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MEMBRANE TRANSPORT AND METABOLISM OF INOSITOL BY HYMENOLEPIS DIMINUTA (CESTODA)

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MEMBRANE TRANSPORT AND METABOLISM OF INOSITOL BY
HYMENOLEPIS DIMINUTA (CESTODA)

by

Yuen-Kwong Ip

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE:

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HOUSTON, TEXAS
MAY 1980
ABSTRACT

Membrane Transport and Metabolism of Inositol

by Hymenolepis diminuta (Cestoda)

by

Yuen-Kwong Ip

Myoinositol and scyloinositol have been identified qualitatively and quantitatively by gas-liquid chromatography in the cestode worm Hymenolepis diminuta. No myoinosos-2 can be detected. Myoinositol is unevenly distributed throughout the worm; the scolex and germinative region contains more free and phosphatidyl-bound inositol than the more posterior proglottids. This region also contains more lipid-bound phosphorus, less lipid and less water. Myoinositol absorption is more rapid in this anterior region. The absorption of myoinositol by Hymenolepis diminuta involves diffusion at high substrate concentrations and mediated transport at low substrate concentrations. The mediated transport process exhibits saturation kinetics with \( V_{\text{max}} \) and \( K_t \) being 0.0105 \( \mu \text{moles/g ethanol extracted dry wt/4 min} \) and 0.0067 mM, respectively. It is sensitive to changes in temperature, \( \text{pH} \) and sodium ion concentration. D-glucose is a non-competitive inhibitor of myoinositol transport but myoinositol has no effect on D-glucose absorption. Phlorizin interacts competitively with the myoinositol transport system. Various sugar alcohols and amino acids examined have no effect on myoinositol transport. Absorbed myoinositol is incorporated into lipid as phosphatidylinositol but not metabolized to form any other water soluble component. The possible function of inositol in Hymenolepis diminuta is discussed.
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INTRODUCTION

Scherer (1850) isolated an optically inactive isomer of cyclohexane- \text{hexol} and christened the compound inosit, after the Greek \textit{is, ios}. With the suffix -\text{ol} added in English and French, the name was applied to the other isomers as they were discovered, so that it is now a generic term. The original isomer was called meso- or i- or myo-inositol. Bouveault (1894) showed that there were nine inositols (Fig. 1)—seven meso forms and one DL pair.

**Physical Properties of Inositols**

The inositols are beautifully crystalline, high-melting substances, markedly stable to heat, acids and alkalis. Neo-inositol and scyllo-inositol have surprisingly low solubilities in water (0.1\% and about 1\%, respectively, in the cold). Among organic solvents, only the very polar ones such as formamide, \text{N,N-dimethylformamide}, and methyl sulfoxide dissolve inositols to an appreciable extent.

Myoinositol can be obtained as anhydrous, non-hygroscopic crystals from water or acetic acid above 80\(^\circ\)C. It has a sweet taste. The density and melting point are 1.752 gm/cm\(^3\) and 225–227\(^\circ\)C, respectively. Its solubility in water at 25\(^\circ\)C is approximately 148 g/100 ml solution. Aqueous solutions are neutral to litmus. When myoinositol is crystallized from water below 50\(^\circ\)C, a dihydrate form is obtained, having a density of 1.524 gm/cm\(^3\) and melting point at 218\(^\circ\)C. These crystals effloresce and rapidly lose their water of crystallization at 100\(^\circ\)C. Myoinositol is optically inactive, even in the presence of borax. It remains optically inactive after the action of microorganisms (Penicil-
lium) thus possessing a symmetrical structure (Posternak, 1965).

**Occurrence of Inositols**

The most widely distributed inositol isomer is myoinositol. It appears to be present, both free and combined, in the tissues of nearly all living species, except a few bacteria (Posternak, 1965).

Myoinositol is found in nature in the following forms: a) as free inositol; b) as inositol-phosphate ester; c) as inositol phospholipids (phosphoinositides); d) as a constituent of simple and complex glycosides; and e) as methyl ethers (mono- or di-O-methylated derivatives). The bound forms b) and c) are those which are most frequently found; they are probably what was once called "inositogens", since they give free inositol on enzymic degradation.

Myoinositol has been isolated from various organs of a number of different mammals. Some animal tissues and secretions have very high inositol content. Van Heyingen (1957) reported that all the lens inositol was in the free state with a concentration varying from 0.5 mg/g wet wt in rat to 5.0 mg/g wet wt in sheep and man. This is, in fact, the highest inositol concentration observed in animals. Mann (1951) discovered a high proportion of inositol in the secretions from pig seminal vesicles, which may contain up to 2-3 g/100 ml. The seminal vesicle, coagulating glands, dorsal prostate gland, ventral prostate gland and cowper's gland of male adult rats contain 5544, 4397, 6984, 1646 and 657 μg of inositol/g tissue (Posternak, 1965), respectively. In thyroid and pituitary glands, the free myoinositol concentration is nearly 100 times higher than that of blood plasma. These high concentrations of inositol in glands are possibly related to the role of phosphoinosit-
ides in excretion phenomena. Intracellularly, a large amount of inositol is found in the mitochondria and microsomes (Herken et al., 1958). Marinetti et al. (1958) reported a particularly high content of inositol phosphatidies in the mitochondria from pig heart.

The major inositol phosphate, from a quantitative viewpoint, is phytic acid, or myoinositol hexaphosphate. Salts of this acid (phytate), accompanied by smaller amounts of related inositol polyphosphate salts, are found in plants and in soil. Phytates are present generally in plant tissues, but they accumulate particularly in seeds until, at maturity, 65 to 90% of the phosphate of the seed is in this form. Calcium magnesium phytate is the native form of phytate found in plant. The only reported occurrence of phytate in animal tissues is in avian and reptilian erythrocytes. According to a recent study, the principal component of chicken erythrocyte phytate is myoinositol-1, 3, 4, 5, 6-pentaphosphate (Johnson and Tate, 1969).

Isolation of myoinositol and myoinositol phosphates from hydrolyzates of phospholipids led to the recognition, around 1930, of the inositol lipids or phosphoinositides (Posternak, 1965). The simplest and most widely distributed of these is phosphatidylinositol which typically constitutes a small portion (usually less than 10%) of the total phospholipid from animal and plant tissues. Substantial amounts of more highly phosphorylated inositide were found in brain, and the original preparation was termed diphosphoinositide by Folch (1949), its discoverer. Subsequently, brain phosphoinositide was shown to be a mixture of phosphatidylinositol, phosphatididylinositol monophosphate (diphosphoinositide) and phosphatididylinositol diphosphate (triphosphoinositide). Trace
amounts of the latter two lipids accompany phosphatidyl inositol in other animal organs. Phosphoinositides having sugar residues attached to the inositol have been reported in a number of instances, and the materials from Mycobacteria (Lee and Ballou, 1965) and from seeds (phytoglycolipids) (Carter et al., 1969) have been investigated in some detail.

The best studied simple glycoside of inositol is galactinol, 1L-1-0-D-galactopyranosylmyoinositol. O-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→2L)-myoinositol has been isolated from the seeds of the vetch Vicia sativa (Petek et al., 1966). A β-D-glucopyranosyl-myoinositol was found among the oligosaccharides of potato tubers after two months' storage (Urbas, 1968), and other glycosylmyoinositols have been obtained as degradation products of lipids. A very interesting discovery is that the seeds of corn contain indoleacetic acid in the form of esters with myoinositol and an arabinosyl-myoinositol (Nicholls, 1967).

A number of myoinositol methyl ethers have been found in plants (Posternak, 1965).

The next most abundant inositol is the optically active isomers, D-(+) and L-(−)-chiroinositol. These occur in higher plants, predominantly as the methyl ethers D-(+)-pinitol and L-(−)-quebrachitol, respectively.

Scyllo-Inositol has been isolated from plants (Plouvier, 1963), and from animals, notably plagiostomous fish (Sherman et al., 1978). It has been detected in several species of insects (Candy, 1967) and in mammalian urines (Posternak, 1965). Mammalian tissues that have been subjected to careful analysis have 2 to 10% as much scylloinositol as
myoinositol (Sherman et al., 1968).

Of the remaining inositols, two have been detected in nature—neoinositol in soil phytate and mucoinositol as a methyl ether in many plant sources. The other three (epi, allo, cis) are so far strictly synthetic products.

**Physiological Actions of Inositol**

In 1901, Wildiers had introduced the idea of "Bios", referring to a substance that was essential for the growth of yeast in a synthetic medium and different from the compounds in the medium providing matter and energy. Since the identification of myoinositol as one of the Bios complex of yeast growth factor (Esotcott, 1928), there have been numerous studies on the role of this isomer in the nutrition of living organisms. Ridgway and Douglas (1958) reported that an inositol deficient nutrient induces inhibition of fermentation and oxidizing power, as well as a considerable reduction in the nucleotide coenzyme and cytochrome content in *Saccharomyces carlsbergensis*. Myoinositol hypovitaminosis also induces morphological changes in *Schizosaccharomyces pombe* (Posternak, 1965), namely, a notable lengthening of the cells. Katznelson and Lochhead (1944) reported that *Bacillus polymyxa* requires a mixture of biotin, pyridoxine, pantothenic acid, nicotinic acid, riboflavin and myoinositol as accessory growth factors. In general, however, bacteria are autotrophic for inositol. It is, in fact, known from animal nutrition studies, that the intestinal bacteria, by virtue of their synthetic powers, can supply a certain amount of vitamins, including myoinositol, to their host (Posternak, 1965).

Eagle et al. (1956, 1957) have examined the requirements of cul-
tures of 20 species of cells—eight derived from normal human tissue, seven from cancerous human cells, three from cells of human bone marrow of indeterminate cancerous nature, one of mouse sarcoma cell, and one of mouse fibroblast cell. In the myoinositol deficient medium, all cell species except the mouse fibroblast, exhibited cessation of growth after varying times, together with cytopathological modifications leading ultimately to death. Addition of inositol to the nutrient allowed normal growth. The authors hypothesized that inositol was used mainly for phospholipid synthesis. Contrary to earlier statements that inositol possesses anti-tumor activity \textit{in vivo} (Laszlo and Leuchtenberger, 1943), cancerous cells appeared to require myoinositol to the same extent as normal ones. In 1960, Eagle \textit{et al.} showed that, out of 22 strains of mammalian cells, only mouse fibroblast L-929 can be cultivated indefinitely on an inositol-free medium. This strain, in fact, synthesizes the cyclitol, which can finally be observed at a concentration of $10^{-6}$ M in the culture medium. The specificity of myoinositol in tissue culture was shown to be very great. None of the six other stereoisomers examined (scyllo, cis, neo, chiro +, chiro −, and muco) turned out to be active.

Maintaining mice on a diet without inositol, Woolley (1940) observed inadequate growth and especially a characteristic baldness (alopecia); hair remained on the head, tail, and hind legs below the knees, but disappeared almost completely from the rest of the body in symmetrical bilateral patches. Usually the death of the animal occurred 2 to 3 weeks after the onset of these symptoms. He finally found that if 100 mg of myoinositol or phytic acid was incorporated into 100 g of diet, the deficiency symptoms disappeared (Woolley, 1941), and he considered myo-
inositol to be a new vitamin of the B complex. Martin (1941), however, found only slight alopecia in mice fed on an inositol-free diet, the deficiency being somewhat remedied, moreover, by pantothenic acid. Stimulation of the growth of higher animals by myoinositol has indeed been observed (Posternak, 1965), but the effect in most cases appears to have been indirect, for the basal diets used have usually been deficient in other components, or unbalanced in some way. In the most careful experiments with diets low in myoinositol but adequate in all other respects, no retardation of growth or other deficiency symptoms were seen (McCormick et al., 1954; Reid, 1954; Anderson et al., 1958). Hamilton and Hogan (1944) reported reproductive difficulties in hamsters fed on an inositol-deficient diet, where several mothers died during parturition, or gave birth to stillborn young, or to sanguinolent formless masses. Addition of inositol to this diet removes these symptoms but does not produce any growth effect.

Although many organisms do not require an external source of myoinositol, it is clear that this compound is an essential item in the life economy of almost all cells. Many functions have been suggested, but so far no biological response to myoinositol has been demonstrated to result from the action of the free cyclitol at the molecular level (Anderson, 1972).

It is known that all the vitamins grouped together represent a functional unit and none of them exerts its activity in a fashion completely independent from the rest. In animals, as in microorganisms, myoinositol can exert a synergistic action when mixed with other B complex vitamins. Woolley (1941) observed a relationship between pantothenic acid and myo-
inositol when examining the alipoecia effect on mouse. Martin (1942) reported a synergistic action between inositol and p-aminobenzoic acid in rats, manifested by a favorable effect on growth, on the appearance of the fur, and on lactation. Dam and Glavind (1942) showed that addition of 1.5% myoinositol to the diet protected chickens from increased capillary permeability resulting from vitamin E deficiency.

Wiebelhaus et al. (1947) have demonstrated an anti-ketogenic effect of myoinositol in rats fed on a diet that is poor in proteins and rich in fats. The anti-ketogenic effect is explicable by the conversion of myoinositol to glucose which, in turn, is transformed into oxaloacetic acid, a necessary Kreb cycle intermediate needed during fatty acid degradation.

Best et al. (1951) reported that myoinositol exerted a limited but clear-cut lipotropic effect when added to a hypolipotrophic diet devoid of fat. The lipotropic activity of inositol, even under favorable conditions, is well below that of choline. Slight synergism of the two factors is found; if the total lipid content in a fatty liver is 22-25%, the action of inositol produces a reduction to 13-17%, choline to 5-7%, and both factors together to 4-5%. Since myoinositol is a normal component of lipid, its absence would be expected to cause disorders in lipid metabolism. Yagi and Kotaki (1969) studied the influence of high doses of myoinositol on hepatic phospholipid metabolism and found considerable differences in the formation of phosphoinositides in microsomes and mitochondria during the first period of synthesis from myoinositol.

Martin et al. (1941) reported increases of peristaltic activity in the stomach and small intestine of dogs in the presence of myoinositol
which may produce a transitory cathartic effect (Bly et al., 1943).

One of the major functions of myoinositol is to serve as a precursor of more complex molecules, such as glycosides, phytates and phosphoinositides.

Galactinol appears to be the galactosyl donor in the synthesis of oligosaccharides of the raffinose series (raffinose, stachose, verbascose) in plants (Tanner and Kandler, 1968; Lehle et al., 1970). The function of myoinositol is that of a coenzyme.

Phytic acid can bind with calcium, iron and magnesium in the intestine, thereby hindering the absorption of these ions. Phytate clearly serves as a storage form of phosphate, and the possibility is being investigated that a portion of this phosphate has a sufficiently high $\Delta G^\circ$ of hydrolysis to act directly as an energy source in metabolism (Morton and Raison, 1963). Furthermore, phytic acid immobilizes cations and can be considered as a reserve of metallic elements in plants.

The phosphoinositides, along with other phospholipids, are components of cellular-membrane structures. The phosphoinositides are found in significant amounts in nervous and secretory tissues. Their turnover is more rapid than that of any other phospholipid moiety and can be increased with appropriate stimulation or excitation of the tissue (White et al., 1974; Abdel-Latif et al., 1977).

Phospholipids seems to be involved in at least three ways in the physiological activity of nerve tissue. Firstly, phosphatidylinositol and its precursor, phosphatic acid, take part in the process of ganglionic transmission. Secondly, the polyphosphoinositides are concerned in some way with the functioning of the neuronal plasma membrane, includ-
ing the axolemma. Thirdly, phospholipid is needed for the activity of the sodium pump ATPase in nervous tissue (Hawthorne, 1973). Durell and Garland (1969) reported an interesting hypothesis on the role of phosphoinositides in membrane depolarization, based on the observation that acetylcholine stimulated the hydrolysis of phosphoinositides by phosphodiesterase action. The high affinity of the polyphosphoinositides for Ca\(^{++}\) (Hauser and Dawson, 1967) lends support to the speculation that these lipids may have a role in the control of membrane permeability possibly via binding and release of calcium (Kai and Hawthorne, 1969). Furthermore, the increased turnover of phosphatidylinositol is associated with transport processes in a wide variety of tissues, such as phagocytosis by polymorphonuclear leucocytes (Karnovsky et al., 1964), denervation of rat gastrocnemius muscle, secretion of hormones by thyroid gland and zymogen secretion by the pancreas. It also accompanies secretion by salivary glands and by salt-glands of sea birds. It is not seen, however, in the secretion of bicarbonate induced in pancreas by secretion, nor in the secretion of steroid hormone by the adrenal cortex (Hawthorne, 1973). The increased synthesis of phosphatidylinositol (and phosphatidic acid) accompanying transport processes leads to the hypothesis that the membrane is opened up at specific areas rich in phosphatidylinositol by an enzyme of the phospholipase C type specific for inositol lipids (Atherton and Hawthorne, 1968). This would be more economical than the destruction of all the membrane phospholipids. Closure of the membrane presumably requires a supply of phosphatidylinositol transported from its site of synthesis on the endoplasmic reticulum. Examining KB cells cultured in myoinositol deficient medium, Lembach and Charalampous (1969)
indeed reported decreases of the initial influx rate and $V_{\text{max}}$ of the absorption of three amino acids examined. The magnitude of this decrease depends on the severity of inositol deficiency.

Phosphoinositides has been reported to possess anti-thromboplastin properties (Overman and Wright, 1948).

Liver mitochondria swollen and pre-aged for several hours or days at 2°C in 0.25 M sucrose are not able to contract below pH 7.4 upon addition of ATP, Mg$^{++}$ and serum albumin unless protein fractions from mitochondria are added. These fractions lose their activity after extraction of their lipid components. The only lipid capable of restoring the ATP-linked contraction is phosphatidylinositol (Vignais et al., 1963).

**Absorption and Metabolism of Inositols**

Active transport of myoinositol has been demonstrated in bacteria (Deshusses and Reber, 1978), central nervous system of mammal (Spector, 1978), hamster small intestine (Caspar and Crane, 1970), rat kidney cortex (Hauser, 1969) and Ehrlich ascites cells (Johnstone and Sung, 1967). Biosynthesis of myoinositol through the cyclization of D-glucose with the chain intact has been reported in yeast (Chen and Charalampous, 1964; Kindl et al., 1965), higher plants (Loewus, 1963; Kindl et al., 1966), and mammalian systems (Eisenberg et al., 1964). The key enzyme involved is D-glucose-6-phosphate cycloaldolase, an enzyme catalyzing the NAD-dependent transformation of D-glucose-6-phosphate to L-1-O-phosphomyo-inositol which can then be hydrolyzed to myoinositol (Eisenberg, 1967; Chen and Charalampous, 1966; Pina, 1969). D-Glucose-6-phosphate labeled in various ways with deuterium or tritium has been used in several
studies of the enzymic cyclization. The results support the hypothesis that enzyme-bound D-xylo-hexos-5-ulous-6-phosphate or its enol is the cyclizing species (Sherman et al., 1969).

The catabolism of myoinositol can follow at least two different pathways. The myoinositol oxygenase, which brings about the formation of D-glucuronate through an oxidative cleavage between C-1 and C-6 of the myoinositol molecule, had originally been found in kidney tissues by Charalampous (1960). Later, an enzyme of similar action has been demonstrated in yeast and higher plants. Further catabolism of myoinositol in higher animals follows the glucuronate-xylulose-pentose pathway (Dworsky and Hoffmann-Ostenhof, 1965). The second catabolic pathway for myoinositol is restricted to bacteria. Here, the first step is an oxidation of the axial hydroxyl group leading to 2,4,6/3,5-pentahydroxycyclohexanone, followed by a dehydration to D-3,5/4-trihydroxylicyclohexanedione, which then is cleaved nonoxidatively to L-threo-2-deoxy-5-hexosulonic acid and further to dihydroxyacetone phosphate and malonic semialdehyde (Hoffmann-Ostenhof, 1969).

Direct methylation of myoinositol, catalyzed by position specific enzymes, appears to be the route of biosynthesis of the methyl ethers sequoyitol, D-ononitol, and D- and L-borneitol in plants (Kindl and Hoffmann-Ostenhof, 1966; Wagner et al., 1969).

The epimerization of the myo- to the scyllo and D- and L-chiro-configuration is accomplished by dehydrogenation to intermediate inososes, and then rehydrogenation of these inososes in the opposite steric sense. The formation of scylloinositol by this process has been demonstrated in animals (Sherman et al., 1978) and in an actinomycete
Studies with cell-free enzyme systems have indicated that myoinositol is incorporated into phosphatidyl inositol by reaction of the free cyclitol with a cytidine 5'-(diglyceride pyrophosphate) (Agranoff et al., 1958). Diphosphoinositide and triphosphoinositide appear to be formed in brain, by the stepwise phosphorylation of phosphatidylinositol (Brockerhoff and Ballou, 1968; Prottey et al., 1968).

**Inositol in Parasites**

Myoinositol has been identified by gas chromatography in hydrolyzed tissues of helminths, such as, *Clonorchis sinensis*, *Schistosoma japonicum*, *Paragonimus westermani*, *Necator americanus* and *Dirofilaria immitis* (Ueda and Swada, 1968). Inositol phosphatides are fairly widely distributed in parasites. They have been identified in parasitic protozoa as *Crithidia fasiculata* (Meyer and Holz, 1966; Carter et al., 1966), *Trypanosoma rhodesiense* (Dixon and Williamson, 1970), *Plasmodium knowlesi* (Ginger, 1967; Rock et al., 1971); cestodes as *Hymenolepis diminuta* (Ginger and Fairbairn, 1966), *Spirometra mansonoides* (Meyer et al., 1966); nematodes as *Ascaris lumbricoides* (Beams, 1964; Subrahmanyam and Venkatesan, 1968), *Dirofilaria immitis* (Hack et al., 1962); and acanthocephala as *Macracanthorhynchus hirudinaceus* and *Moniliformis dubius* (Beams and Fisher, 1964). There is no information on the presence of any other inositol isomers in parasites and one of the objectives of this study is to illustrate the qualitative and quantitative deposition of inositol in the cestode, *Hymenolepis diminuta*.

**Morphology and Body Wall of Cestodes**

The body of the typical cestode can be divided into three regions:

1) the scolex with holdfast organs at the anterior end; 2) the unsegment-
ed, poorly differentiated, germinative region giving rise to immature proglottids; and 3) the strobila (chains of proglottids) constituting the main bulk of the body. The most anteriorly situated proglottids are generally immature. Proglottids posterior to the immature ones are sexually mature, while those toward the posterior end of the body are usually gravid, that is, filled with eggs. In some species, the gravid proglottids drop off from time to time, thus permitting the eggs to escape from the host along with fecal wastes. During development and maturation of reproductive organs, the tapeworms demonstrate protandrous hermaphroditism; the male organs become functional before the female ones.

When observed with the light microscope, the body wall of cestodes is made up of several layers. The outermost layer of the body surface, formerly called the cuticle, is more appropriately designated as the tegument. When studied with the electron microscope, minute projections (microtriches or microvilli) can be observed projecting from the outer membrane of the tegument. The dimensions of these projections vary, depending on the species and location. Those found on the strobila of *H. diminuta* are 750 nm long, while those of *Calliobothrium* and *Lacistorhynchus* may reach 2 μm in length (Lumsden, 1966). Rothman (1963) has suggested that the distal portion of each microthrix, which is solid, is concerned with two functions: 1) it may serve as a means of resisting the intestinal current, since the body surface is in intimate contact with the microvilli of the striated border of the cells lining the host's small intestine, and 2) it may serve to agitate the microhabitat in the vicinity as the worm moves, thus stirring up the intestinal fluids so that nutrient materials as well as waste products are always in a state
flux. The proximal portion of each microthrix is medullated and could very well serve as sites of absorption.

The cytoplasmic layer from which the microvilli arise is a syncytium containing mitochondria, Golgi apparatus, ribosomes and endoplasmic reticulum. Glycogen granules also occur in this region in many species. Some of the vesicles found in this layer are believed to be endocytotic vesicles. Others may be secretory and concerned with the membrane digestion phenomenon.

The external level of the tegument is joined with the internal level by cytoplasmic bridges. Unlike the external level, the internal level consists of discrete cells known as cytons which are rich in glycogen deposits and often also include lipid globules.

Immediately beneath the external level of the tegument is a structure of connective tissue known as the basal lamina. Mediated to the basal lamina is found the body wall musculature, consisting of a circular and a longitudinal layer.

The space enclosed by the body wall, except that occupied by the reproductive organs, osmoregulatory structures, muscle fibers and nervous tissue, is filled with a spongy type of tissue, the parenchma. Cestodes do not possess any digestive tract. They must obtain all of their nutrients from the luminal contents of the host intestine by absorption across their body surfaces which are therefore functionally equivalent to the brush border of the vertebrate intestine mucosa.

The arthropod-vertebrate life cycles of these helminths are easily maintained in the laboratory. The size of individual worms can be controlled by manipulating the intensity of infection, and it is possible
to obtain large numbers of worms of uniform size at predictable stages of development. They are, therefore, ideal tools for the study of membrane transport phenomena.

*Hymenolepis diminuta* is absolutely dependent on host dietary carbohydrate for their growth and energy metabolism (Read, 1959; Roberts, 1966). The present investigation was undertaken to characterize the site and mechanism of inositol transport and their relationships to those of other carbohydrates in *H. diminuta*.

**The Nature of Absorptive Mechanisms**

The passage of molecules through membranes can occur by either a process of simple diffusion or mediated absorption. In some cases, the mechanism of permeation may be extended to include mass flow through pores and endocytosis. Diffusion involves no specific structural relationship between the membrane and the permeating material and is described by Fick's law. Mediated absorption involves a specific structural relationship between the membrane and the permeating material and deviates from Fick's law in that the unidirectional flux of permeant becomes saturated with increasing substrate concentration. The kinetics of the mediated system resemble those of classical enzyme or adsorption phenomena and are characterized by the following properties: 1) saturation kinetics, 2) greater transfer rates than predicted for simple diffusion, 3) stereospecificity involving competitive inhibitions by chemically similar compounds, and 4) inhibition by poisons of energy metabolism. Mediated systems are divided into two categories, facilitated diffusion and active transport. The primary difference between the two is the accumulation of a substrate against its electrochemical potential difference.
as a result of expenditure of energy by the cell by the active system and the lack of accumulation by the facilitated system.

The absorption of compounds by some parasitic helminths has been attributed to facilitated diffusion, but in almost all instances apparently involving this mediated process, the solute being absorbed is metabolized at an extremely rapid rate and not accumulated against a concentration gradient (Pappas and Read, 1975). Some parasitic helminths may absorb a compound by a combination of mediated uptake and diffusion. In such cases, the rate of absorption is not linear with respect to substrate concentration at low substrate concentrations, but becomes linear at higher substrate concentrations. The fact that mediated uptake can be inhibited by structurally similar compounds, while diffusion cannot, has been used extensively in discerning the presence of two distinct transport mechanisms for the absorption of a single solute.
MATERIALS AND METHODS

Maintenance of Infections

_Hymenolepis diminuta_ were obtained from South Carolina Biological Supply Co. as cysticercoïds in adult *Tenebria* sp. Ten or thirty cysticercoïds were force-fed to each rat host. Male albino rats of Tex SD strain weighing 100-125 grams at the time of infection were used as definitive hosts in all experiments. Before and after infection, the rats were provided with water and food (Purina Laboratory Chow) _ad libitum_. Parasites were flushed from the excised guts with saline consisting of: NaCl 120 mM, KCl 5 mM, CaCl₂ 1 mM, MgCl₂ 1 mM, NaHCO₃ 10 mM, Na₂HPO₄ 7.5 mM, KH₂PO₄ 2.5 mM at pH 7.4, 275-280 mosmo/kg and equilibrated with 5% carbon dioxide in nitrogen (see Appendix). Flushed parasites were washed in several changes of the same medium before experiments were initiated.

Tissue Preparation

Samples of parasites were homogenized in 19 volume (w/v) of chloroform:methanol (2:1) according to Folch et al. (1959) and the extract washed as described by those authors. The saline wash solution used was 0.05% CaCl₂ and the solvent upper phase contained 0.02% of the same salt. The residue was extracted with distilled water three times. The water washes from the Folch procedure as well as the water extract of the residue were combined and dried under nitrogen at room temperature. This fraction contained the free carbohydrates.

The washed total lipid fraction was evaporated under nitrogen and the residue dissolved in 1 ml of 6N HCl which was then incubated for 48 hr in a sealed ampule at 110°C. Hydrochloric acid was removed by
evaporation and the residue dissolved in small volume of water before passage through attached columns of Dowex 50 (H⁺) and Amberlite IRA 400 (OH⁻). The resultant sample was reduced to dryness on a rotary evaporator in vacuo.

In order to confirm the identity of inositol in the extract, procedures of Agranoff et al. (1958) were employed. All reducing sugars were converted to their corresponding acids by heating with 0.3 N barium hydroxide. After precipitation of barium with zinc sulphate (5%), the acid moieties were removed with ion-exchange resins as above. Extracts thus purified contained only cyclitols and open-chain alditols.

**Preparation of Trifluoroacyl (TFA) Derivatives**

Dried samples were processed according to the method of Tamio et al. (1967). One mg of a free sugar dry sample or extract from 200 mg wet weight (ww) of worm sample and 0.3 mg of sodium trifluoroacetate were dissolved in 0.05 ml of formamide by heating. The solution was cooled to room temperature and 0.2 ml of trifluoroacetic anhydride (TFAA) was gradually added with cooling in an ice bath. The mixture was incubated for 10 min at room temperature (26°C) and diluted with acetonitrile according to Zenzo et al. (1967) immediately before analysis by gas-liquid chromatography (GLC). This procedure prevented any loss of sample due to degradation of the derivatives.

**Preparation of Trimethylsilyl (TMS) Derivatives**

The carbohydrates from dried samples were converted to their TMS ethers by addition of 1 µl of a mixture of pyridine-hexamethyldisilazane-trimethylchlorosilane (17:2:1, v/v/v) per mg of original tissue (ww). Such samples were then stored in a desiccator for 48 hr at room tempera-
ture and diluted ten times with hexane prior to analysis by GLC (Sherman et al., 1968).

**Gas Chromatography**

A Varian Aerograph Model 2100 equipped with electron capture detectors (tritium) and Model 20 recorder were employed for all analyses. Glass columns of 6 ft by 2 mm (I.D.) were used in all analyses. The column packings utilized were: 3% nitrile silicone (XE-60) on Gas Chrom Q, 5% DC-200 on Gas Chrom Q, and 5% QF-1 on Gas Chrom Q. All packings were purchased precoated from the Applied Science Laboratory, State College, PA. Inositol standards were obtained from the following sources: Myo-inositol, Aldrich Chemical Co.; scyloinositol, California Biochemical Research; and myoinosos-2, Sigma Chemical Co. All other chemicals used were of reagent grade. Pyridine was dehydrated by storage over molecular sieve 4A. Peak area was determined by triangulation.

**Chemical Analysis**

Worms were dried at 95°C under vacuum. Total worm water was estimated from wet weight and dry weight comparisons.

Dry weights of total lipid were determined from aliquots of washed chloroform extracts dried under nitrogen and stored over KOH for 24 hr.

Quantities of phosphorus present in the lipid extracts were estimated according to Ames (1966).

**Absorption Experiments**

Worms from three or four rats were randomized and sorted into pools of 5, each pool constituting a sample. Each sample was preincubated in 5 ml of saline at 37°C for 30 min prior to the experimental incubation. Standard incubation periods were 4 min at 100 oscillations/min and 37°C
in $^{3}$H-labelled substrate with a specific activity adjusted from 0.05 μCi/μmole depending on the nature of the experiment. Upon removal from the incubation medium, the worm sample was rinsed rapidly in 3 changes of saline (50 ml each), blotted free of excess moisture on filter paper, and placed in 5 ml of 70% ethanol. Tubes were agitated several times during the subsequent 24-hr extraction period. Radioactivity in the alcohol extracts was assayed by use of a Packard Tri-Carb liquid scintillation spectrometer with Biofluor (New England Nuclear). Dry weights of alcohol-extracted samples were obtained by heating worms at 100°C for 24 hr.

To study the metabolism of myoinositol by *Hymenolepis diminuta*, ten worms were incubated in 10 ml of 0.1 mM $^{14}$C-myoinositol (2 μCi/μmole) for 1 to 4 hrs. After washing through three changes of saline, free and bound myoinositol were extracted, separated by chromatographic methods, and the radioactivity analyzed as above.

Myo-$(2-^3$H)-Inositol and myo-$(U-^{14}$C)-Inositol were obtained from Amersham Corporation. Uniformly labelled $^{14}$C-glucose was obtained from New England Nuclear Corporation. Reagent grade chemicals and unlabelled carbohydrates of the highest purity were obtained from commercial sources. Uptake velocities were expressed in terms of μmoles inositol absorbed/g ethanol extracted dry wt/4 min.

**Chromatographic Methods**

Ethanol extracts of radioactive myoinositol were examined by paper chromatography on 3 MM paper in acetone:water (9:1) (descending) or propanol:water:pyridine:acetic acid (8:4:8:1) (ascending). High voltage paper electrophoresis on Whatman #1 paper was carried out in 0.05 M sod-
ium borate at pH 9.2 and 20 V/cm for 1.5 hr. Stable carbohydrate standards were mixed with comparable amount of extract to compensate for the interference of any water-soluble components. Carbohydrates were visualized with the silver nitrate method of Trevelyan et al. (1950). Electrophoretograms were treated with hydrogen fluoride in acetone (Britton, 1959) before detection. Radioactive spots were localized by using a fas-flow radiochromatogram scanner. Radioactivity was quantitatively determined by liquid scintillation counting. Counting efficiency was determined by the channel ratio method.

Phospholipids were separated on precoated silica gel G plates (Applied Science Laboratory) using the two-dimensional system of Rouser et al. (1967): chloroform:methanol:conc. ammonia (65:35:5, v/v) and chloroform:methanol:acetone:acetic acid:water (5:1:2:1:0.5, v/v). Lipid spots were detected by exposing the developed plates to iodine vapor and identified by comparing with phospholipid standards purchased from Sigma Chemical Company. Identified spots were scrapped from the plates and radioactivity determined by liquid scintillation counting in a cocktail containing 5 g PPO, 0.3 g POPOP, 130 ml of methanol, and 100 ml of Bio-solv solubilizer in 1 liter of toluene.

**Statistical Method**

Student's "t" test was employed as analysis of variance for the difference between means. Data presented graphically were plotted as least square regression lines where applicable.
RESULTS

Quantitative Determination of Inositol in Hymenolepis diminuta

The presence of free inositol in *H. diminuta* was first demonstrated by paper chromatographic analysis of ethanol extract of worm samples using the solvent system as described. Further examination of TFA derivatives of worm extracts on QF-1 and DC-200 by GLC confirmed this finding and suggested the presence of both myoinositol and scylloinositol (Fig. 2).

Preliminary studies demonstrated a linear relationship between the mass of TFA derivatives of myoinositol and the height of the resulting peak over a range of 0.1 to 0.42 ng. The procedure used was tested for the ability to recover added myoinositol. Four groups of five 10-day-old worms were each homogenized in 1 ml of water and the free sugar contents of 0.4 ml of the resulting solutions were extracted and analyzed. To another 0.4 ml aliquot, 50 µg of standard myoinositol was added prior to analysis. The amount of added inositol recovered, after correction for the tissue content, was equivalent to an average of 96.5% (range: 94.3%–102.4%).

The amount of free myoinositol, scylloinositol and phosphatidyl-bound inositol in 8-day to 18-day-old worms is presented in Table 1. The young worm contains more free myoinositol and phosphatidyl-bound inositol than the aged worms while the quantity of scylloinositol is relatively constant in all age groups. These results suggest that the distribution of these compounds is correlated with the growth pattern.

In order to investigate further, 18- to 22-day-old worms were pooled from three to four rats, extended on wet filter paper and cut in-
TABLE 1

Quantitative analysis of myoinositol, scylloinositol and phosphatidyl-bound inositol in *Hymenolepis diminuta* of different age.

<table>
<thead>
<tr>
<th>Age</th>
<th>Free myoinositol % wet wt.</th>
<th>Free scylloinositol % wet wt.</th>
<th>Phosphatidyl-bound inositol % wet wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 day</td>
<td>0.0260 ± 0.0011</td>
<td>0.0043 ± 0.0010</td>
<td>0.0063 ± 0.0009</td>
</tr>
<tr>
<td>10 day</td>
<td>0.0241 ± 0.0013</td>
<td>0.0047 ± 0.0014</td>
<td>0.0059 ± 0.0013</td>
</tr>
<tr>
<td>13 day</td>
<td>0.0216 ± 0.0007</td>
<td>0.0045 ± 0.0012</td>
<td>0.0054 ± 0.0009</td>
</tr>
<tr>
<td>15 day</td>
<td>0.0186 ± 0.0012</td>
<td>0.0041 ± 0.0008</td>
<td>0.0045 ± 0.0011</td>
</tr>
<tr>
<td>18 day</td>
<td>0.0193 ± 0.0005</td>
<td>0.0044 ± 0.0012</td>
<td>0.0052 ± 0.0007</td>
</tr>
</tbody>
</table>
to four sections corresponding to: 1) scolex and germinative region (anterior 2 cm), 2) immature region, 3) mature region, and 4) gravid proglottids. These different regions were extracted and the inositol, lipid and water content were analyzed. The results are shown in Table 2. The anterior region contains the highest content of free myoinositol, phosphatidyl-bound inositol and lipid-bound phosphorus, but less water and lipid.

The dietary contribution of myoinositol and scylloinositol was determined by two methods. Powdered diet (0.2 gm) (Purina Laboratory Chow) was dissolved in 2 ml of water with heating at 100°C for 10 minutes. The suspension was adjusted to 5 mM with ZnSO₄ and centrifuged. The supernatant fluid was then dried under nitrogen and derivatized by TFAA. The diet was found to contain 0.38 gm/kg myoinositol but no scylloinositol. Another portion of diet was hydrolyzed by heating 0.5 gm of powdered diet in 2 ml of 6 N HCl at 110°C for 48 hrs. The hydrolysate was centrifuged and the supernatant fluid reduced to dryness. The dried residue was dissolved in a small amount of water, deionized and derivatized as above. After hydrolysis, the myoinositol content was 0.86 gm/kg; no scylloinositol could be detected.

Myoinosos-2 was eluted together with a fructose component on QF-1, but in the analysis of TFA derivatives of worm extracts no myoinosos-2 peak could be found on DC-200. In order to confirm this, the procedure of Sherman et al. (1968) was followed. According to their technique, TMS myoinosos-2 can be separated from other TMS sugars on 3% XE-60 and detected with great sensitivity by electron capture detectors due to its structure. The results were still negative for H. diminuta when this
<table>
<thead>
<tr>
<th>Region</th>
<th>Free myo-inositol</th>
<th>Free scyllo-inositol</th>
<th>Scyllo myo</th>
<th>Conc. of myo-inositol</th>
<th>Lipid content</th>
<th>Lipid-P % of lipid</th>
<th>Lipid bound inositol</th>
<th>Bound inositol lipid-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>0.0318 ± 0.0049</td>
<td>0.0045 ± 0.0013</td>
<td>14.05 ± 1.99</td>
<td>68.71 ± 2.58</td>
<td>0.4628 ± 0.3126</td>
<td>4.3079 ± 0.0481</td>
<td>1.1166 ± 0.0027</td>
<td>0.0076 ± 0.0001</td>
</tr>
<tr>
<td>2 cm region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature region</td>
<td>0.0226 ± 0.0029</td>
<td>0.0044 ± 0.0005</td>
<td>21.05 ± 1.06</td>
<td>72.29 ± 3.35</td>
<td>0.3126 ± 0.0029</td>
<td>5.6889 ± 0.0389</td>
<td>0.6838 ± 0.0064</td>
<td>0.0065 ± 0.0007</td>
</tr>
<tr>
<td>Mature region</td>
<td>0.0213 ± 0.0046</td>
<td>0.0049 ± 0.0016</td>
<td>24.33 ± 2.09</td>
<td>73.83 ± 3.21</td>
<td>0.2885 ± 0.0035</td>
<td>6.4677 ± 0.0355</td>
<td>0.5489 ± 0.0061</td>
<td>0.0046 ± 0.0004</td>
</tr>
<tr>
<td>Gravid region</td>
<td>0.0130 ± 0.0059</td>
<td>0.0036 ± 0.0012</td>
<td>34.92 ± 2.96</td>
<td>66.43 ± 2.40</td>
<td>0.1701 ± 0.0063</td>
<td>6.9130 ± 0.0325</td>
<td>0.4701 ± 0.0063</td>
<td>0.0040 ± 0.0010</td>
</tr>
</tbody>
</table>

* Each value, except lipid content, represents average of three to seven determinations.
technique was used (detection limit: 0.01 ng). From these studies we feel confident that myoinositol-2 is not present in *H. diminuta*.

Ginger and Fairbairn (1966) reported synthesis of water soluble components of lipid, such as glycerol and inositol from glucose in *H. diminuta*. Since the Purina Laboratory Chow was found to contain myoinositol, *H. diminuta* might also be able to absorb it in vivo. Further investigation was therefore undertaken to characterize the sites and mechanism of inositol transport and their relationships to those of other carbohydrates in this parasite.

**The Membrane Transport of Inositol by *Hymenolepis diminuta***

The rate of absorption of radiomyoinositol from a 0.01 mM solution was found to be constant over a period of twenty minutes (Fig. 3). Four minutes was therefore chosen as a convenient incubation period for all kinetic studies.

Since myoinositol was sparingly soluble in alcohol, the efficacy of the extraction method was tested. After incubation, the worms were extracted with 5 ml of 70% ethanol overnight. An aliquot of the ethanol extract was counted. The worms were washed thoroughly, blotted and solubilized by perchloric acid-hydrogen peroxide oxidation (Mahin and Lofberg, 1966). The amounts of radioactivity recovered from the ethanol extracts were then compared with those recovered from the ethanol extract plus the activity remaining in the worms as measured from the solubilized tissue. The percentage recovery was calculated to be 96.57 ± 0.93 (n=7). Paper chromatography of the ethanol extract confirmed that only a single component identified as myoinositol was present.

**Effect of substrate concentration on the uptake of myoinositol:**

The absorption process was linear in the substrate concentra-
tion range of 0.03 mM to 30 mM (Fig. 4) suggesting that diffusion might be involved. At low substrate concentrations (0.002 mM to 0.02 mM), however, uptake was non-linear with respect to substrate concentration and, in part, appeared to follow the kinetics of a catalyzed reaction (Fig. 4). To establish the diffusion rate, it was assumed that at high substrate concentrations any mediated process of uptake was saturated and increases in rate with increase in concentration was a result of diffusion. Thus, the slope at high substrate concentrations of a V vs. S plot was an indication of diffusion rate. A curve for the mediated process, corrected for diffusion is also presented in Figure 4, from which the V\text{max} and Kt are calculated to be 0.0105 μmoles/g ethanol extracted fry wt/4 min and 0.0067 mM, respectively (Figs. 5, 6).

As the concentration of stable myoinositol in the incubation medium was increased, the initial rate of uptake of labelled myoinositol (0.006 mM) decreased rapidly and levelled off, supporting the hypothesis that mediated transport was involved and further demonstrating a component of uptake which was not inhibited by increases in inhibitor concentration (Fig. 7).

**Effect of various carbohydrates and amino acids on myoinositol transport:**

Information concerning the specificity of the transport site was obtained by testing the effect of various compounds upon uptake of \(^3\)H-myoinositol. Labelled myoinositol absorption was inhibited by stable myoinositol, scyllinositol, myinosos-2, inositol-2-phosphate, sodium phytate, streptomycin, glucose, galactose and 6-deoxyglucose. But, none of the sugar alcohols or amino acids tested had effect on myo-
inositol transport, indicating its substrate specificity (Table 3). The inhibition by D-glucose appeared to be non-competitive (Figs. 5, 6). In order to determine the inhibitor constant (K_i) of D-glucose, constant concentrations of myoinositol were incubated with different concentrations of D-glucose (Fig. 8). An K_i of 0.56 mM was obtained. To investigate if the glucose effect was internal, worms were preincubated in 2.5 mM glucose for 30 minutes, rinsed quickly in 3 changes of saline and then incubated with 0.01 mM \(^3\)H-myoinositol. Instead of inhibition, a 33.97% stimulation of the mediated inositol uptake was obtained when compared with the control.

Effect of metabolic poisons on the uptake of myoinositol:

Several compounds commonly regarded as metabolic inhibitors were studied to determine whether they would affect myoinositol uptake by \textit{H. diminuta} (Table 4). The addition of phlorizin and parachloromercuribenzoic acid decreased the uptake of myoinositol (0.01 mM) markedly even without preincubation in the specific inhibitor. All inhibitors tested, except phloretin, inhibited myoinositol transport after 30 minutes preincubation with their presence. Phlorizin acted as a competitive inhibitor with a K_i of 22 \(\mu\)M (Fig. 9). To study if the effect of phlorizin was reversible, worms were preincubated in phlorizin (0.5 mM) for 30 minutes, rinsed quickly in three changes of saline and incubated in 0.01 mM \(^3\)H-myoinositol with or without the inhibitor. The degree of inhibition of the mediated process were 75.38% and 27.32%, respectively.

Effects of pH and temperature on the uptake of myoinositol:

To study the effect of pH on the uptake of inositol by \textit{H. diminuta}, worms were preincubated in balanced salt solution at pH 7.2
TABLE 3

Effects of various carbohydrates and amino acids on the uptake of

0.01 mM $^3$H-myoinositol by *Hymenolepis diminuta*.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibitor concentration</th>
<th>% Inhibition of mediated process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoinositol</td>
<td>2.5 mM</td>
<td>100</td>
</tr>
<tr>
<td>Scyloinositol</td>
<td>2.5 mM</td>
<td>100</td>
</tr>
<tr>
<td>Myoinosos-2</td>
<td>2.5 mM</td>
<td>98</td>
</tr>
<tr>
<td>Inositol-2-P0$_4$</td>
<td>2.5 mM</td>
<td>61.40</td>
</tr>
<tr>
<td>Sodium phytate</td>
<td>2.5 mM</td>
<td>71.12</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.5 mM</td>
<td>57.45</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5 mM</td>
<td>50.58</td>
</tr>
<tr>
<td>Galactose</td>
<td>2.5 mM</td>
<td>43.20</td>
</tr>
<tr>
<td>6-Deoxyglucose</td>
<td>2.5 mM</td>
<td>49.73</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Adonitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Arabitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Ducitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Presitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Xylitol</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.0 mM</td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2.5 mM</td>
<td>0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.0 mM</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 4

Effect of metabolic poisons on the uptake of 0.01 mM 

$^3$H-myoinositol by *Hymenolepis diminuta*.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>n</th>
<th>Inhibitor concentration</th>
<th>Preincubation in inhibitor</th>
<th>% Inhibition of mediated process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloretin</td>
<td>4</td>
<td>0.01 mM</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>4</td>
<td>0.50 mM</td>
<td>+</td>
<td>75.38</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>2</td>
<td>0.50 mM</td>
<td>--</td>
<td>81.13</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>3</td>
<td>0.10 mM</td>
<td>+</td>
<td>59.97</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>3</td>
<td>0.10 mM</td>
<td>--</td>
<td>8.51</td>
</tr>
<tr>
<td>Iodoacetamide</td>
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<td>+</td>
<td>33.10</td>
</tr>
<tr>
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<td>--</td>
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</tr>
<tr>
<td>Oubain</td>
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<td>+</td>
<td>37.58</td>
</tr>
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<td>0.01 mM</td>
<td>--</td>
<td>9.82</td>
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<tr>
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<td>75.64</td>
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</tr>
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<tr>
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<td>--</td>
<td>0</td>
</tr>
</tbody>
</table>
for 30 minutes; uptake of inositol was then determined in the standard 4-minute incubation with labelled inositol at 0.01 mM in the pH range of 6 to 8 (Fig. 10). The velocity of the absorption process dropped gradually with increasing pH in the range studied and the absorption maximum appeared to be at some lower pH which was beyond the buffering capacity of the saline.

After preincubation at 37°C, the parasites were incubated for 4 min in 0.01 mM labelled myoinositol at a specific temperature ranging from 20°C to 60°C. The velocity of uptake rose gradually with increasing temperature to a maximum at about 45°C and decreased thereafter (Fig. 11).

**Effect of Na⁺ ion on the uptake of myoinositol:**

Worms were preincubated in the balanced salt solution for 30 minutes, rinsed in three changes of saline with a specific amount of sodium replaced by choline, blotted dry and then incubated in 0.01 mM labelled myoinositol in the same sodium deficient saline. Myoinositol uptake by *H. diminuta* was Na⁺-dependent and a hyperbolic function of Na⁺ concentration of the ambient medium (Fig. 12).

**Study of the efflux of myoinositol:**

Worms were preincubated in labelled myoinositol (0.01 mM) for 1 hr, rinsed in three changes of saline, blotted dry and then incubated in 4 ml normal saline for 4 min. After incubation, an aliquot of the incubation medium was assayed for radioactivity. The total radioactivity in the worms was determined after ethanol extraction. The amount of radioactive inositol accumulated by the worm was $0.1876 \pm 0.0565 \mu$moles/g ethanol extracted dry wt/hr (n=3). The rate of efflux was estimated to be $0.0060 \pm 0.0013 \mu$moles/g ethanol extracted dry wt/4 min (n=3).
the presence of 0.5 mM glucose and 0.5 mM myoinositol in the incubation medium, the rate of efflux were 0.0066 ± 0.0008 (n=3) and 0.0058 ± 0.0001 (n=3) μmoles/g ethanol extracted dry wt/4 min, respectively.

**Effect of myoinositol on glucose absorption:**

When 10 mM myoinositol was tested as inhibitor of the transport of 0.001 mM labelled glucose in standard 2-min incubations, the rate of glucose uptake obtained was 0.02517 ± 0.00281 μmoles/g ethanol extracted dry wt/2 min (n=6) which was not significantly different from the control value of 0.02711 ± 0.00333 (n=6).

**Rate of myoinositol uptake in different region in Hymenolepis diminuta:**

By dividing the mature worms (22 days old) into different segments, the rate of myoinositol uptake (0.01 mM) in the scolex and germinative region and gravid segments were found to be 0.00955 ± 0.00049 (n=4) and 0.00450 ± 0.00035 (n=4) μmoles/g ethanol extracted dry wt/4 min, respectively.

**Metabolism of Absorbed Myoinositol**

In order to ascertain the fate of the absorbed myoinositol, experiments were designed to study the metabolism of myoinositol by *H. diminuta*.

A sample of 5 worms (18 days old) was preincubated in 10 ml of saline containing penicillin 150 μg/ml, streptomycin 150 μg/ml and mycostatin 10 μg/ml for 30 min. The worms were rinsed in 3 changes of saline (50 ml each) and incubated in 10 ml of 0.1 mM myo-(U-14C)-inositol (2 μCi/μmole) for 4 hrs. After incubation, the worm sample was rinsed and extracted according to Folch et al. (1959).

Radioactive inositol was incorporated into lipid in *H. diminuta* at
a rate of 0.0051 μmole/g wet wt/hr. Extracted lipid was separated by thin layer chromatography and 92% of the radioactivity was recovered as phosphatidylinositol.

The water washes from the Folch procedure and the ethanol (70%) extract of the residue were combined, dried under nitrogen at room temperature, redissolved in 70% ethanol and centrifuged. The resulting extract was analyzed by paper chromatography and high voltage paper electrophoresis. Only a single radioactive component identified as myoinositol was present.
DISCUSSION

The amount of free myoinositol present in *H. diminuta* (0.0241% wet wt in 10-day-old worms) is comparable with that of the host skeletal muscle 0.013% (ww), brain 0.056% (ww) and kidney 0.088% (ww) (Posternak, 1965). Posternak et al. (1963) observed that the inositol content of rats fell as the age increased. That this cyclitol decreases with age in *H. diminuta* suggests that it may be related to the growth of the worm. By dividing the parasites into different developmental portions, it was observed that the scolex and germinative region contains more free myo-inositol (at the 95% probability level) than the more mature posterior segments both in terms of percent wet weight and gram per liter of worm water. This is reasonable considering that it is in the region of proliferation that phosphatidylinositol is incorporated into new proglottids. This distribution may be due to either a difference in rate of absorption or rate of removal of inositol along the length of the worm. The absorption of inositol is indeed greater in the anterior portion of the parasite. The distribution of glucose in the worm was observed to be similar to that of myoinositol with 0.2367 ± 0.0414% ww and 0.030 ± 0.0063% ww for the anterior and gravid regions, respectively. Glucose absorption was reported to be more rapid in the anterior region of *H. diminuta* (Phifer, 1960). The fact that the gravid segments contain less myoinositol may explain why there is a decrease in quantity of this compound in the aged worms.

The function of myoinositol has been suggested as a vitamin, a lipotropic agent, a phosphate storage agent, a phosphagen and, the most important of all, as a component of phospholipid. The function of myo-
inositol in *H. diminuta* other than taking part in phospholipid formation is obscure. Addition of it can cause a slight, though perhaps insignificant, decrease in the stunting effect of a vitamin C-deficient diet on rats (Addis and Chandler, 1946), an observation which deserves further study.

In agreement with the previous findings, the anterior portion of *H. diminuta* contains less lipid than the posterior segments (Mettrick and Cannon, 1970). But, the distribution of lipid phosphorus and inositol is opposite to this observation. Phospholipid, especially phosphoinositides and phosphatidic acid has long been shown to be related to membrane transport phenomenon. Phagocytosis is accompanied by the increased incorporation of phosphate into phosphatidic acid, phosphoinositides and phosphatidylinerine (Karnovsky et al., 1961). Phospholipid is reported involving in sugar transport in microorganisms (Milner and Kaback, 1970) and amino acid transport in KB cells is significantly affected by the presence of inositol in the culture medium (Lembach and Charalampous, 1967). It is possible that changes in these lipids, especially phosphoinositides, could regulate ion transport indirectly by altering the properties of cell membranes. The plasma membrane adenosine triphosphatase (ATPase), which is activated by Na⁺ and K⁺ and undoubtedly participates in translocation of these cations, is dependent on the presence of certain phospholipid in a number of tissues (Tanaka et al., 1971). The phospholipid requirement for sarcoplasmic reticulum Ca²⁺ ATPase activity was noted by Martonosi (1968). Using artificial membranes consisting of phosphatidylinerine, which has an approximate Ka for Ca²⁺ of $10^6 - 10^4$ l/mol (Hauser et al., 1976) and phosphatidylcholine, it has
been shown that Ca\(^{++}\) induces a phase separation of calcium-chelated phosphatidylserine aggregates in a fluid phase of phosphatidylcholine (Ohnishi and Ito, 1974). Such alternations may greatly affect lipid properties such as ion permeability and influence protein activation as well.

Adenosine triphosphatase activation (Na\(^{+}\) and K\(^{+}\)) has been demonstrated to be present in *H. diminuta* (Galogly, 1972) and there is evidence indicating that membrane transport processes in *H. diminuta* may be coupled with ATPase function (Dike and Read, 1971; Read et al., 1974; Pappas et al., 1974). The anterior portion of *H. diminuta* accumulates glucose at a rate over three times that of the posterior portion of the parasites (Phifer, 1960). The rate of myoinositol absorption is also more rapid in this region. The distribution of lipid phosphorus, being two times greater in the anterior region, suggests that there may be some relationship between phospholipid and membrane transport in this parasite. Other than having a higher phosphorus content, the lipid of this region also contains more phosphatidyl-bound inositol, giving a higher value both in terms of percent wet weight and in terms of inositol:phosphorus ratio although this region actually contains less lipid. The rate of incorporation of P\(^{32}\)-inorganic phosphate into phosphatidylinositol is faster than in any other phospholipid component except phosphatidic acid (Webb and Mettrick, 1971). Studying the incorporation of label from \(^{14}\)C-glucose into lipid of *H. diminuta* (Ginger and Fairbairn, 1966) showed that phosphatidylinositol has the highest specific activity in comparison with the other phospholipid components. These observations suggest that the rate of turnover of phosphatidylinositol inside the
worm is very fast and alludes to the lability of this active component. On the other hand, scylloinositol levels are quite constant throughout the worm. The scylloinositol:myoinositol ratio increases towards the gravid portion of the worm due to decrease in myoinositol content. The function of scylloinositol in this parasite as well as other animals is unknown.

The food fed to the rats contained a considerable amount of myoinositol. The increase in the level of myoinositol in the diet following hydrolysis of the diet can be explained by release from inositol phosphates that are normally present in plant tissues which represent a substantial portion of the diet.

Since scylloinositol was not present in the diet, its presence in the worm suggests that 1) the worm may be able to synthesize it, 2) scylloinositol is synthesized by the rat host and become available to gut dwelling parasites, or 3) the intestinal bacterial flora synthesize scylloinositol. LABELLED myoinositol administered to the rat could be recovered as scylloinositol in homogenates of the whole animal (Posternak et al., cited in Sherman et al., 1968).

The presence of scylloinositol and myoinositol in organisms is usually accompanied by myoinosos-2 (Sherman et al., 1968, 1978) which is suggested to be an intermediate formed in the interconversion of the above cyclitols (Sherman et al., 1968; Hips et al., 1973, 1976). We were unable to demonstrate the presence of myoinosos-2 in H. diminuta, hence, this metabolic pathway must either be absent or is different from that has been suggested for other organisms.

Ginger and Fairbairn (1966) reported synthesis of inositol from glucose in H. diminuta. The present investigation demonstrates that the
worm can also accumulate inositol from its ambient surroundings.

Active myoinositol transport has been reported in bacteria (Deshusses and Reber, 1978) and a number of mammalian tissues. The myoinositol transport system within the choroid plexus is saturable, concentrative, and energy dependent (Spector, 1978). The uptake of myoinositol against a concentration gradient by kidney cortex slices (Hauser, 1969 a, b) and hamster small intestine (Caspar and Crane, 1970) has been observed and characterized as Na+-dependent, energy dependent and inhibited by phlorizin. The absorption of myoinositol in *H. diminuta* involves two processes: diffusion at high substrate concentrations, and mediated transport at low substrate concentrations (Fig. 4). At substrate concentrations above 0.03 mM, diffusion appears to be the major component of myoinositol transport. In this dual mode of absorption, myoinositol transport resembles the uptake of uracil (MacInnis et al., 1965), proline (Kilejian, 1966), palmitate (Chappell et al., 1969), acetate (Arme and Read, 1968) and glycerol (Pittman and Fisher, 1972) in the same organism. In comparison with hamster small intestine (Kt=0.14 mM), *H. diminuta* has a lower Kt value for myoinositol transport (0.0067 mM) which may have certain advantage for competition with the host when inositol is present in very low concentrations. After preincubation in labelled myoinositol (0.01 mM) for 1 hr, the amount of radioactive inositol accumulated inside the worm was 0.1876 ± 0.0565 μmoles/g ethanol extracted dry wt. Estimating the ratio of wet weight:dry weight:ethanol extracted dry weight to be 100:23:18, the concentration of radioactive myoinositol inside the worm is calculated to be 0.044 mM assuming that water associated with *H. diminuta* exists in a free state and solutes are
in true solution. It appears, therefore, that this mediated process is concentrative.

The inhibition of $^3$H-myoinositol uptake by stable myoinositol was to be expected if a mediated system was involved in the absorption process. As the inhibitor concentration increased, a greater number of inhibitor molecules would be present to compete with the substrate molecules for the membrane transport sites. Scylloinositol differs from myoinositol in the orientation of the hydroxyl group at the C-2 position, but the degree of inhibition to $^3$H-myoinositol uptake is comparable with stable myoinositol. Also, myoinosos-2, which differs from scylloinositol and myoinositol by having a carbonyl and not a hydroxyl group on the C-2 position, has significant inhibitory effect. However, when the hydroxyl groups are replaced by more bulky phosphate groups as in inositol-2-phosphate and sodium phytate, this inhibitory effect decreases. The antibiotic, streptomycin, also inhibits myoinositol uptake, possibly through the action of streptidine.

Transport of myoinositol is inhibited by glucose and galactose. Glucose acts as a non-competitive inhibitor and its effect is external, either during the binding of myoinositol to specific sites or during the translocation step. Caspary and Crane (1970) demonstrated that interference of glucose and analogs with myoinositol transport in hamster small intestine occurred at the level of translocation. In the present investigation, the two possibilities are not differentiated. The stimulation of myoinositol uptake by preincubation with glucose indicates that the mediated process may be energy dependent or exchange diffusion occurs between the two carbohydrates. However, the lack of effect of glucose
on myoinositol efflux suggests that exchange diffusion is not involved. Using worms from starved rats, Phifer (1960) also showed that preincubation of worms in a metabolizable monosaccharide (e.g., glucose or galactose) resulted in a significant increase in radioactive glucose uptake over that of controls which were not preincubated. In spite of its high affinity for transport, myoinositol does not inhibit transport of glucose. Glucose may sterically hinder myoinositol binding but not the reverse. If interference occurs, considering _V_{\text{max}}_ of glucose transport (27.78 µmoles/g ethanol extracted dry wt/4 min) (McCracken and Lumsden, 1974) to be 2500 times greater than myoinositol transport (0.01053 µmoles/g ethanol extracted dry wt/4 min) and assuming a 1:1 relationship between binding and translocation, it is clear that myoinositol can effect less than 0.1% inhibition of glucose absorption. The myoinositol absorptive site appears to be distinctly different from those of glycerol, sugar alcohols, and amino acids.

Similar to hamster small intestine (Caspary and Crane, 1970) and kidney cortex slices (Hauser, 1969a), the mediated transport of myoinositol in _H. diminuta_ is inhibited by phlorizin and parachloromercuribenzoic acid. Phlorizin is a competitive inhibitor of glucose uptake in _H. diminuta_. McCracken and Lumsden (1974) suggested that phlorizin competed via its glucose moiety with the sugar solute for a common binding site on a mobile carrier, but was not transported. The apparent competitive inhibitory nature of phlorizin on myoinositol transport may be a result of both adsorption of phlorizin to the glucose site through the glucose moiety and formation of secondary bonds between the aglycone portion of the phlorizin molecule and the surface membrane of _H. diminuta_. The
adsorbed molecules can be released by brief rinsing in balanced saline. The hypothesis that phlorizin and phloretin are not competing for the same site on a carrier (McCracken and Lumsden, 1973) is consistent with the observation that phloretin, at the concentration examined, has no effect on myoinositol uptake. All the other metabolic poisons examined have no significant inhibitory effect unless the parasite is preincubated in their presence. A similar preincubation requirement has been reported for iodoacetate and 2,4-dinitrophenol inhibition of glucose uptake by Hymenolepis (Phifer, 1960a,b). The effects of these inhibitors suggest that there may be an energy requirement for mediated myoinositol transport and sulfhydryl bonds may be involved.

The absorption of myoinositol in *H. diminuta* is sensitive to changes in pH and ambient temperature. The absorption maximum for myoinositol appears to be around pH 6. Podesta and Mettrick (1974) demonstrated that pH of parasitized rat intestine was lowered indicating that certain homeostatic mechanisms had been impaired by *H. diminuta* or the acidic secretions of the worms had overwhelmed the intestinal buffering mechanisms. Maximum uptake occurs at about 45°C. At higher temperatures, the rate of myoinositol uptake decreases possibly due to damage of the surface of the parasite.

Dike and Read (1971) showed that glucose transport in *H. diminuta* is Na⁺-dependent and there are evidence indicating that glucose transport may be coupled with ATPase function (Read et al., 1974; Pappas et al., 1974). However, the necessity of preincubating the worms in low Na⁺ medium in these studies led to the argument that the effect of Na⁺ on hexose transport in the parasite might be internal (Podesta and
Mettrick, 1976). Myoinositol transport in *H. diminuta* is also *Na*⁺-dependent, but preincubation in *Na*⁺-deleted medium is unnecessary. Brief rinses of the worms in the specific low sodium saline after preincubation are important in minimizing transfer of *Na*⁺ into the incubation medium. The *Na*⁺ requirement, analogous to that of glucose absorption, indicates that myoinositol and sodium may be cotransported in *H. diminuta*, conceivably by a mobile carrier system similar to that proposed by Crane (1965) for glucose in intestine.

The slow leakage of myoinositol into the medium during 4-min incubation was to be expected if a symmetric diffusive component was involved in the absorption process. The presence of stable myoinositol or glucose in the incubation medium has no significant effect on the rate of myoinositol efflux.

Absorbed myoinositol is rapidly incorporated into phosphatidyl-inositol. Webb and Mettrick (1971) reported the presence of mechanisms in *H. diminuta* for the *de novo* synthesis of phosphatidic acid, phosphoinositide, phosphatidylserine, lecithin, lysolecithin and phosphatidylethanolamine. They suggested that the major pathway utilized by *H. diminuta* in fatty acid incorporation was via phosphatidic acid.

Myoinositol is the precursor of the other inositols and inositol methyl ethers found in plants (Kindl *et al.*, 1966). Inositol-degrading yeasts, higher plants and mammals are equipped with an inositol oxygenase (Charalampous, 1960), which effects a cleavage between C-1 and C-6 according to the equation

\[
\text{myoinositol} + \text{O}_2 \rightarrow \text{D-glucuronate} + \text{H}_2\text{O} + \text{H}^+.
\]
D-glucuronate is converted into D-xylulose and then, via the pentose phosphate sequence, to the intermediates of glycolysis (Hollmann and Touster, 1964; Dworsky and Hoffmann-Ostenhof, 1965). Myoinositol is thus channeled into the mainstream of carbohydrate metabolism. Laurie (1957) reported that _H. diminuta_ could metabolize glucose and galactose but not myoinositol. In agreement with this observation, we were unable to demonstrate synthesis of any organo-soluble components from myoinositol in _H. diminuta_ during the 4 hours of incubation.

Inositol appears to be an important carbohydrate for _H. diminuta_ since the organism is synthesizing (Ginger and Fairbairn, 1966) and actively absorbing it. Preliminary experiments were therefore designed to examine the effect of deleting myoinositol from the diet on the growth and establishment of the parasite in the host.

The standard host diet used in the experiments had the following components: starch (Sigma Chemical Co.) 30%, casein (Nutritional Biochemical Co.) 30%, cellulose (Sigma Chemical Co.) 30%, corn oil (Mazola) 6%, and salt mix #2 (Nutritional Biochemical Co.) 4%. To each 100 g of diet the following was added: thiamine 1.0 mg, riboflavin 5.0 mg, nicotinamide 5.0 mg, folic acid 0.1 mg, pyridoxine-HCl 0.1 mg, choline 10.0 mg, and vitamin _B_12 1 μg. For a diet containing inositol, 0.1 g of myoinositol was added to every 100 g of the basic diet. Water was added to the requisite amount of the dry materials to make a thick paste which was fed to the rats _ad libitum_.

Worm lengths were determined after relaxing the worms in tap water for 45 min.

Two groups of three rats each were fed with the controlled diet
with or without myoinositol. After two days, each rat was force-fed with 30 cysticercoids. The specific controlled diet was fed for another 5 days followed by 5 days of normal diet (Purina Laboratory Chow). The rats were then sacrificed and the number of infections determined. An average of 26.00 ± 1.00 and 24.67 ± 3.05 infections were found in rats fed with and without myoinositol, respectively.

To examine the effect of deleting myoinositol from the diet on the growth of *H. diminuta*, rats infected with 30 cysticercoids were fed with Purina Laboratory Chow during the first 5 days of infections followed by 5 days of controlled diet with or without inositol. Wet weight and length of these 10-day-old worms were determined (Table 5).

Deletion of myoinositol from the diet appears to have no effect on the establishment of the parasite in its host. However, worms recovered from rats fed with controlled diet with or without myoinositol showed differences in wet weight and average length per gram wet weight. This indicates that myoinositol can affect the growth of *H. diminuta* possibly through its functions discussed above. By analyzing the inositol content of these worms, determining their rate of egg production after maturation and comparing the nature of their membrane transport processes, future experiments can be performed to elucidate further the role of inositol in this parasite.
TABLE 5

Effect of controlled diet on growth of *Hymenolepis diminuta*.

<table>
<thead>
<tr>
<th>Myoinositol in diet</th>
<th>Number of infections</th>
<th>Total wet wt. of worms (g)</th>
<th>Average length per worm (cm)</th>
<th>Average length per g wet wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. +</td>
<td>34</td>
<td>0.9968</td>
<td>18.56 ± 3.38</td>
<td>633.07</td>
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<tr>
<td>2. +</td>
<td>29</td>
<td>0.6384</td>
<td>15.41 ± 1.92</td>
<td>700.02</td>
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<tr>
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<td>17.11 ± 2.35</td>
<td>588.52</td>
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<tr>
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<tr>
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<tr>
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<td>30</td>
<td>1.7752</td>
<td>29.38 ± 2.77</td>
<td>496.56</td>
</tr>
</tbody>
</table>
APPENDIX

Chandler et al. (1950) reported that H. diminuta gained in body weight in diluted Tyrode solution, loss weight in concentrated solution and remained in equilibrium with normal strength solution. Many in vitro studies on this tapeworm have been performed in the balanced saline (Krebs-Ringer-Tris-maleate buffer) described by Read et al. (1963). In agreement with Webster's observation (1970), this saline was found to be hyperosmotic to the worms at normal strength, leading to drastic losses of their body weight during the initial thirty minutes of incubation (Fig. 13). Webster (1970) concluded that H. diminuta is an osmoconformer. It loses sodium to Hank's saline, diluted with respect to its sodium content, in direct proportion to the dilution factor, while water loss from the worm depends upon the osmotic pressure of the saline (Webster, 1971).

Pappas et al. (1974) demonstrated that glucose absorption in H. diminuta is accompanied by an increase in body wet weight, indicating absorption phenomena and volume regulation in the parasite might be closely related. Sodium absorption by H. diminuta is closely associated with the hexose transport system. Bicarbonate is absorbed via a cation exchange mechanism (Podesta and Mettrick, 1974), which, in turn, accelerates the absorption of sodium ions. The use of Krebs-Ringer-Tris-maleate buffer for membrane transport experiments might, therefore, introduce problems in interpretation of data.

The saline used throughout this study consists of both phosphate and bicarbonate ions as buffer. The osmolality is around 275 mosmo/kg. Worms incubated in this solution lost weight gradually with time (Fig. 14) indicating that an energy source may be needed. The concentra-
tion of glucose in the gut of rats fed *ad libitum* fluctuates between 1.5 mM and 12 mM during the nocturnal feeding cycle (Starling, 1975). With the presence of 5 mM glucose in the incubation medium, there is practically no change of weight in *H. diminuta* during the 4-hr incubation period (Fig. 15). After incubation, worms were demonstrated to be still alive by transplanting their scolex and neck regions into the guts of uninfected rats with the recovery of longer worms ten days later.
REFERENCES


Wildiers, E. 1901. Cellule. 18: 313.


Figure 1: Molecular structure of the nine inositolis.
Molecular Structure of the nine inositols

cis
epi
allo
myo
muco
neo
D-chiro(+)  L-chiro(-)  scyllo
Figure 2: A gas chromatogram of free sugars in *Hymenolepis diminuta* on QF-1 at 146°C, gas flow rate, 30 ml/min.

- a = α-D-fructose;
- b = myo-inositol;
- c = scyllo-inositol;
- d = α-D-glucose.
A Gas Chromatogram of Free Sugars in *H. diminuta*
on QF-1 at 146°C, gas flow rate 30 ml/min.
Figure 3: Absorption of 0.01 mM H$_2$-myoinositol by 10-day-old *Hymenolepis diminuta*. $V = \mu$moles/g ethanol extracted dry wt.
Absorption of 0.01mM $^3$H-myoinositol by 10 days old *H. diminuta*
Figure 4: Absorption of $^3$H-myoinositol as a function of inositol concentration. $S = \text{inositol in molarity} \times 10^3$; $V = \text{micromoles inositol absorbed per gram ethanol extracted dry weight per 4 minutes.}$

Inset = Observed value for rate of inositol uptake at higher substrate concentrations (slope = 0.25).

- $\bullet$ = Total uptake
- $O$ = Mediated component
- $\circ$ = Diffusive component
Figure 5: Lineweaver-Burk plot of $^3$H-myoinositol absorption in the presence and absence of D-glucose.

$V = \text{uptake of } ^3\text{H-inositol in } \mu\text{mole/g ethanol extracted}$

$\text{dry wt/}4 \text{ min;}$

$S = \text{inositol concentration in molarity } \times 10^3;$

$\bullet = \text{uninhibited curve;}$

$\circ = \text{inhibited curve (0.5 mM D-glucose).}$
V_max = 0.0105 umoles/g ethanol extracted dry wt./4 min

K_t = 0.0067 mM
Figure 6: V versus V/X plot of $^3$H-myoinositol absorption in the presence and absence of D-glucose.

$V$ = uptake of $^3$H-inositol in $\mu$mole/g ethanol extracted dry wt/4 min;

$S$ = mM myoinositol;

$\bullet$ = uninhibited curve;

$O$ = inhibited curve (0.5 mM D-glucose).
● = myoinositol
○ = myoinositol + 0.5 mM D-glucose
Figure 7: Effect of increasing stable myoinositol concentration (●) and stable scylloinositol (○) on the uptake of 0.01 mM $^3$H-myoinositol by *Hymenolepis diminuta*.

\[ V = \text{uptake of } ^3\text{H-inositol in } \mu \text{mole/g ethanol extracted dry wt/4 min;} \]

\[ I = \text{stable inhibitor concentration in molarity } \times 10^3. \]
Effect of increasing stable myoinositol concentration (●) and stable scylloinositol (○) on the uptake of 0.01 mM ³H-myoinositol by *H. diminuta*
Figure 8: Effect of increasing D-glucose concentration on the absorption of 0.002 mM (○), 0.004 mM (●) and 0.005 mM (●) $^3$H-myoinositol.

$V$ = uptake of $^3$H-inositol in μmole/g ethanol extracted dry wt/4 min.
Figure 9: Effect of increasing phlorizin concentration on the absorption of 0.002 mM ($\circ$) and 0.006 mM ($\bullet$) $^3$H-myoinositol.

$$V = \text{uptake of } ^3\text{H-inositol in } \mu\text{mole/g ethanol}$$

extracted dry wt/4 min.
Figure 10: Effect of temperature on rate of uptake of 0.01 mM $^3$H-myoinositol by *Hymenolepis diminuta*.

\[ V = \text{uptake of } ^3\text{H-inositol in } \mu\text{mole/g ethanol extracted dry wt/4 min.} \]

Figure 11: Effect of pH on rate of $^3$H-myoinositol (0.01 mM) absorption by *Hymenolepis diminuta*. Samples were preincubated for 30 min in buffered saline at the appropriate pH.

\[ V = \text{uptake of } ^3\text{H-inositol in } \mu\text{mole/g ethanol extracted dry wt/4 min.} \]
Figure 12: Effect of Na⁺ on the absorption of 0.01 mM ³H-myoinositol by *Hymenolepis diminuta*.

$$ V = \text{uptake of } ^3\text{H}-\text{inositol in } \mu\text{mole/g ethanol extracted dry wt/4 min.} $$
Figure 13: Percent change in weight of *Hymenolepis diminuta* incubated in Krebs-Ringer-Tris-maleate buffer.
Read's saline
310-315 mosmo
Figure 14: Percent change in weight of *Hymenolepis diminuta* incubated in the bicarbonate-phosphate buffered saline.

Figure 15: Percent change in weight of *Hymenolepis diminuta* incubated in the bicarbonate-phosphate buffered saline incorporated with 5 mM glucose.
TIME (hr)

PER CENT INITIAL Wt

1  2  3  4

275 mosmo saline

→ = 5 mM glucose added

TIME (hr)

PER CENT INITIAL Wt

1  2  3  4

○ = 275 mosmo saline + 5 mM glucose
● = 275 mosmo saline