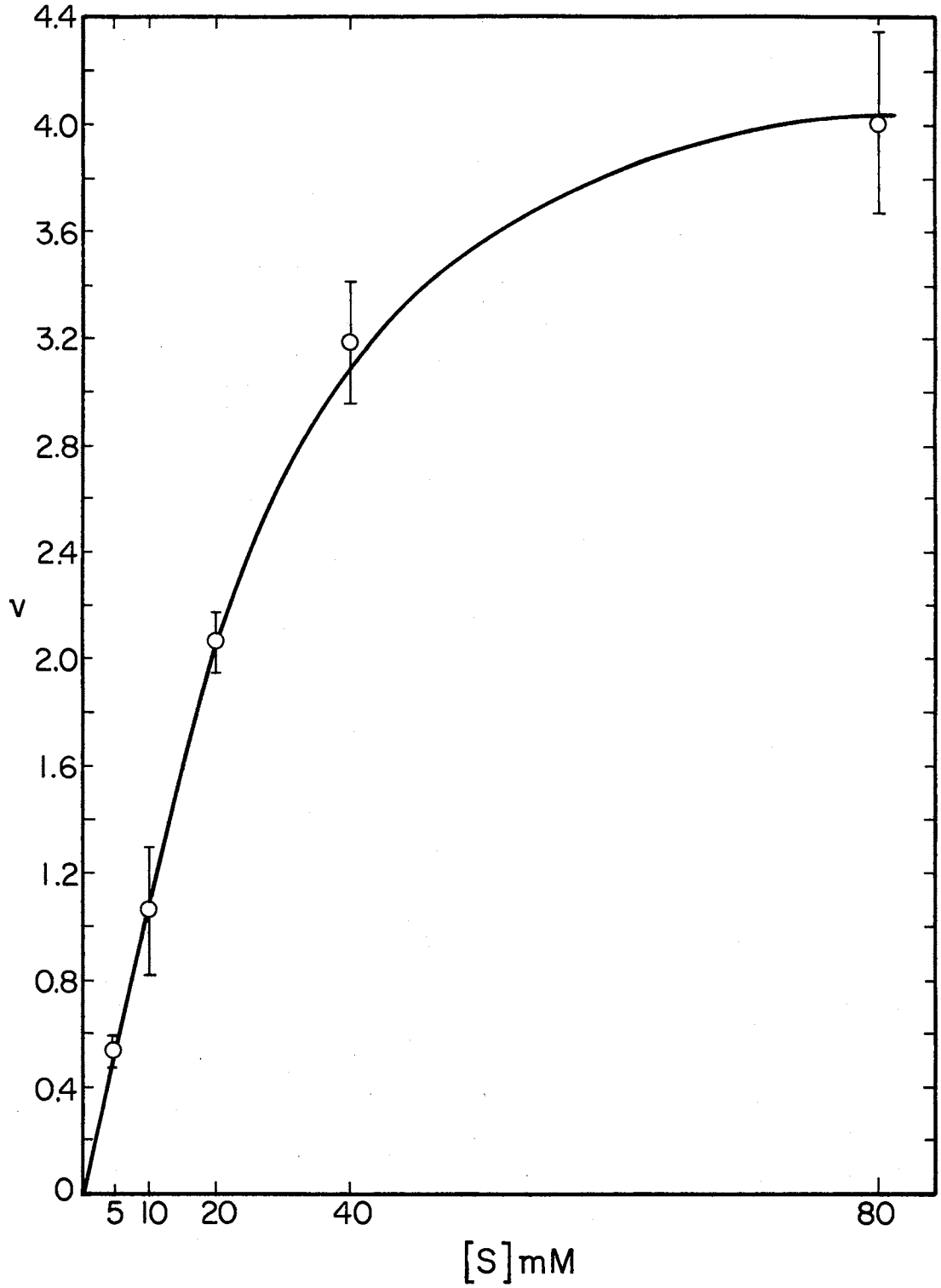

Figure 6. Substrate Saturation Curve for the Movement of 3-O-MG Across the Intestine

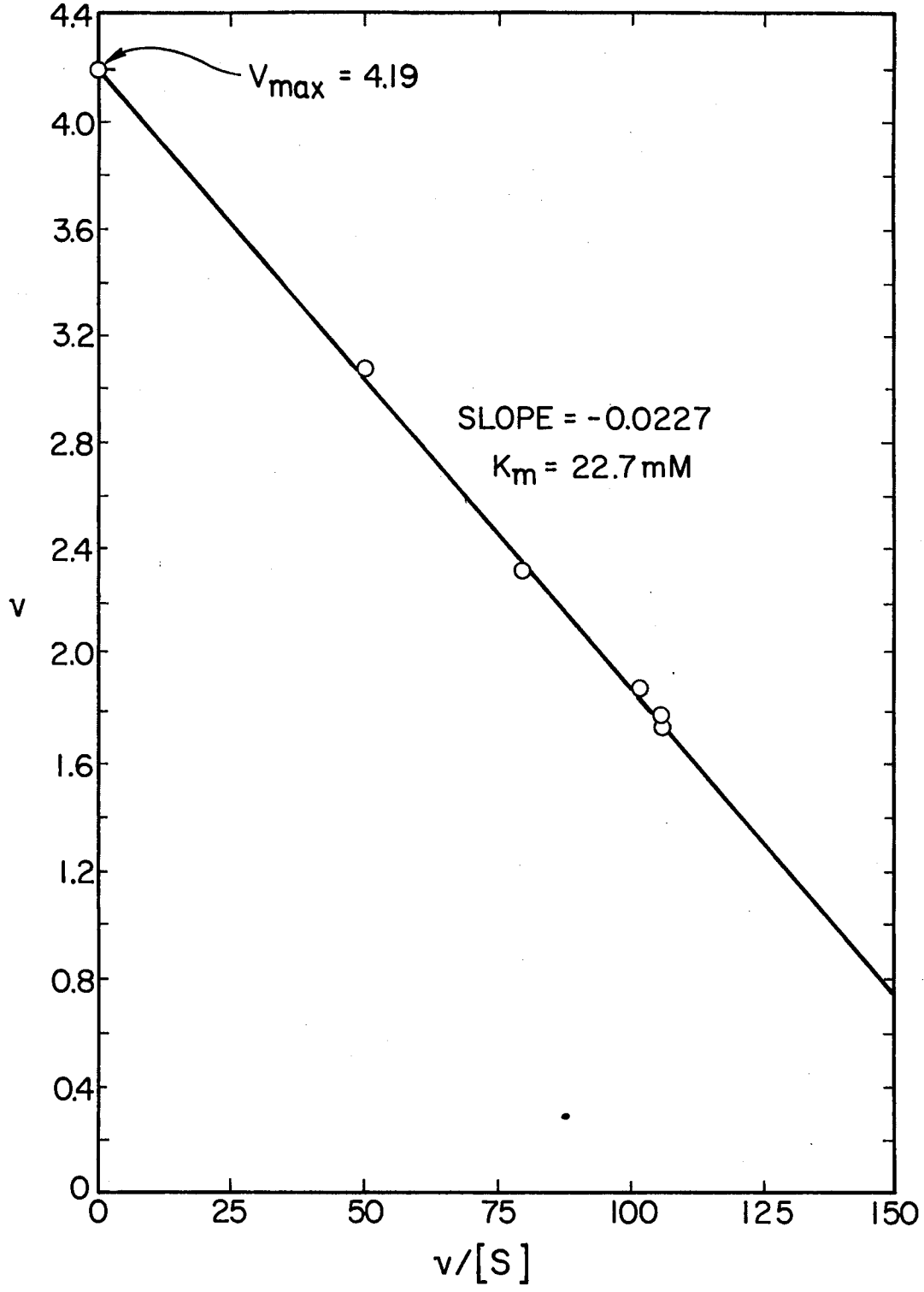

Figure 7. Hofstee Method of Plotting the Substrate Saturation Curve

through the points is determined by linear regression analysis. The slope of the line is -0.0227 and the K_m for the 3-O-MG transport system is 22.7 x 10^{-3} M 3-O-MG. The intercept of the line with the ordinate is 4.19 μmoles/cm^2/hr and is the V_{max} for the system.

Competitive Sugar Studies

Since the system for moving 3-O-MG across the intestine of Ascaris shows saturation kinetics, a series of experiments in vitro were run to determine the effect of unlabeled sugars upon the movement and uptake of ^{14}C-3-O-MG from the lumen. The effect of unlabeled sugars at an inhibitor-to-substrate ratio (I/S) of 1.0 and 5.0 was tested.

The Effect of Unlabeled Sugars Upon the Movement of 3-O-Methyl-^{14}C-D-Glucose Across the Intestine

The results of the effect of unlabeled sugars upon the movement of ^{14}C-3-O-MG across the intestine are presented in Table V. To serve as a control, sac preparations without unlabeled sugars in the luminal solution gave a mean rate of ^{14}C-3-O-MG movement of 0.89 μmoles/cm^2/hr. In general, these data show that the addition of inhibitor sugars to the luminal solution result in a decreased rate of ^{14}C-3-O-MG movement and that movement of ^{14}C-3-O-MG decreased further by increasing the inhibitor sugar concentration. The percent inhibition of ^{14}C-3-O-MG movement is greater with some sugars than with others. At an I/S ratio of 1.0 the movement of ^{14}C-3-O-MG is inhibited some 55% with unlabeled D-xylose. It is inhibited some 35-45% with unlabeled D-fructose, D-glucose, 3-O-methyl-D-glucose, and D-ribose, and some 10-24% by unlabeled D-glucosamine or D-mannose. Unlabeled D-galactose at the lower concentration did not

TABLE V

THE EFFECT OF UNLABELED SUGARS UPON THE MOVEMENT OF 3-O-METHYL-^{14}C-D-GLUCOSE ACROSS THE INTESTINE[a]

Inhibitor	I/S = 1		I/S = 5	
	µmoles/cm^2/ hr ± SE	Percent Inhibition	µmoles/cm^2/ hr ± SE	Percent Inhibition
D-Fructose	0.58 ± .15[b]	35	0.17 ± .03	81
D-Glucose	0.49 ± .15	45	0.18 ± .03	80
3-O-Methyl-D-Glucose	0.49 ± .10	45	0.30 ± .09	66
2-Deoxy-D-Glucose	0.62 ± .09	30	0.32 ± .05	64
D-Xylose	0.40 ± .17	55	0.37 ± .17	59
D-Glucosamine	0.80 ± .20	10	0.46 ± .10	48
D-Mannose	0.68 ± .13	24	0.49 ± .05	45
D-Galactose	1.01 ± .10	0	0.64 ± .11	28
D-Ribose	0.59 ± .08	44	0.73 ± .09	18

[a]The luminal solution was either 10 mM unlabeled sugar plus 10 mM 3-O-methyl-^{14}C-D-glucose (I/S = 1), or 50 mM unlabeled sugar plus 10 mM 3-O-methyl-^{14}C-D-glucose (I/S = 5) in buffered saline (pH 7.1). Sacs were prepared from the posterior region of the intestine and incubated in 1.0 ml of buffered saline (pH 7.1) containing 0.04 M glucose. Each sac was incubated for 1 hour at 37 C in a 95% N_2-5% CO_2 gas atmosphere. The specific activity of the luminal solution was 22,275 dpm/µmole 3-O-MG.

[b]Each value represents the mean of 6 observations ± standard error.

inhibit the movement of ^{14}C-3-O-MG across the intestine. When the I/S concentration was increased to 5.0 the rate of movement of ^{14}C-3-O-MG was inhibited some 80% with unlabeled D-glucose or D-fructose, and by some 60% with either unlabeled 3-O-methyl-D-glucose, 2-deoxy-D-glucose or D-xylose. The presence of unlabeled D-glucosamine or D-mannose in the luminal solution at high inhibitor concentrations only inhibited the rate of ^{14}C-3-O-MG movement by some 40%, while unlabeled D-galactose and D-ribose only inhibited the rate of movement of ^{14}C-3-O-MG some 28% and 18%, respectively.

The Effect of Unlabeled Sugars on the Uptake of 3-O-Methyl-^{14}C-D-Glucose in Intestinal Tissue

In the same series of experiments the intestinal tissue was extracted with ethanol after incubation and the total radioactivity was used to determine the effect of unlabeled sugars on the absorption of ^{14}C-3-O-MG. Table VI shows the results of adding unlabeled sugars to the luminal solution on the uptake of ^{14}C-3-O-MG in the tissue. When only 10 mM ^{14}C-3-O-MG was present in the luminal solution the tissue concentration is 7.5 mM 3-O-MG. The presence of unlabeled D-mannose or D-glucose at an I/S concentration of 1.0 decreased the uptake of ^{14}C-3-O-MG some 50 to 75%, respectively. At the same low inhibitor concentration, the addition of either unlabeled D-fructose or D-galactose only inhibited the uptake of ^{14}C-3-O-MG from the lumen of the intestine some 11-29%, respectively. At an I/S concentration of 5.0, unlabeled 3-O-MG decreased the uptake of ^{14}C-3-O-MG some 55% while D-mannose, D-glucose, D-fructose and 2-deoxy-D-glucose results in a much greater reduction in the uptake of ^{14}C-3-O-MG. Increasing the concentration of unlabeled D-galactose in the luminal

TABLE VI

THE EFFECT OF UNLABELED SUGARS ON THE UPTAKE OF 3-O-METHYL-^{14}C-D-GLUCOSE IN INTESTINAL TISSUE[a]

Inhibitor	I/S = 1		I/S = 5	
	Tissue Concentration mM 3MG	Percent Inhibition	Tissue Concentration mM 3MG	Percent Inhibition
D-Fructose	6.7 ± 1.6[b]	11	2.4 ± 0.4	68
D-Glucose	1.9 ± 0.4	75	2.1 ± 0.4	72
3-O-Methyl-D-Glucose	4.0 ± 0.5	47	3.4 ± 0.3	55
2-Deoxy-D-Glucose	4.5 ± 1.0	40	2.7 ± 0.3	64
D-xylose	4.6 ± 0.4	39	4.5 ± 1.2	40
D-Glucosamine	3.8 ± 0.8	49	3.8 ± 0.8	49
D-Mannose	3.1 ± 0.5	59	1.6 ± 0.3	79
D-Galactose	5.3 ± 1.0	29	5.1 ± 0.9	32
D-Ribose	4.1 ± 0.9	45	4.3 ± 0.3	43

[a]See Table V for details on experimental conditions.

[b]Each value represents the mean tissue concentration (mM) of 6 observations ± standard error.

solution only inhibited the uptake of ^{14}C-3-O-MG in the tissue by some 32%. D-galactose was the least effective hexose inhibitor added to the luminal solution. Furthermore, unlabeled D-fructose and D-glucose in the luminal solution are the most effective inhibitors of the 3-O-MG transport system. These hexoses drastically reduced the transport as well as the uptake of ^{14}C-3-O-MG more than unlabeled 3-O-MG at the same concentrations.

The Effect of Various Inhibitors on the Movement of 3-O-Methyl-D-Glucose Across the Intestine

The effect of iodoacetamide, sodium fluroide, 2,4-dinitrophenol, phlorizin or ouabain on the movement of 3-O-MG across the intestine was determined, and the results are shown in Table VII. For comparisons, sac preparations were incubated in the absence of inhibitors as a control and the rate of movement of 3-O-MG was calculated to be 1.36 μmoles of 3-O-MG moved/cm^2/hr. When sacs were incubated immediately with 3 x 10^{-4} M iodoacetamide in the system the rate of 3-O-MG movement was reduced to 60% of the control value. Statistical analysis by Students t test indicated that the difference between means was not significant ($P > 0.05$). On the other hand, when sac preparations were preincubated in 3 x 10^{-4} M iodoacetamide for 15 minutes, and then incubated with 3 x 10^{-4} M iodoacetamide for 1 hour, the rate of movement of 3-O-MG was significantly ($P < 0.005$) reduced to 0.28 μmoles 3-O-MG/cm^2/hour, or one-fifth the rate of the control. In the presence of 3 x 10^{-4} M dinitrophenol the movement of 3-O-MG is not altered. However, with the addition of 0.15 M sodium fluoride to the system the rate of 3-O-MG movement was significantly

TABLE VII

EFFECT OF VARIOUS INHIBITORS ON THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE INTESTINE[a]

Addition	Movement of 3-O-MG (μmoles/cm^2/hr$\pm$SD)	% Relative Rate
None	1.36 $\pm$ 0.28[b]	100
Iodoacetamide (3×10^{-4} M)	0.80 $\pm$ 0.11	59
Iodoacetamide (3×10^{-4} M) (Preincubated 15 min)	0.28 $\pm$ 0.10**	21
Dinitrophenol (3×10^{-4} M)	1.48 $\pm$ 0.30	109
NaF (0.15 M)	0.11 $\pm$ 0.04***	8
Phlorizin (4×10^{-4} M)	0.50 $\pm$ 0.06*	37
Ouabain (1×10^{-4} M)	1.38 $\pm$ 0.35	101
Ouabain (1×10^{-3} M)	1.31 $\pm$ 0.40	96

[a]Sac preparations were incubated at 37 C for 1 hour in a 95% N_2-5% CO_2 gas atmosphere. The luminal solution was buffer saline (pH 7.2) containing 0.04 M 3-O-methyl-^{14}C-D-glucose (14,032 dpm/μmole). Sacs were prepared from the posterior region and incubated in 1.0 ml of 0.04 M glucose in buffered saline (pH 7.2). All inhibitors except phlorizin were added to both the luminal and incubation medium at a final concentration as indicated. Phlorizin was added only to the luminal solution.

[b]Each value represents the mean of 6 observations.

*$P < 0.025$.

**$P < 0.005$.

***$P < 0.001$.

($P < 0.001$) reduced to only 8 percent of the control value. Furthermore, the system for moving 3-0-MG across the intestine is partially inhibited with 4×10^{-4} M phlorizin since the relative rate of 37% is significantly ($P < 0.025$) lower than the control value. The presence of ouabain in the system at two different concentrations does not effect the rate of 3-0-MG movement from the luminal to the pseudocoelomic side of the intestine.

The Influence of Certain Cations and EDTA on the Movement of 3-0-Methyl-D-Glucose

The effect of omitting certain mono- or divalent cations, or the presence of the chelating agent, ethylenediaminetetracetate (EDTA) was determined. Sac preparations were prepared and incubated in a specific electrolyte-free solution, or with EDTA, and the results of these experiments are presented in Table VIII. For comparisons, six sac preparations were incubated in a complete system. A control rate of 1.31 µmoles of 3-0-MG/cm^2/hr was calculated. When Na^+ were omitted from the system and replaced with the respective potassium salts the rate of 3-0-MG movements was significantly ($P < 0.025$) reduced to 0.64 µmoles of 3-0-MG/cm^2/hr. This low rate of movement represents 49 percent of the rate calculated for the control. When potassium was omitted and the potassium salts replaced with the appropriate sodium salts, a significant ($P < 0.05$) increase in the rate of 3-0-MG movement was calculated. This increase is 166% above the rate determined for the control. Furthermore, statistical analysis by Students t tests indicated that the deletion of Ca^{++} from the system resulted in a significant ($P < 0.05$) decrease in the rate of 3-0-MG movement to 55% of the rate calculated for the complete system. Unlike Ca^{++}, the omission of Mg^{++} or the addition of 5.0 mM EDTA to both

TABLE VIII

THE INFLUENCE OF CATIONS AND EDTA ON THE MOVEMENT OF 3-O-METHYL-D-GLUCOSE ACROSS THE INTESTINE[a]

System	n	Movement of 3-O-MG (μmoles/cm^2/hr$\pm$SD)	Percent of Complete System
Complete	6	1.31 $\pm$ .21	100
Minus Na^+	12	0.64 $\pm$ .14**	49
Minus K^+	12	2.18 $\pm$ .27*	166
Minus Ca^{++}	11	0.72 $\pm$ .14*	55
Minus Mg^{++}	8	1.21 $\pm$ .13	92
Plus 5 mM EDTA	6	1.72 $\pm$ .29	131

[a]Sac preparations were prepared from the posterior region and rinsed in 3 changes of the appropriate electrolyte-free solution. The luminal solution was buffered saline (pH 7.1) containing 0.04 M 3-O-Methyl-^{14}C-D-glucose (15,141 dpm/μmole). Each sac was incubated in 1.0 ml of 0.04 M glucose in buffered saline (pH 7.1) for 1 hour at 37 C in a 95% N_2-5% CO_2 gas atmosphere. Each electrolyte was omitted from both sides of the sac and the osmolarity maintained by replacing the K^+, Ca^{++} and Mg^{++} with the appropriate sodium salts. Sodium ions were replaced by the appropriate potassium salts. n represents the number of observations.

*$P < 0.05$.

**$P < 0.025$.

sides of the system did not significantly ($P > 0.05$) alter the rate of movement of 3-O-MG across the intestine.

CHAPTER V

DISCUSSION

The results of this study indicate that the rate of 3-0-MG movement progressively increases from the anterior to the posterior region of the intestine of Ascaris. It is interesting that statistical analysis of these data indicate that the differences between mean rates of 3-0-MG movement across various regions of the intestine are distinctly different when the data is expressed on the basis of mg dry weight of tissue. Although the length of each region of the intestine remained constant for each worm, the width of the intestine increases progressively from the anterior to the posterior region. When one compares the means for different regions of the same intestine real differences that exist may become apparent only when a more accurate quantitation is made of the tissue involved. Measurements for determining the surface area are more subjective and can only be used for comparisons of measurements obtained from the same region of intestine since the variation due to different regions of the intestine is eliminated and does not confound the data.

The rate of movement of 3-0-MG across the intestine is some 2 times greater in the mid region and some 3 times greater in the posterior region than it is in the anterior region. These results support the suggestion (Lee, 1965) that the mid and posterior regions of the intestine of Ascaris function more in the absorption of nutrients than does the anterior region.

From a structure-function standpoint, it is interesting that the transport gradient for moving 3-0-MG increases along the length of the intestine. The presence of such a gradient would greatly facilitate extracellular digestion of carbohydrates (Carpenter, 1952) and assure efficient removal of hydrolyzed products such as glucose from the lumen. Further, the reproductive system is closely associated with the posterior two-thirds of the intestine. The ovary-oviduct tissue has a high anabolic rate (Entner and Gonzales, 1959) and undoubtedly requires a generous supply of nutrients. The close relationship of the reproductive system and the regions of the intestine that are most active in the absorption of nutrients assures the ovary-oviduct tissue of a ready access to nutrients from the intestine.

In comparison, Fisher and Parsons (1950) show that the mucosal surface area per unit length of intestine increases from the ileum to the duodenum of the rat. They also report that a linear gradient for glucose absorption from the lumen exists along the length of the intestine such that the absorption in the duodenum is some four times that in the ileum. The density of villi increases from 10 to 40/mm^2 in the ileum and duodenum, respectively, and this increase could account for the four-fold increase in glucose absorption between these regions. Since changes in both surface area and mg dry weight are accounted for in this study, it is difficult to explain the differences in the movement of 3-0-MG across various regions of the intestine of Ascaris that occurred. However, it does seem possible that changes in the absorptive surface could occur which increase the density of microvilli in various regions of the intestine and that these changes could not be accounted for with the measuring techniques employed. It would be interesting to look for differences in

the density and height of the brush border microvilli in various regions of the intestine of Ascaris with the electron microscope to see if such changes exist. If such differences do not occur along the length of the intestine of Ascaris then differences in the rate of movement of material could be explained on the basis of an increased permeability of the cells lining each region, or the presence of a more active transport system.

The rapid depletion of the TCA soluble carbohydrate in intestinal tissue incubated in vitro without glucose supports the suggestion (Beames, 1971) that intestinal tissue from Ascaris rapidly utilizes its endogenous carbohydrate reserves. During the first 10 minutes of incubation the tissue utilizes 68% of its TCA soluble carbohydrate and during the remaining 70 minutes only an additional 13% is lost. Fairbairn and Passey (1957) show that the total carbohydrate of this tissue is only 0.84% of the wet weight, and that endogenous glycogen and trehalose make up 83 and 17% of the total carbohydrate content, respectively. The results of the present study show that the total carbohydrate of intestinal tissue incubated without glucose does not fall below 19% after 80 minutes incubation. The failure to demonstrate total depletion of endogenous carbohydrate after 80 minutes incubation may be attributed to the inability of this tissue to utilize its endogenous trehalose since the enzyme, trehalase, is specifically located on the brush border and not in the cytosol of the cell (Gentner, et al, 1972). Therefore, the depletion of TCA soluble carbohydrate of intestinal tissue is due to the rapid and complete utilization of endogenous glycogen reserves. Since glycogen is the only utilizable carbohydrate in intestinal tissue the results of this study suggest that some 84% of the endogenous glycogen is depleted within the first 10 minutes of incubation, and at the end of 80 minutes

incubation the endogenous glycogen reserves are totally depleted.

The endogenous carbohydrate of intestinal tissue measured in this study is approximately one-half the value of the total alkali-stable carbohydrate value reported by Fairbairn and Passey (1957). The results of the present study show that the TCA soluble carbohydrate of the intestine of Ascaris is 0.31% of the tissue wet weight. This lower value cannot be attributed to the loss of carbohydrates during the time the worms were transported to the laboratory since analysis of intestinal tissue collected and maintained on ice was 0.32 ± 0.2% of the wet weight. Therefore, the difference must be due to different techniques used to extract tissue carbohydrates. Alkali digestion not only frees the endogenous free carbohydrates but the membrane bound material as well. Beames (unpublished data) showed that it is possible to account for the alkali-stable carbohydrate by measuring the anthrone positive material in the TCA precipitate. It is quite possible that the majority of the anthrone sensitive material in the TCA precipitate is not an available carbohydrate source and that the TCA soluble carbohydrate represents the soluble endogenous carbohydrate available for metabolism. If so, then the lower value of 0.31 mg carbohydrate/g wet weight of tissue strongly emphasizes the need for exogenous carbohydrate in the incubation system. Added glucose would maintain rapid endogenous carbohydrate metabolism and facilitate such physiological processes as the movement of materials across the intestine that requires metabolic energy.

Both Sanhueza, et al. (1968) and Castro and Fairbairn (1969) observed rapid uptake and metabolism of glucose by intestinal tissue from Ascaris incubated in vitro. After incubating the tissue in glucose for one hour their results indicate that most of the glucose is incorporated

into glycogen. The results of the present study indicate that during the first 5-10 minutes of incubation the increase in the TCA soluble carbohydrate is due to the rapid uptake of glucose by the tissue. These results also show that after 10 minutes of incubation the reducing sugar remains fairly constant but that the glycogen steadily increases. At the end of 80 minutes of incubation the glycogen accounted for some 50% of the TCA soluble carbohydrate and increased some 4 times the initial quantity present. Preliminary experiments run in this laboratory indicate that the intestine of Ascaris rapidly equilibrates with 0.04 M glucose placed on the pseudocoelomic side. Since strips of intestinal tissue were incubated on a 0.04 M glucose media, rapid movement of glucose across the basement membrane could account for the rapid increase in the TCA soluble carbohydrate observed in the first 10 minutes of incubation. Nevertheless, the endogenous carbohydrate of the intestine can be regulated by incubating the tissue in the presence or absence of glucose.

The results also indicate that the intestine utilizes its endogenous carbohydrate reserves to facilitate the movement of 3-0-MG across sac preparations in vitro. When the endogenous carbohydrate level is high at the start of the incubation the rapid movement of 3-0-MG is maintained for only the first 10 minutes. At this point the endogenous carbohydrate becomes critical and the movement of 3-0-MG is limited throughout the remainder of the incubation. When the endogenous carbohydrate of the system is depleted the movement of 3-0-MG is drastically reduced. On the other hand, if the endogenous carbohydrate is maintained in the tissue, 3-0-MG moves rapidly across the intestine and maintains a linear rate of movement up to 80 minutes of incubation. These observations explain the failure of Sanhueza, et al. (1968) to demonstrate movement of 3-0-MG

across the intestine, and further, supports the observation (Beames, 1971) that the movement of hexoses across the intestine of Ascaris requires an exogenous energy source such as glucose.

The observation that exogenous glucose in the incubation media is the only sugar tested that significantly facilitates the movement of 3-0-MG across the intestine is interesting. Fairbairn and Passey (1957) report that the predominate sugars in the body fluids of Ascaris are trehalose and glycogen. The enzyme trehalase is found only in the intestinal tissue of this parasite (Feist, et al., 1965). With this in mind, it was thought that these two substrates would serve as an energy source for supporting movement of 3-0-MG across the intestine. Similarly, amylase is present in the hemolymph (Fukushima, 1967) and it was thought that added maltose, a hydrolysis product of amylase, would serve as a possible sugar to support the 3-0-MG transport system. However, these sugars did not significantly stimulate 3-0-MG movement across the intestine.

There appears to be several possibilities to help explain these results. One possibility is that trehalose and matose may cross the basement membrane and enter the cytosol. If this is the case, then these sugars are not hydrolized in the cell, and suggest that trehalase and maltase are not associated with the cytosol. These enzymes may be compartmentalized and not readily available to hydrolyze their substrate within the cell. The recent finding (Gentner, et al., 1972) that trehalase and maltase are tightly bound to the brush border, and that the specific activity of these enzymes is some 10 times lower in the cytosol than in brush border preparations suggests that these enzymes are compartmentalized. On the other hand, if these sugars do not penetrate the

intestine from the pseudocoelomic side they would not be avilable to the cells. Since the incubation media did not contain amylase, and glycogen is an extremely large molecule, this substrate or its hydrolytic products would not be available to the intestinal preparation.

The addition of glycogen, trehalose, or maltose did result in a slight increase in the movement of 3-0-MG as compared to sac preparations incubated with the substrate omitted. The presence of small amounts of glucose as a contaminant in these sugars could account for this slight increase since both trehalose and maltose contained 0.5 and 1.0% glucose, respectively.

The results of altering the pH of the luminal solution between 5.5 and 8.0 show that the system for moving 3-0-MG across the intestine is sensitive to pH. If the process for moving this glucose derivative was simple diffusion, the rate of 3-0-MG movement would not be altered by slight changes in the pH of the luminal solution over the range employed for this study. These results indicate that the 3-0-MG transport system involves a catalytic process and that the greatest rate of movement occurs at pH 6.5. This optimum pH of 6.5 is close to the pH of 6.74 determined on the luminal contents from several worms. Since these two values are close, this observation suggests that the environment of the lumen of the intestine is within the optimal pH range of the transport system and would greatly facilitate the absorption process.

The addition of glucose to the incubation system results in an overall increase in the rate of 3-0-MG movement as well as increased sensitivity of the transport system to pH changes. Although the trend in the rate of movement is the same with or without glucose in the incubation system, it is quite possible that alterations in the pH of the

luminal solution not only effect changes in the activity at the absorptive surface, but also influence the rate of carbohydrate metabolism. Since most transport systems involve several enzyme systems, it is difficult to interpret from these results that the effect of pH is specifically localized to the absorptive surface. The movement of 3-0-MG is dependent upon metabolic energy from the catabolism of carbohydrate (Beames, 1971), and slight alterations in this process could limit the availability of metabolic energy essential for driving the transport system.

Ascaris must be kept warm from the moment of collection at the slaughterhouse. At temperatures lower than 20 C these animals die quickly (Fairbairn, 1967). The reason for this unusual sensitivity of Ascaris to moderately lowered temperatures is not known, but it has been suggested that this phenomenon is associated with defective metabolism (Fairbairn, 1957). Temperatures above 40 C are also lethal to these parasites and on certain occasions this was observed in the laboratory. With this information a temperature range between 20 and 45 C was selected to determine the effect of temperature upon the movement of 3-0-MG across the intestine of Ascaris.

The results of this study indicate that with glucose in the system the movement of 3-0-MG across the intestine is greatly enhanced by temperature. The fact that exogenous glucose greatly facilitates the movement of 3-0-MG across the intestine indicates that metabolic energy participates in this process, and without glucose little movement occurs. It is interesting that between 20 and 25 C the addition of glucose to the system markedly increases the rate of 3-0-MG movement across the intestine. A large Q_{10} value of 72.0 associated with this 5 C rise suggests that the transport system is placed in a more reactive state by

increasing the thermal energy of the system and that the participation of metabolic energy may facilitate this process by specifically altering the affinity of enzymes for their substrate.

Most enzymes are denatured irreversibly at temperatures between 50 and 80 C. The blanched and hardened appearance of intestinal tissue incubated at 60 C suggests that protein denaturation had occurred at this temperature, and that the integrity of the tissue was disrupted. The low net rate of movement determined at 60 C indicates that the transport system was nonfunctional. At 60 C the rates of 3-0-MG movement are approximately the same with or without glucose in the system. This indicates that the permeability barrier was disrupted at high temperature. Further, these studies indicate that the system for moving 3-0-MG across the intestine is not a process of simple diffusion. A simple diffusion process shows a linear response to temperature changes and has a Q_{10} value near 1.0. In the intestine of Ascaris, an exponential increase in the rate of 3-0-MG movement occurs between 25 and 45 C, and the Q_{10} values associated with this function are all greater than 2.0. Q_{10} values greater than 2.0 are characteristic of the involvement of a catalytic reaction in most biological systems. In this instance the participation of metabolic processes as well as the activation of a specific transport system could account for the increased sensitivity of the 3-0-MG transport system to temperature.

The high energy of activation value of 19,938 calories per mole of 3-0-MG moved across the intestine is within the range of most biologically catalyzed systems and indicates that a large amount of kinetic energy is involved in the movement of this material across the intestine. Phifer (1960) reports that the absorption of glucose by the tapeworm,

Hymenolepis diminuta, is temperature sensitive and that the energy of activation for this absorption process requires 20,910 calories per mole of glucose absorbed.

The results of the kinetic study indicate that the system for moving 3-0-MG across the intestine of Ascaris is saturable with high substrate concentrations in the luminal solution. The saturation curve obtained for 3-0-MG movement across the intestine of Ascaris is characteristic of a Michaelis saturation curve for an enzyme mediated reaction and suggests that 3-0-MG transport system involves reactive sites. The initial velocity of 3-0-MG movement is typical of a first-order reaction up to a substrate concentration of 20 mM 3-0-MG. At higher concentrations the velocity of 3-0-MG movement becomes more or less independent of the substrate concentration and approaches a zero-order reaction rate. The response of 3-0-MG movement to increased substrate concentrations is similar to the change in rate of glucose movement across the intestine of the rat (Fisher and Parsons, 1949) and guinea pig (Riklis and Quastel, 1958) intestine which follows Michaelis-Menton saturation kinetics. These data indicate that the system for moving 3-0-MG across the intestine of Ascaris is a carrier-mediated process.

Initial attempts to analyze these data by the double reciprocal plot method of Lineweaver and Burk (1934) indicated that the calculated linear regression line did not fit the plotted values. It was apparent from this information that the calculated K_m of 51 mM 3-0-MG did not agree with the value extrapolated from the saturation curve. Read (1973) has experience similar problems using this method for determining glucose absorption kinetics in the tapeworm H. diminuta. He attributes this biased effect to weighting, due to an increased error term associated with

reciprocal plots of low velocities and small substrate concentrations. A more unbiased analysis may be obtained by the Hofstee method (Christensen and Palmer, 1967). Application of this method to the 3-0-MG transport system results in a linear regression line that closely fits the plotted values and gives a K_m value of 22.7 mM 3-0-MG and V_{max} value of 4.19 moles 3-0-MG/cm^2/hr. These kinetic parameters closely correspond to the expected values from the saturation curve. The K_m represents the substrate concentration that gives one-half the maximum velocity and is an index of the affinity of the transport system. Sanhueza, et al. (1968) reports that the luminal concentration of glucose necessary to yield half the maximum rate of glucose absorption as 9 mM glucose. Since the K_m for 3-0-MG movement is higher than that reported by Sanhueza, et al. (1958) for glucose, it suggests that the system for moving sugars across the intestine has a higher affinity for glucose than 3-0-MG.

To demonstrate saturation kinetics in this system it is necessary to determine the net rate of 3-0-MG movement since a large diffusion component greatly contributes to the absolute movement of 3-0-MG at high concentrations. It is interesting that Pappas, et al. (1973) observes a similar process in the absorption of glucose and galactose by the tapeworm larvae Taenia crassiceps. In this parasitic larvae, they show that at substrate concentrations of 10 mM or higher the absorption of both glucose and galactose is by a combination of rapid diffusion and a mediated process, and that saturation of the transport system can only be demonstrated by correcting for the diffusion component.

The results of the effect of unlabeled sugars upon the movement and absorption of 3-0-MG by the intestine of Ascaris indicate that modifications in the structure of the inhibitory sugar greatly effects the

affinity of the transport system for its substrate. Furthermore, the transport system has a higher specificity for some sugars as inhibitors than others, and the reactive site of the transport system that recognizes these differences is located on the luminal surface of the intestine.

The affinity of the transport system can best be explained by comparing the percentage of inhibition of the various sugars relative to the inhibitory effect of unlabeled 3-O-MG. Hence, an inhibitory effect greater than unlabeled 3-O-MG represents a higher affinity of the transport system for the inhibitor sugar than for 3-O-MG, and an inhibitory effect less than unlabeled 3-O-MG indicates a lower affinity of the transport system for the inhibitor sugar than for 3-O-MG. At an I/S ratio of 5.0 comparison of the relative inhibitory effect of unlabeled sugars upon the rate of movement of ^{14}C-3-O-MG across the intestine indicates that the transport system has a greater affinity for both D-glucose and D-fructose (80% inhibition) than for unlabeled 3-O-MG (66% inhibition). This suggests that the substitution of a methyl group in the C-3 position decreases the affinity of the transport system for the substrate. The high inhibitory effect of the keto-sugar, D-fructose, is difficult to explain, but it does indicate that the pyranose ring structure is not essential for recognition at the reactive site. Since the K_m of 9 mM for glucose transport (Sanhueza, et al., 1968) is much lower than the K_m of 22.7 mM for 3-O-MG, one would expect glucose to have a higher affinity for the transport system than 3-O-MG. The results of this study strongly support this relationship.

The results also indicate that modifications in the C-2 position are not as critical as the rotation of the hydroxyl group about C-4. Changes

about the C-2 position (2-deoxy-D-glucose, D-glucosamine, D-mannose) result in an inhibitory effect equal to or slightly less than the inhibitory effect of unlabeled 3-O-MG. On the other hand, the low inhibitory effect of D-galactose, an isomer of glucose differing at the position of the hydroxyl group at the C-4 position, indicates that the position of the hydroxyl group at C-4 is critical for recognition and binding to the reactive site. The difference in the inhibitor effect of the pentose sugars on the transport system indicates that D-xylose, a homomorphic isomer of D-glucose lacking the 6th carbon, elicits a slightly greater inhibitory effect than does D-ribose. It is interesting that the percent inhibition of either the movement or absorption of 3-O-MG by D-xylose or D-ribose is not increased by increasing their concentration in the luminal solution. It is not clear from the results of the present study to determine the nature of this unaltered response unless the sugar transport system has little affinity for these pentoses. Alvarado (1965) and Salmon, et al. (1961) have shown that D-xylose is actively transported by the glucose transport system in the hamster small intestine and indicate that the requirement for the pyranose ring structure is no longer tenable for the glucose transport system. This observation agrees with the results of these studies, in that D-fructose greatly alters the activity of the 3-O-MG transport system.

The explanation offered for the inhibitory effects of unlabeled sugars upon the absorption of 3-O-MG is essentially the same as described above. The only exceptions are 2-deoxy-D-glucose and D-mannose. These sugars have a greater inhibitory effect on absorption than unlabeled 3-O-MG. This difference may be indicative of the structural orientation of the functional groups about carbons 2, 3 and 4 and that the affinity

of the transport system is altered by modifications at carbons 2 and 3, and especially sensitive to alteration at carbon 4. Thus the structural requirements of the transport system for the intestine of Ascaris are quite different than those proposed by the glucose transport system in the vertebrate intestine (Crane, 1960).

The results of the competitive sugar studies agree with the information available on the absorption and utilization of sugars by Ascaris. Beames (1971) has shown that both D-fructose and 3-O-MG but not D-galactose, are rapidly moved across the intestine of Ascaris. Furthermore, studies by Cavier and Savel (1952) show that D-fructose and D-glucose, but not D-galactose, stimulate glycogen synthesis in intact worms. Castro and Fairbairn (1969) report that glucose moves into intestinal tissue against a concentration gradient and is incorporated into glycogen in the intestinal tissue of Ascaris. Interestingly, those sugars which are utilized, absorbed or moved by this parasite have a high affinity for the transport system while D-galactose, a sugar that is not moved across the intestine, has a low affinity for the transport system; an observation that could explain the inability of Ascaris to utilize D-galactose.

The results also show that 3-O-MG is not concentrated in the intestinal tissue. After an hour incubation the tissue/luminal solution concentration is 0.75. Castro and Fairbairn (1969) show that glucose is concentrated in intestinal strips from Ascaris. They report that the final tissue/incubation media concentration is 3.22. Since the tissue/luminal solution concentration is 1.0, 3-O-MG is not accumulated in the tissue, even though it moves from the luminal to the pseudocoelomic side of the intestine against a concentration gradient (Beames, 1971). One

possible explanation for the failure to demonstrate accumulation of 3-0-MG in the tissue is that the rapid entry of glucose from the pseudocoelomic side may decrease the intracellular pool of 3-0-MG. A more likely explanation is that 3-0-MG is actively concentrated in the extracellular space which represents one-fifth the total tissue water. If the majority of the ethanol extractable radioactivity is in this compartment then the extracellular tissue fluid/luminal solution concentration of 3-0-MG could be as high as 3.5. Under this condition the active transport process would be located on the plasma membrane between two adjacent cells and allow rapid diffusion of 3-0-MG down its concentration gradient.

Earlier studies by Wilbrandt and Lastz (1933) indicate that iodoacetate greatly impairs the absorption of glucose in the intestine of the rat. Phifer (1960) reports that it is necessary to preincubate H. diminuta in iodoacetate before glucose absorption is impaired. In the present study preincubation of intestinal sac preparations in iodoacetamide resulted in further reduction in the movement of 3-0-MG across the intestine. These results indicate that glycolytic energy production is necessary to facilitate the movement of 3-0-MG across the intestine, and that the system for moving 3-0-MG is greatly impaired when glycolytic metabolism is blocked by allowing the inhibitor to penetrate the tissue prior to incubation.

Sodium fluoride also produced a rapid and significant reduction in the rate of 3-0-MG movement across the intestine. This finding strongly indicates that the availability of metabolic energy (ATP) is drastically reduced and thus limits the energy dependent transport of 3-0-MG. Both iodoacetamide and sodium fluoride are effective inhibitors of glycolysis. In addition to blocking glycolysis, the inhibitory effect of sodium

fluoride is rather nonspecific and may also effectively block some ATPase dependent enzyme systems (Bucher, 1965) that are directly involved in membrane transport processes. Besides the fact that sodium fluoride rapidly penetrates tissue, its greater inhibitory effect may be due to an additional inhibitory effect on ATPase systems. Nevertheless, these studies suggest that the transport of 3-0-MG is highly dependent upon metabolic energy derived from substrate phosphorylation processes, and that glycolytic carbohydrate metabolism is involved in this process.

The inability of 2,4-dinitrophenol to inhibit the movement of 3-0-MG across the intestine suggests that metabolic energy produced from mitochondrial oxidation is not essential for driving the transport process. This finding agrees with what is known of anaerobic carbohydrate metabolism in Ascaris. These parasites utilize a mitochondrial flavoprotein system for oxidative substrate (ATP) production (Kmetec and Bueding, 1961). This provides one-third of the total ATP produced from anaerobic carbohydrate metabolism. Saz (1972) shows that 1.5×10^{-4} M dinitrophenol uncouples oxidative phosphorylation and inhibits the uptake of labeled inorganic phosphate ($^{32}P_i$) some 67% in Ascaris mitochondria. Van den Bossche (1972) also shows that the anaerobic malate-induced incorporation of $^{32}P_i$ into organic phosphate is inhibited by dinitrophenol without blocking malate utilization. These studies conclusively demonstrate that dinitrophenol is an effective uncoupler of Ascaris mitochondrial oxidative phosphorylation. Glycolysis supplies two-thirds of the available metabolic energy (ATP) to the tissues of Ascaris. Since 2,4-dinitrophenol does not alter substrate phosphorylation (Lehninger, 1970), then the metabolic requirements of the transport process are fully met through glycolysis to drive the system.

When 4 x 10^{-4} M phorizin is present in the luminal solution movement of 3-0-MG across the intestine is inhibited some 63%. Phlorizin is a well known competitive inhibitor of the glucose transport system of the vertebrate gut (Alvarado and Crane, 1962), the tapeworm, H. diminuta (Phifer, 1960) and the tapeworm larvae, Taenia crassiceps (Pappas, et al, 1973). Phlorizin acts as a competitive inhibitor of glucose absorption by reducing the affinity of the transport system for its substrate of the binding site. The fact that phorizin in the luminal solution inhibits the movement of 3-0-MG suggests that the transport system is a carrier-mediated process located on the luminal surface of the intestinal epithelium. This finding supports the conclusion of Sanhueza, et al. (1968) that specific processes for glucose transport exist on the luminal surface of the epithelial cells.

The observation that 1 x 10^{-3} M ouabain had no effect on the movement of 3-0-MG across the intestine of Ascaris is not consistent with most energy-dependent Na^+-activated transport systems. These results indicate that the 3-0-MG transport system in the intestine of Ascaris is ouabain insensitive and suggest that the participation of the Na^+-K^+ activated ATPase system (if such an enzyme exists in Ascaris) is not inhibited. Pappas, et al. (1973) report that absorbed ouabain is without effect on the uptake of glucose in Taenia crassiceps larvae. They suggest that the inability of ouabain to alter glucose uptake may be attributed to compartmentalization of absorbed ouabain in a region separated from Na^+-K^+ activated ATPase. If this is the case, then ouabain would effectively disrupt the sodium efflux without altering glucose accumulation in this larvae. This could be the case in the intestine of Ascaris. However, as is shown in this study, 3-0-MG does not apparently

accumulate in the intestine and the intracellular sodium efflux may not be coupled to the glucose transport system. Another possibility is that at the concentrations used ouabain does not enter the intestine. If so, this would explain its inability to inhibit the movement of 3-0-MG across the intestine.

The influence of cations upon the movement of 3-0-MG across the intestine indicates that the transport system is partially dependent upon the presence of Na^+. In a Na^+-free system where Na^+ is replaced with K^+ the movement of 3-0-MG across the intestine is significantly reduced. This effect could be due to either the absence of Na^+ or the presence of K^+. However, the present study shows that K^+ will not substitute for Na^+ to restore the full activity of the transport system. Other studies indicate that increasing concentrations of K^+ reduced the Na^+-dependent glucose transport process in both the vertebrate intestine (Crane, 1962), and in tapeworm larvae (Pappas, et al., 1972). The inhibitory effect on both systems is the result of competitive inhibition of K^+ on the Na^+-activated glucose transport system. In a K^+-free system where K^+ is replaced with Na^+, the movement of 3-0-MG across the intestine of Ascaris is greatly enhanced. This suggests that the 3-0-MG transport system is activated by the presence of high levels of Na^+ or that the inhibitory effect of K^+ is absent. Under these conditions the transport system has a much higher affinity for 3-0-MG and moves it at a faster rate when K^+ is omitted. These results suggest that transport of 3-0-MG across the intestine is enhanced by increasing the extracellular Na^+ concentration. This suggests that the entry of 3-0-MG into the cell is a Na^+-coupled process.

In the vertebrate small intestine the Na^+-K^+ ATPase dependent pump

requires the presence of both K^+ and Na^+ for activity. Crane (1968) indicates that the activity of the Na^+-K^+ pump is drastically reduced in the absence of either of these ions. If the intestine of Ascaris has a Na^+-K^+ pump for maintaining the balance of electrolytes that is inactivated by omitting either of these ions, its activity is not directly coupled to the movement of 3-0-MG across the intestine under the conditions employed. It is unlike the ouabain sensitive Na^+-K^+ pump described for the mammalian intestine and may explain the inability of ouabain to effect the movement of 3-0-MG across the intestine of Ascaris.

The system for moving 3-0-MG across the intestine is dependent upon the presence of Ca^{++} and uneffected by the removal of Mg^{++}. If the system is coupled to an ATPase dependent process, it was thought that the absence of Ca^{++} would stimulate movement, and the removal of Mg^{++} would result in a reduced rate of movement of 3-0-MG across the intestine. The results are not consistent with this concept, and cannot explain this relationship.

It does seem possible that Ca^{++} are essential for maintaining the integrity of the cell membrane. The addition of EDTA to the system would effectively remove all divalent cations. In the presence of EDTA the system appears to move 3-0-MG at a somewhat greater rate. This effect may be attributed to the disruption of the permeability barrier and result in rapid diffusion across the intestine.

The results of this study provide a better understanding of the requirements for moving sugars across the nematode intestine. They show that specific deviations from optimum conditions for moving 3-0-MG across the intestine of Ascaris drastically alter the transport process. Unlike the intestine of its mammalian host, these intestinal parasites rely

almost exclusively upon the catabolism of carbohydrates which they acquire from the carbohydrate rich diet of their host. The results of the present study indicate that the specificity and metabolic requirements of the intestinal sugar transport process of Ascaris are uniquely different from those described in the vertebrate small intestine. These findings provide a basis for future studies to develop parasite specific chemotherapeutic agents and determine their effect under certain conditions in vitro.

Although 3-0-MG is actively transported against a concentration gradient (Beames, 1971), it is not concentrated within the epithelial cells and raises some question as to the type mechanism involved. This transport process is quite different than the glucose transport system described in the vertebrate intestine and deserves further study. Such studies may reveal that sugars are actively moved via the extracellular fluids and not through the basal region of the epithelial cell. The latter process would explain the inability of the tissue to concentrate 3-0-MG.

CHAPTER VI

SUMMARY

Experiments *in vitro* were designed to study the mechanism and requirments for moving 3-O-methyl-D-glucose across the intestine of *Ascaris lumbricoides*.

The results of these experiments indicate that the rate of 3-O-MG movement progressively increases from the anterior to the posterior region of the intestine. The movement of 3-O-MG is some 2 times greater in the mid region and some 3 times greater in the posterior region than in the anterior region. These findings indicate that the mid and posterior regions of the intestine have a greater ($P < 0.01$) capacity for absorption of this compound than does the anterior region.

Incubation of intestinal tissue with or without glucose showed that the intestine can rapidly utilize its endogenous carbohydrate reserves. The addition of glucose to the incubation system prevents a loss of endogenous carbohydrates, and in fact, maintains the glycogen and reducing sugar content of the tissue throughout 80 minutes incubation. The results of this study also indicate that the intestine utilizes its endogenous carbohydrates to facilitate the movement of 3-O-MG across *in vitro* sac preparations. When the endogenous carbohydrate of the intestine is maintained at a low level the movement of 3-O-MG is drastically reduced. By maintaining the endogenous carbohydrate level in the tissue with exogenous glucose, a rapid linear rate of movement

of 3-O-MG was shown. However, when the endogenous carbohydrate reserves become critically reduced after 10 minutes incubation without glucose, the movement of 3-O-MG is limited. It was also observed that exogenous glucose, but not glycogen, trehalose or maltose, is the only sugar studied that significantly ($P < 0.05$) enhanced the movement of 3-O-MG. This indicates that neither glycogen, trehalose nor maltose serves as an energy source.

Studies on the effect of pH, temperature, and substrate concentration indicate that the system for moving 3-O-MG across the intestine is a carrier-mediated process that has an optimal pH of 6.5 and is saturable. Saturation of the system could only be demonstrated by correcting for a diffusion component. Kinetic studies on the movement of 3-O-MG indicate that the transport system has a K_m = 22.7 mM 3-O-MG and a V_{max} = 4.19 μmoles 3-O-MG/cm^2/hr.

The results of the competitive sugar studies show that the system for moving sugars across the intestine elicits specificity and recognized slight structural changes on the sugar molecule. Of the naturally occurring sugars studied, D-glucose and D-fructose show the greatest inhibitory effect (80% inhibition) on 3-O-MG movement at higher inhibitor concentrations (I/S = 5). The addition of D-galactose to the luminal solution at a high concentration (I/S = 5) only results in a slight reduction (28 to 39% inhibition) in the movement or absorption of 3-O-MG, respectively. All other sugars studied show an intermediate inhibitory effect on the movement and absorption of 3-O-MG. These studies indicate that a specific process for moving sugars across the intestine is located on the luminal surface. Furthermore, the system does not necessarily show a specific requirement for the pyranose ring

structure and alterations about carbons 2, 3, 4 and 6 alter the affinity of the system for a substrate. The tissue does not concentrate 10 mM 3-O-MG after 1 hour incubation with glucose in the system.

Studies on the effect of metabolic poisons indicate that the expenditure of metabolic energy is necessary to facilitate the movement of 3-O-MG across the intestine. The addition of either 3×10^{-4} M iodoacetamide or 0.15 M NaF to the system significantly ($P < 0.005$ and $P < 0.001$) reduces the movement of 3-O-MG. This observation suggests that glycolytic carbohydrate metabolism is either directly or indirectly involved with the transport process. 2,4-Dinitrophenol has no effect on the movement of 3-O-MG across the intestine, and unlike the other metabolic poisons it does not block substrate phosphorylation.

The influence of cations, and ouabain at two different concentrations, shows that the movement of 3-O-MG is partially dependent upon the presence of Na^+ and insensitive to ouabain. K^+ can not be substituted for Na^+ to restore full activity of the transport system. The intestine also shows a requirement for Ca^{++}, and the addition of 5 mM EDTA to the incubation system results in a slight increase in the rate of movement of 3-O-MG. The latter result is attributed to the disruption of the permeability barrier and an increased rate of diffusion.

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APPENDIX

TABLE IX

DETERMINATION OF THE LUMINAL pH OF INTESTINAL CONTENTS[a]

Intestine No.	Intestinal pH
1	6.24
2	6.34
3	6.75
4	6.10
5	7.28
6	6.95
7	7.05
8	6.65
9	6.03
10	6.89
11	7.00
12	7.81
13	6.28
14	6.83
15	6.88
16	6.72
Average ± SE	6.74 ± 0.08

[a]The pH was determined from the luminal contents in the posterior two-thirds of the intestine.

2

VITA

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