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Carbohydrate Transport and Accumulation in the Intestinal Parasite Moniliformis dubius (Acanthocephala)

by

Jane A. Starling

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

DOCTOR OF PHILOSOPHY

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The informal exchange of ideas is invaluable to the development of personal and professional philosophies. I have been extremely fortunate during my years at Rice in having associates and mentors who were willing to share over coffee or beer long hours of discourse on every imaginable topic. The dynamic character of Professor Clark P. Read as a scientist and as a humanist and the all too few hours spent with him in learning to appreciate not only the exciting diversity of biological systems but also the unbounded capacity of human endeavor shall always be inspirational to me.

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INTRODUCTION

The success of a parasite-host relationship depends upon the degree to which the physiology of the host allows the establishment and maintenance of the parasite. Read (1968) has suggested that the basis of parasitism be regarded as the dependence of the parasite upon the homeostatic mechanisms of the host. Of paramount importance among these mechanisms is the maintenance of a biochemical environment in which the parasite can satisfy its nutritional needs. Indeed, no organism can successfully exploit an ecological niche in which it is unable to satisfy its nutritional requirements.

In his review of the feeding mechanisms of selected parasites, Read (1968) described three categories of adaptations for food acquisition among parasites: 1) internal or external digestive capacity coupled with mechanical specializations for biting or sucking, 2) digestive capacities without mechanical feeding devices, and 3) neither mechanical feeding devices nor digestive capacities. Parasites displaying either of the first two types of adaptations have the potential for utilizing high molecular weight and even particulate food sources; they may also be capable of obtaining soluble nutrients available to them in the host environment.

Organisms described by the third category must depend entirely on the trans tegumentary absorption of organic solutes for the satisfaction of their nutritional requirements. The most conspicuous of this group are the cestodes and the acanthocephalans, almost invariably parasites of the vertebrate intestine in their adult stages. Their digestive capacities are, at most, limited. The failure to demonstrate
any digestive capacities in the hemoflagellates has led Read (1968) to suggest that they, too, are solely dependent on absorption for the acquisition of nutrients.

The obvious importance of surface absorption among these groups makes an understanding of their absorption mechanisms essential to our attempts to define the strategies by which they cope with the pressures of existence. The similarities in life cycles and environments of cestodes and acanthocephalans offer the opportunity for comparative studies adding to our understanding of what generalizations can and cannot be made about parasites living in comparable habitats. From the standpoint of comparative physiology in general, it is important that mechanistic information be obtained on widely diverse systems if valid unifying models are to be derived for a phenomenon as cosmopolitan as cellular absorption.

In 1966, Read said "more is known of the processes involved in absorption of low molecular weight compounds by tapeworms than is known of any group of invertebrate metazoans, parasitic or free-living." Although there has been a tendency in recent years to broaden our research efforts in this area, this statement is equally applicable in 1972. Information from systematic examinations of the mechanisms of nutrient absorption in acanthocephalans is almost non-existent. The study of the nature of the hexose absorption systems of the acanthocephalan _Moniliformis dubius_ described in this dissertation is a much needed step toward the expansion of our knowledge concerning the diversity of nutrient absorption mechanisms among animal parasites.
The Nature of Absorptive Mechanisms

The general and theoretical considerations of cellular absorption have been reviewed extensively by Stein (1967). Absorption phenomena can be separated into two types: simple diffusion and mediated absorption. Simple diffusion is described by Fick's law: the flux of a substrate across a membrane of defined area is a linear function of the trans-membrane concentration difference of that permeant. Mediated absorption deviates from Fick's law in that the unidirectional flux becomes saturated with increasing permeant concentration.

Mediated absorptive systems are formally divided into two categories based on the thermodynamic reversibility of the overall process. The term facilitated diffusion refers to absorption in which there is no net accumulation of permeant. Active transport connotes the accumulation of a substrate against its electrochemical potential; the ultimate expenditure of energy by the cell is necessary in order to overcome a thermodynamic barrier. Stein (1967, pp. 177-241) has further subdivided active transport systems according to their proximity to the site of energy expenditure: primary active transport systems, such as the sodium pump, involve the direct consumption of energy; secondary (and perhaps higher order) active transport systems function as a result of a thermodynamic driving force accruing from the operation of a primary system.

A third mechanism for the accumulation of organic permeants is included by some workers in the category of transport mechanisms. "Group translocation", in particular, "vectorial phosphorylation", has been demonstrated to be the mediating mechanism for the accumulation of
hexose carbon in a broad spectrum of bacteria (reviewed by Kaback, 1970). This mechanism, however, involves a chemical alteration of the permeant; the alteration, in this case, the phosphorylation of hexoses, appears to be effected by a complex of enzymes which is an integral part of the translocation site itself. Since group translocation does not actually involve the movement of a single molecular species across a permeability barrier, it should be regarded as a phenomenon mechanistically distinct from the mediated absorption of permeants.

The available evidence strongly suggests that active transport of organic solutes by animal cells is a secondary process, differing from facilitated diffusion only in the maintenance of a low probability for efflux of absorbed permeants as a result of the operation of a primary active transport system (see Stein loc. cit.). Two mechanisms through which this low probability for efflux can be attained have been described. In sodium-dependent cotransport systems, which have been exhaustively reviewed for vertebrate tissues by Schultz and Curran (1970), the high ratio of external to internal sodium ion concentration maintained by the sodium pump provides the driving force for permeant accumulation; the relatively lower rate of sodium binding at the internal membrane face, resulting from the much lower local sodium concentration, can reduce either the carrier-permeant equilibrium or the kinetics of membrane transit, or both. In counterflow, the accumulation of permeant is driven by the efflux of a second permeant which shares the same carrier (discussed in Stein, 1967, pp. 145-148).

Several properties of mediated absorptive systems are phenomenologically comparable to enzyme catalysis: 1) saturation kinetics,
2) greater transfer rates than thermodynamically predicted for simple diffusion, 3) stereospecificity in varying degrees with respect to substrate, 4) inhibition by substrate analogs, and 5) inhibition by metabolic poisons. Because of these similarities, it is postulated that absorption is mediated through physically distinct components of the cell membrane, analogous in their substrate specificity and kinetic properties to enzymes. A physical model for mediated absorption and the kinetics predicted by that model are discussed in the THEORETICAL section of this dissertation.

Mediated Absorption in Animal Parasites

A review of the literature concerning absorption in animal parasites in general is beyond the province of this dissertation. The absorption of low molecular weight nutrients by intestinal helminths has been reviewed thoroughly by Read (1966, 1968).

The absorption of glucose by adult cestodes appears to be mediated and concentrative in all cases where the question has been examined (literature cited in Read, 1966). In both larval and adult *Taenia taeniaformis*, the absorption of glucose is sodium dependent and sensitive to the glycoside phlorizin; on the other hand, glycerol uptake, though mediated, appears to be only slightly affected by the deletion of sodium from the incubation medium and is phlorizin insensitive (von Brand et al., 1964, 1966; von Brand and Gibbs, 1966).

Fisher and Read (1971) have reported that the concentrative absorption of glucose by *Calliobothrium verticillatum* is both sodium dependent and extremely sensitive to phlorizin. Similarly, phlorizin
has been shown to inhibit the absorption and metabolism of glucose by *Hymenolepis diminuta* (Laurie, 1957; Phifer, 1960a). Recent studies by Gallogly (1972) demonstrate that glucose transport is also sodium dependent in this tapeworm. Phlorizin sensitive glucose uptake has been reported in a number of other cestodes, but nothing is known of the relationship of sodium to these systems (see Read, 1966, for literature citations).

Phifer (1960b) was unable to demonstrate any effect of 4 mM galactose, 3-O-methylglucose, mannose, or 2-deoxyglucose on the absorption of 2 mM glucose by *Hymenolepis*. Using higher inhibitor to substrate ratios, Read (1961) showed that glucose transport is inhibited by galactose, allose, α-methylglucoside, 6-deoxyglucose, 3-O-methylglucose and 1-deoxyglucose, but not by fructose, fucose, or 1,5-anhydromannitol. Read also studied the mediated absorption of galactose by *Hymenolepis*, reporting a close agreement of the \( K_t \) (5.1 mM) for galactose absorption and the \( K_i \) for galactose inhibition of glucose transport. The \( K_t \) for glucose found by Read (1.6 mM) is in close agreement with values obtained by Phifer. These studies correlate well with earlier findings that glucose and galactose support metabolic gas production in *Hymenolepis*, whereas fructose does not (Read, 1956). Both glucose and galactose are accumulated by *Calliobothrium* (Fisher and Read, 1971), and it is known that fermentation is supported almost exclusively by these two hexoses (Read, 1957; Laurie, 1961).

Although the metabolic specificities for sugar appear to be similar for these two cestodes, the membrane transport properties differ. 3-O-Methylglucose does not inhibit glucose absorption in *Calliobothrium*,
but maltose and cellobiose do. Furthermore, ouabain, which Gallogly (1972) has found ineffective in inhibiting glucose transport in *Hymenolepis*, appears to affect transport in *Calliobothrium* both by interfering with the maintenance of the sodium distribution and by competitively interacting with the transport locus (Fisher and Read, 1971). Read (1961) has pointed out the similarity of the hexose transport system of *Hymenolepis*, in terms of specificity for inhibitors, to that of the vertebrate intestine (Crane, 1960); he further speculated in 1966 that it would be of interest to compare the specificities of the system in *Calliobothrium* to those of the dogfish intestine.

Studies of carbohydrate absorption among various hemoflagellates indicate a great diversity in the specificities of the uptake systems. In 1965, Seed et al., reported that 2-deoxyglucose inhibits the metabolism of glucose in *Trypanosoma gambiense* and *T. rhodesiense*. Finding that this hexose did not reduce the oxygen consumption of cell lysates, the authors concluded that its primary effect was upon the entry of glucose and glycerol into the cells. Von Brand and his collaborators (1967) measured the absorption of glucose and glycerol in nine trypanosomatid species in terms of the disappearance of chemically measured substrate from the incubation medium. They reported varying degrees of mutual inhibition of glucose and glycerol absorption when both substrates were present in the incubation media, and suggested that their entry was effected through a common transport locus. The failure of phlorizin or of sodium deletion to inhibit absorption suggested to the authors that glucose and glycerol uptake in hemoflagellates does not occur by "active transport".
Sanchez and Read (1969) have shown that the absorption of glucose by blood-stream forms of *T. lewisi* exhibits saturation kinetics. It was found that sorbose did not inhibit the uptake of glucose (or of any other substrate against which it was tested), nor was its meager absorption inhibited in reciprocal experiments. The authors concluded that sorbose is not transported in *T. lewisi*, and they remarked that this is not the case in *Crithidia luciliae*, for which Min (1966) demonstrated that sorbose absorption was subject to inhibition by glucose.

Graphical analysis of the effects of mannose, fructose, galactose, glucosamine, and 3-O-methylglucose at several concentrations on the absorption of glucose revealed kinetics similar to those generated by partially competitive inhibition of enzyme reactions displaying Michaelis-Menten kinetics. Taking this as presumptive evidence for two transport sites, Sanchez and Read examined the mutual inhibitory effects of all 36 possible pair-wise combinations of these hexoses at a fixed substrate to inhibitor ratio. An inspection of the results of this examination makes clear the impossibility of assigning relative orders of affinity if one assumes that all enter through the same transport locus. The authors postulated a "glucose site" through which all sugars are absorbed, and a "glucosamine" site, for which fructose, mannose, galactose, and 3-O-methylglucose, but not glucose, have a high affinity. As further evidence for two transport loci, it was shown that the inhibition of mannose absorption by a combination of glucose and glucosamine at 4 mM concentrations was greater than the effect exerted by either sugar alone at an 8 mM concentration.

Southworth and Read (1969, 1970) have examined the specificity of carbohydrate absorption in blood-stream forms of *T. gambiense* in some
detail. They reported that glucose, fructose, mannose, and glycerol, the four carbohydrates metabolized by this protozoan, were absorbed through a saturable mediated process. In addition, the non-metabolized hexoses 2-deoxyglucose and glucosamine exhibited mediated absorption kinetics. When tested as inhibitors of the absorption of one another, all six carbohydrates were found to interact competitively to varying degrees. N-acetylglucosamine, whose entry appears to be non-mediated, was found to inhibit the absorption of both glucose and fructose. In contrast to the results obtained by Sanchez and Read for T. lewisi, 3-O-methylglucose is neither absorbed nor capable of inhibiting the uptake of other sugars in T. gambiense.

In order to account for the low degree to which fructose, whose experimentally determined transport constant of 1.58 mM only slightly exceeds that of glucose, inhibited the absorption of other sugars, as compared to the reciprocal effects of these sugars on fructose, Southworth and Read proposed that carbohydrate absorption in T. gambiense is mediated through two distinct sites. They suggested a "glucose site" through which all sugars except fructose are transported, and a "fructose site" through which only fructose enters, but with which the other carbohydrates will interact.

Southworth (1971a) was unable to demonstrate any significant effects of ouabain, phlorizin, or sodium deletion on glucose uptake. Additional kinetic studies using inhibitors at varying concentrations showed that the change in the reciprocal of the absorption velocity was a non-linear function of inhibitor concentration for all substrates and inhibitors tested. Although Sanchez and Read showed that this relationship can be
indicative of the action of two transport systems, the universality of the phenomenon caused Southworth to offer another interpretation. He concluded that the interactions of carbohydrates in *T. gambiense* were "partially competitive" in nature, and suggested that, in the light of this interpretation, the inhibition data could most reasonably be explained in terms of a single transport locus.

Studies of carbohydrate absorption in acanthocephalans are few. In 1957, Laurie presented presumptive evidence for the absorption of several sugars by *Moniliformis dubius*. He measured the fermentation of glucose, galactose, mannose, fructose, and maltose under a nitrogen-carbon dioxide atmosphere, manometrically determining the carbon dioxide liberated from a bicarbonate buffer by acidic end products excreted during 30 minute and one hour incubations. Both male and female worms from hosts that had been starved for 24 hours fermented all of the above sugars to some degree when the substrates were tested at initial concentrations of 30 mM. Laurie found no evidence for metabolism of cellobiose, lactose, sucrose, trehalose, turanose, glycerol, rhamnose, glucosamine, 3-O-methylglucose, 2-deoxyglucose, sorbose, or fructose diphosphate, among other compounds. Neither phlorizin nor iodoacetic acid had any effect on fermentation; p-chloromercuribenzoic acid, on the other hand, was found to be a potent inhibitor.

Crompton and Lockwood (1968) measured the absorption of glucose by *Polymorphus minutie* in long term incubations. When the data for the amount of glucose removed from the medium at various concentrations were plotted as a function of mean substrate concentration, the absorption kinetics appeared to saturate at a concentration of two mg/ml. An
attempt was made to determine whether the absorption was concentrative: worms preloaded for one hour in radioglucose at high concentrations were subsequently incubated for 30 minutes in dilute labelled glucose; using the distribution of total radioactivity between the worms and the medium as an index of the glucose concentration, the authors concluded that glucose had been accumulated!

Rothman and Fisher (1964) studied in vitro the absorption of several neutral amino acids by both males and females of two acanthocephalan species: *Macracanthorhynchus hirudinaceus* and *Moniliformis dubius*. Methionine uptake was shown to become saturated at high substrate concentrations; chemical determinations of methionine levels in worms and incubation media following 10, 20, and 30 minute incubations in 1 mM methionine demonstrated that the mediated absorption occurred against a chemical concentration difference. One mM concentrations of leucine, isoleucine, serine, and alanine competitively inhibited the uptake of methionine in both species. Reciprocal experiments indicated that the transport of each of these amino acids was competitively inhibited by methionine in *Moniliformis*, while, under the conditions examined, serine and alanine uptakes were inhibited in male *Macracanthorhynchus*, but not in females.

Edmonds (1965) measured in vivo the incorporation of radioactive leucine into *Moniliformis*. He confirmed the findings of Rothman and Fisher by demonstrating in vitro that the incorporation of leucine was inhibited by valine, serine, and methionine.

In 1970, Branch re-examined the absorption of leucine by *Moniliformis* (Branch, 1970). The data indicate an apparent plateau in the
substrate concentration kinetic profile at two mM leucine, the highest concentration tested by Rothman and Fisher, but at higher concentrations the uptake rate appears to be linear with respect to substrate. Failing to demonstrate any effect of 1 mM methionine on the uptake of 4 mM leucine, the author suggested the presence of a second transport site because "this absence of competitive inhibition would only be expected had the amino acid transport system remained unsaturated at 4 mM leucine"! The large standard errors in individual data points, resulting from the use of only three samples at each concentration and from variability in the size of the worms ("six- to eight-week-old worms"), make quantitative interpretation of the data difficult, but it would appear that Brach has demonstrated a diffusional component for the entry of amino acids at high concentrations.

**Moniliformis dubius**: Life History

*Moniliformis dubius* (Meyer, 1933) is an acanthocephalan parasitic in the small intestine of the rat. The phylum Acanthocephala is a small one consisting of three orders: the Palaeacanthocephala, the Eoacanthocephala, and the Archiacanthocephala, of which *Moniliformis* is a member. The early studies on members of this phylum were reviewed by Meyer (1932-1933). Hyman (1951) presents an excellent treatment of the morphology and embryology of the group. For treatments of the biology and physiology of Acanthocephala, the reader is referred to recent reviews by Nicholas (1967) and by Crompton (1970).

The life history of *Moniliformis* was described partially by Burlingame and Chandler (1941) and in detail by Moore (1946). Nicholas
reviewed the cycle in parallel with other members of the phylum; his nomenclature differs from that of Burlingame and Chandler and that of Moore, but is more generally accepted, and is used in the following abbreviated summary.

The dioecious adults reside in the anterior half of the small intestine (the "zone of viability" described by Burlingame and Chandler), the hook-armed proboscis embedded in the intestinal mucosa. Following copulation and maturation of the embryos, the mature acanthors, encased in a hardened, four-layered shell, pass into the uterus, from which they are extruded to pass out of the rat with the feces.

When the dormant acanthor, protected against environmental extremes by its hardened shell, is ingested by the intermediate host, *Periplaneta americana*, hatching begins. The activated acanthor penetrates the midgut of the cockroach and comes to lie in the serosal connective tissues. Within about 12 days, the much enlarged acanthor breaks free of the serosal wall into the haemocoel of the cockroach where, encased in several layers of haemocytes (Robinson and Strickland, 1969), it differentiates into an acanthella. The acanthella, which very much resembles the adult in general appearance, is found approximately six weeks after ingestion of the acanthor, although the rate of development varies with environmental temperature.

Within two weeks, the acanthella matures into a cystacanth. The vesicular mass, derived from the host haemocytes surrounding the acanthella, is transformed into a capsule by the deposition of an "amorphous material of host origin" (Mercer and Nicholas, 1967). Concomitantly, the proboscis and neck region are inverted by muscular
contraction, partially filling the pseudocoel, and fluid accumulates between the parasite and its encapsulating cyst.

When the cockroach is eaten by a suitable definitive host, the dormant cystacanth passes from the stomach, where the cyst wall may have been partially digested by proteolytic enzymes, to the small intestine, where bile salts, potentiated by the presence of carbon dioxide and low oxygen tensions, initiate the activation process (Graff and Kitzman, 1965). The proboscis is everted, and the activated cystacanth attaches to the intestinal mucosa, where the final differentiation of the metasomal musculature occurs within a week (King and Robinson, 1967). The adults grow rapidly for the first four or five weeks; the growth slows with the onset of patency at five to six weeks (Burlingame and Chandler, 1941). Growth continues throughout the life of the adult, the only limitation being the physical restriction of the size of the rat intestine.

The Acanthocephalan Tegument

Read (1966) has emphasized the similarity between the microvillar surfaces of cestodes and the absorptive surfaces of other animal cells, in particular, the apical epithelia of the cells of the vertebrate intestine. In view of the obvious advantage of increased surface area to the efficacy of transmembrane absorption, it is not surprising that microvillar-like surface amplifications are found in a wide variety of tissues in which absorptive or excretory activities occur. Although absorptive function is vitally important in the acquisition of nutrients by acanthocephalans, the structure of the absorptive surface of acanthocephalans is markedly different from that of cestodes.
The syncytial tegument of Moniliformis has been studied at the ultrastructural level by a number of workers. Nicholas and Mercer (1965) have enumerated six layers corresponding to those described for Polymorphus minutus by Crompton and Lee (1965): 1) the fibrillar "epicuticle"; 2) the striped layer, subjacent to the plasmalemma; 3) the vericular layer; 4) the felt layer; 5) the radial layer; and 6) the subtegmentary layer of connective tissue underlying the inner plasma membrane. Internal to the subtegmentary connective tissue lie the circular and longitudinal muscle layers, embedded in a collagenous matrix (see Nicholas, 1967). These muscle layers, together with the tegument, constitute the body wall.

Histochemical studies by Wright and Lumsden (1968) suggest that the "epicuticle" of Moniliformis resembles, both chemically and morphologically, the mucopolysaccharide-glycoprotein glycocalyx found on many vertebrate and invertebrate cell types. The characteristic "texture" of the striped, felt, and radial layers results from the different orientations of the multitudinous fibers present in the cytoplasm within these layers. Poorly defined mitochondria are present predominantly in the vesicular and radial layers. Byram (1971) has demonstrated lysosome-like vesicles within these same two layers. Lacunar canals, considered to be characteristic of all acanthocephalans, have yet to be observed in the tegument of Moniliformis dubius (Nicholas and Mercer, 1965; Byram, 1971).

In his 1967 review, Nicholas described the striped layer as "penetrated by closely spaced narrow extensions of the underlying cytoplasm which reach the surface of the plasma membrane.... Where the
cytoplasm reaches the surface, the plasma membrane is frequently drawn into it as though pinocytotic vacuoles were forming." Wright and Lumsden (1969) more correctly described a system of "pores", or invaginations of the plasmalemma, which are continuous through tubular canals with blind sacs up to four microns in length. The authors interpreted this system as a specialization for the amplification of the surface area available for chemical exchange.

One might question the efficacy of this system as an adaptation for maximizing absorptive capacity. Byram (1971) has demonstrated that the pores, whose maximum diameter is about 85 Å, are partially "selective" in allowing the entry of large molecules. He found that large electron opaque tracers, in particular, ferritin and micelles of thorium dioxide, were excluded almost completely from the canal-sac system (collectively termed "crypts"), while horseradish peroxidase, whose molecular weight is 40,000 daltons, entered the crypts readily. Rothman (1967) has presented cytochemical evidence that alkaline and acid phosphatases are present in the crypts of Moniliformis but absent from the tegumentary surface. The studies by Byram suggest that the alkaline phosphatase activity is bound to the membranes of the crypts. Although the exclusion of high molecular weight molecules from the crypts indicates that there is some degree of impedance of exchange with the "milieu extérieur", it is not unreasonable to assume that low molecular weight solutes have access to the membrane lining the crypts and that some absorption may occur there.
Carbohydrate Metabolism in *Moniliformis dubius*

The importance of carbohydrate in the host diet to the survival of *Moniliformis dubius* was dramatically demonstrated by Read and Rothman (1958a). Starvation of infected hosts for 48 hours was found to effect as much as a ten-fold decrease in the glycogen content of worms. A two-fold diurnal fluctuation in the glycogen content of worms was also demonstrated. In hosts placed on carbohydrate deficient diets during the fifth and sixth weeks post infection, growth of male worms ceased completely and female worms were lost. It was shown that resynthesis of depleted glycogen stores resulted when starved rats were given starch by stomach tube, although oral administration of maltose to rats did not result in the restoration of worm polysaccharide. The authors noted that the striking changes in wet weight following starvation could not be accounted for by glycogen depletion alone.

The first extensive study of carbohydrate metabolism in *M. dubius* was that by Laurie (1957) described earlier in the discussion of sugar absorption. Subsequently, Laurie (1959) examined the aerobic metabolism of *M. dubius* using worms from hosts which had been starved for 24 hours. Following two to four hours incubation in ten millimolar solutions of glucose, fructose, mannose, and maltose (at 5 mM), the glycogen content of both male and female worms (at least six weeks old) increased from about 6.7 to 15-18 percent of the dry weight. Levels of the non-reducing disaccharide trehalose did not increase significantly over control values in these worms.

Graff (1964) studied the aerobic incorporation of radioactive glucose into various endogenous fractions of *M. dubius* in short term
incubations. He found that worms from starved hosts absorbed almost twice as much glucose and incorporated ten times as much glucose into glycogen as did worms from normally fed hosts. Higher rates of incorporation in males were found to be related to the greater surface area to volume ratio in the small male worms. Significant amounts of radioactivity were found in several dicarboxylic acids and amino acids following one-, three-, and five-minute incubations. Aspartic acid and alanine were labelled approximately equally; small levels of radioactivity were found in serine in extracts of female worms, while no radioglutamic acid was demonstrated. Of the dicarboxylic acids recovered, the following were found to be labelled (in descending order): malate, lactate, succinate, and fumarate. In similar studies of carbon dioxide fixation by M. dubius, Graff (1965) showed the incorporation of radioactivity into these same metabolites; the levels of radioactivity in lactate and alanine were much lower than those found when radioglucose was the substrate. In neither study was the incorporation of label into trehalose or excreted acids examined.

In his study of aerobic metabolism, Laurie (1959) examined the acidic end products excreted into the medium. He reported that formic and acetic acids are the major non-volatile end products of aerobic metabolism, with lactic acid accounting for from 3.2% (in the absence of exogenous substrate) to 22% or greater when metabolizable sugars were added to the incubation medium. Crompton and Ward (1967b) were unable to detect any label in formic acid when M. dubius was incubated in radioactive glucose under an atmosphere of nitrogen. The most heavily labelled non-volatile excretory product was lactic acid; some radioactivity was
found in succinic acid, and even smaller levels of radioactivity were demonstrated in acetic acid. The majority of the excreted radioactivity was in a highly volatile compound which they identified as ethanol. The authors contrasted these results to those they had obtained for *Polymorphus minutus* (Crompton and Ward, 1967a); the major end products of glucose metabolism excreted by this acanthocephalan parasite of the duck are succinic and lactic acids.

The results obtained by Crompton and Ward have been confirmed in a recent report by Körting and Fairbairn (1972), who determined by chemical analysis the products excreted by *M. dubius* under an atmosphere of nitrogen-carbon dioxide. In addition to those products found by Crompton and Ward, the authors demonstrated trace amounts of propionic acid. The similarity of the results reported is these two reports suggests that carbon dioxide is not necessary for metabolism in *Moniliformis*. Although Graff (1965) demonstrated the fixation of carbon dioxide into dicarboxylic acids, probably via phosphoenolpyruvate carboxykinase (Horvath and Fisher, 1971), Kilejian (1963) showed that it had no effect on the synthesis of glycogen.

The absence of appreciable excretion of formic and acetic acids under anaerobic conditions cannot be considered a repudiation of the results reported by Laurie. It must be remembered that the worms used in Laurie's experiments had been taken from fasted hosts, and that therefore the carbohydrate reserves of these worms were drastically depleted. The data of Körting and Fairbairn demonstrate that the carbohydrates of ten-week-old female worms were reduced as much as 29 percent during four hours incubation in the absence of substrate.
Laurie reported an increase in the relative amount of lactic acid excreted when starved worms were supplied with exogenous sugar. The discrepancy may therefore be reflective of metabolic changes in the worms which are correlated with the availability of substrates capable of supporting energy production.

Laurie demonstrated that *M. dubius* is capable of some degree of aerobic respiration (1959). Read (1967b) has reported that although oxygen has no effect on the rates of fermentation, glucose absorption, or net glycogen synthesis in the cestode *Hymenolepis diminuta*, the presence of oxygen enhances the fixation of carbon dioxide into glycogen. As the author pointed out, further studies are necessary before any conclusions can be drawn about the nature of the effect of oxygen on carbohydrate metabolism in cestodes; however, it would seem that oxygen may alter the relative importance of different metabolic pathways for carbohydrate utilization and/or resynthesis in *H. diminuta*. In the absence of any evidence to the contrary, similar alterations in metabolism of carbohydrates by *Moniliformis dubius* cannot be ruled out.

Körting and Fairbairn (1972) also measured the occurrence of enzymes for carbohydrate and fatty acid metabolism in both adult worms and cystacanths. Their findings confirm the results of both metabolic studies and biochemical assays reported by a number of workers (see Körting and Fairbairn, 1972, for references). High levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were detected in both adults and cystacanths; neither contained a full complement of enzymes for the β oxidation of fatty acids; enzymes for the metabolism of tricarboxylic acids were absent in adults, but present
in low quantities in cystanths. The authors were unable to detect fumarate hydratase, although it is known that carbon dioxide fixation results in the production of all of the four-carbon dicarboxylic acids. This might suggest that metabolism of dicarboxylic acids occurs through some other pathway than the abbreviated dicarboxylic acid pathway of the citric acid cycle.

In 1958, Fairbairn reported the presence of trehalose (α-1-glucopyranosyl-α-1-glucopyranoside) in the tissues of M. dubius and a broad spectrum of other invertebrates. Fisher (1964) demonstrated the synthesis of trehalose in minces of the body walls of Moniliformis and of Macracanthorhynchus. McAlister and Fisher (1972) have studied the biosynthesis of trehalose using partially purified homogenates of the body walls of female M. dubius. The pathway appears to be similar to that found in insects and microorganisms:

$$ \text{UDPG}^1 + \text{glucose-6-phosphate} \rightarrow \text{UDP} + \text{trehalose-phosphate} $$

$$ \text{trehalose-phosphate} \rightarrow \text{trehalose} + P_i $$

although they were unable to demonstrate a trehalose-phosphate intermediate.

McAlister and Fisher speculated that the biosynthesis of trehalose might be an effective means of promoting the diffusion of glucose into M. dubius, the removal of glucose by conversion to a different molecular species permitting the further passive entry of glucose. Such a system has been demonstrated for the removal of hexoses from the midgut of the locust (Treherne, 1958 a,b). Fairbairn proposed a similar mechanism for the accumulation of glucose carbon across the gut of Ascaris, but

$^1\text{UDPG} = \text{uridine diphospho-glucose;} \text{UDP} = \text{uridine diphosphate.}$
this hypothesis has not been borne out experimentally (discussed in Read, 1966). A somewhat similar relationship between absorption and trehalose synthesis was suggested by Crompton (1970, p. 48), although he did not restrict the mode of glucose entry to simple diffusion.
THEORETICAL

The Mobile Carrier Hypothesis

The currently accepted model for mediated absorption involves the concept of a "mobile carrier" which is a structural component of the cell membrane. The transport process can be represented by a series of association-dissociation reactions involving the permeant and the carrier site coupled with the movement of the carrier-permeant complex from one side of the cell membrane to the other. The following physical model of the mobile carrier is adapted from Stein (1967, pp. 152-157):

\[
\begin{align*}
    \text{SIDE A (OUTSIDE)} & \quad \text{MEMBRANE} & \quad \text{SIDE B (INSIDE)} \\
    & & \\
    CS_A & \xleftarrow{k_1} & S_A & \quad k_2 & \quad CS_B \\
    C_A & \quad k_3 & & \quad k_4 & \quad C_B \\
    & \quad k_{-2} & \quad k_{-1} & \quad k_{-3} & \quad k_{-4} & \quad S_B
\end{align*}
\]

$C_A$ and $C_B$ represent the concentrations of free carrier at sides A and B of the membrane. Similarly $CS_A$ and $CS_B$ are the concentrations of carrier associated with permeating substrate, at concentrations $S_A$ and $S_B$, at their respective solvent-membrane interfaces. $K_A = k_{-1}/k_1$ and $K_B = k_{-4}/k_4$ are the substrate dissociation constants for association with carrier at face A and face B of the membrane. $k_2$, $k_{-2}$, $k_3$, and
$k_3$ are the kinetic constants for the movement of combined and free carrier across the membrane.

The "movement" of carrier across the membrane can be envisioned as any of several more specific mechanisms embodied in nominally different models: the actual movement of a freely motile component of the membrane as pictured in the "ferry boat" model; a conformational change in a structural component of the membrane, such that the binding site for substrate is rotated from one face to the other; or a conformational change in the membrane surrounding a stereospecific pore, such that the probability distribution function for availability of binding sites within the pore is reversed.

Kinetics: The Michaelis-Menten Equation

It is common practice in the study of mediated absorption to describe kinetics in terms of the Michaelis-Menten equation for enzyme catalysis:

$$v = \frac{V_{\text{max}} S}{K_m + S}$$

In examining the validity of this formulation in the treatment of transport kinetics it is important to understand the assumptions made in its derivation.

For the simple reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$
the velocity is dependent upon the concentration of the enzyme-substrate complex, ES. If it is assumed that the breakdown of ES to form free enzyme and product (P) is the rate limiting step in catalysis, ES can be defined in terms of the substrate-enzyme dissociation constant, $K_S$:

$$K_S = \frac{k_{-1}}{k_1} = \frac{(E-ES) \cdot S}{ES}$$

where $(E-ES)$ is the concentration of enzyme not combined with substrate. Initially, the reverse reaction is negligible, and:

$$v = k_2ES = \frac{k_2E\cdot S}{k_{-1} + S} = \frac{V_{\text{max}} \cdot S}{K_S + S}$$

It is possible also to derive a rate function without making the assumption that $k_2$ is rate limiting by considering the conditions prevailing at the steady state (Briggs and Haldane, 1925). At steady state, the rates of formation and breakdown of ES are equal, and:

$$\frac{dES}{dt} = k_1(E-ES) - k_{-1}ES - k_2ES + k_2(E-ES)P = 0$$

The rate equation obtained when $(ES)$ is calculated using the steady state equation is:

$$v = \frac{k_2E\cdot S}{\frac{k_{-1} + k_2}{k_1} + S} = \frac{V_{\text{max}} \cdot S}{K_m + S}$$

The Michaelis constant, $K_m$, is that substrate concentration at which the velocity is half-maximal. It is equivalent to the substrate dissociation constant, $K_S$, only when the rate limiting step is the
formation of product from the enzyme-substrate complex, in which case 
k_1 \gg k_2. The adaptation of the Michaelis-Menten equation for the de-
scription of mediated absorption merely involves the substitution of K_t, 
the "transport constant", for K_m. The transit of the carrier-substrate 
complex across the membrane is assumed to be comparable to the breakdown 
of ES to products, and hence is described by the kinetic parameter k_2.

Kinetics: The Carrier Model

A rate function for mediated absorption can be derived directly from 
the physical model for the carrier. It is assumed that the rates of 
movement of each form of carrier in each direction are equal, so that 
k_2 = k_3 and k_{-2} = k_{-3}. The ratio of the rates of transit of combined 
and free carrier can be represented by \( r = k_2/k_3 \). If C is the "concentra-
tion" of total carrier sites, the solution of the simultaneous equations 
for the steady state relations among the different forms of carrier yields 
the following equation for the unidirectional flux of substrate from 
side A (outside) to side B (inside):

\[
J_{A \rightarrow B} = \frac{k_2}{k_2C} \frac{(K_B + \frac{k_2}{k_4} + rS_B) \cdot S_A + \frac{k_2S_B}{k_4}}{(r+1)K_B + 2 \frac{k_2}{k_4} + 2rS_B}
\]

\[
J_{A \rightarrow B} = \frac{k_2}{K_A(2K_B + 2 \frac{k_2}{k_4} + (r+1)S_B) + 2 \frac{k_2}{k_4}(K_B + S_B)} + S_A
\]

Although this rather formidable equation cannot be arranged to fit the 
Michaelis-Menten format, two simplifying assumptions can be made.
If it is assumed that the rates of transit of free and combined carrier are equal \((r=1)\), equation (1) reduces to:

\[
J_{A \rightarrow B} = \frac{k_2 C}{2} \cdot \frac{S_A + \frac{k_2}{k_1} \cdot \frac{S_B}{K_B + S_B + \frac{k_2}{k_4}}}{S_A + \frac{k_2}{k_1} \cdot \frac{S_B}{K_B + S_B + \frac{k_2}{k_4}}} \tag{2}
\]

If, instead, it is assumed that the rate limiting step is the transport of loaded carrier across the membrane, so that \(k_1 \gg k_2\) (and presumably \(k_4 \gg k_2\)), equation (1) reduces to a form that does describe Michaelis-Menten kinetics:

\[
J_{A \rightarrow B} = \frac{k_2 C \cdot \frac{K_B + rS_B}{(r+1)K_B + 2rS_B} \cdot S_A}{2K_B + (r+1)S_B \cdot \frac{K_A}{(r+1)K_B + 2rS_B} + S_A} \tag{3}
\]

and

\[
V_{\text{max}} = k_2 C \cdot \frac{K_B + rS_B}{(r+1)K_B + 2rS_B} ; \quad K_t = K_A \cdot \frac{2K_B + (r+1)S_B}{(r+1)K_B + 2rS_B}
\]

If both assumptions are made simultaneously, the unidirectional flux becomes independent of the trans-membrane concentration of substrate, and the rate equation is identical to the Michaelis-Menten equation for the case that \(K_m = K_B\):

\[
J_{A \rightarrow B} = \frac{k_2 C}{2} \cdot \frac{S_A}{K_A + S_A}
\]
Although many mediated absorptive systems are adequately described by the Michaelis-Menten equation, it can by no means be assumed that all systems display such kinetics. In a facilitated diffusion system, it must be the case that $K_A = K_B$ (hence, $k_1 = k_4$ and $k_{-1} = k_{-4}$). Equation (2) can then be written:

$$J_{A \rightarrow B} = \frac{k_2 C}{2} \cdot \frac{k_2 (S_A + S_B) + S_A S_B}{(S_A + K_B)(S_B + K_A) + \frac{k_2}{k_1} (S_A + S_B + 2K_A)} \tag{2a}$$

The net flux described by this equation and its counterpart for the flux in the opposite direction is identical to that proposed by Dawson and Widdas (1964) to explain the effects of temperature and pH on glucose transfer in foetal guinea pig erythrocytes which could not be accounted for by true Michaelis-Menten kinetics.

Not only is there evidence that the rate limiting step in transport is not always the transit of loaded carrier, there is also evidence that in some systems the rates of transit of free and loaded carrier are unequal. The phenomena of accelerative exchange diffusion and compulsory exchange diffusion (described in Stein, 1967, pp. 151-152, 156) appear to result from a more rapid movement of loaded carrier than of free carrier.

It remains the case, however, that the most feasible method of examining mediated absorption kinetics is the application of the Michaelis-Menten equation as a first approximation. In this regard, it will be helpful to examine the consequences of inhibition and the methods of determination of kinetic constants using the Michaelis-Menten formulation.
Inhibition

The effects of inhibitors can be divided primarily into two types: competitive inhibition, in which the inhibitor changes the apparent affinity of the carrier for substrate without altering the maximal velocity; and non-competitive inhibition, in which the substrate dissociation constant is unchanged and the maximal velocity is reduced.

A generalized inhibition equation can be derived which describes both competitive and non-competitive inhibition for a system which displays Michaelis-Menten kinetics. Two parameters require definition: \( \alpha \) represents the change in affinity of a carrier site induced by the binding of a competitive inhibitor; \( \beta \) is the change in the kinetic parameter \( (k_2) \) induced by a non-competitive inhibitor (see Webb, 1963, p. 56). Webb gives the following equation for the inhibition of a single step enzyme catalysis in which \( k_2 \) is rate limiting:

\[
V_i = \frac{V_{\text{max}} \cdot \frac{\alpha K_i + \beta I}{\alpha K_i + I}}{\frac{K_s \cdot \frac{\alpha K_i + \alpha I}{\alpha K_i + I} + S}{K_m' + S}} = \frac{V'_{\text{max}} S}{K_m' + S}
\]

where \( K_i \) is the inhibitor dissociation constant. From this formulation, expressions for various inhibitory situations are easily obtained.

**Competitive Inhibition:**

By definition, competitive inhibition effects a change only in the affinity of the carrier for substrate: \( \beta \) equals unity, \( 1 < \alpha \leq \infty \),

\[
V'_{\text{max}} = V_{\text{max}}', \quad \text{and:} \quad K_m' = K_m \cdot \frac{K_i + I}{\frac{K_i + I}{\alpha}}
\]
When the inhibitor totally prevents the binding of a substrate molecule, \( \alpha \) is infinite, the inhibition is completely competitive, and

\[
K'_m = K_m \left( 1 + \frac{I}{K_i} \right)
\] (4)

If \( \alpha \) has a finite value, the inhibitor only partially reduces the binding of substrate, and the inhibition is partially competitive. Partially competitive inhibition of enzyme reactions frequently arises when the inhibitor binds at some location other than the active site, or when it replaces a co-factor whose primary function is to increase the affinity of the enzyme for substrate. Partially competitive inhibition of mediated absorption by substrate analogs implies the presence of two distinct substrate binding sites. If both sites function in transport, the kinetics of uptake must be second order with respect to substrate.

**Non-competitive inhibition:**

Non-competitive inhibition demands that \( \alpha \) equal unity and \( 0 \leq \beta < 1 \).

If \( k_2 \) is rate limiting, \( K'_m = K_m \) and

\[
V'_{\text{max}} = V_{\text{max}} \frac{K_i + \beta I}{K_i + I}
\]

When \( k_2 \) is not rate limiting, \( K'_m \) is a function of \( \beta k_2 \) and \( K'_m \neq K_m \).

When \( \beta = 0 \), the inhibition is completely non-competitive and

\[
V'_{\text{max}} = V_{\text{max}} \frac{K_i}{K_i + I}
\]
Methods of Graphical Analysis

Algebraic manipulation of the standard Michaelis-Menten equation allows the formulation of three linear functions in velocity and substrate from which $K_m$ and $V_{max}$ can be determined:

a) \[ \frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{S} \]

b) \[ \frac{S}{V} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \cdot S \]

c) \[ V = V_{max} - K_m \cdot \frac{V}{S} \]

Dixon and Webb (1964, pp. 69-70) have discussed briefly the relative merits and limitations, in their opinions, of each of the functions for the determination of kinetic parameters. The formulation which they prefer, and which is by far the most commonly used, is the double reciprocal plot of Lineweaver and Burk\(^2\) (equation a). Hofstee (1959) has argued that the plot of $v$ against $v/S$ (equation c) gives a more equal distribution of points; such a plot is therefore more likely to show any deviations from linearity which might be overlooked when either of the reciprocal plots is used. In defense of the Lineweaver-Burk method, Dixon and Webb have argued that data points ought to be taken over logarithmic increments, and that this will give a more even distribution of points in a double reciprocal plot. In criticism of the $v$ versus $v/S$ plot, they point out that since both the ordinate and the

\(^2\)Webb (1963, p. 152) points out that this method was originally suggested by B. Woolf, who was quoted in a book by Haldane and Stern in 1932, prior to the publication of the paper by Lineweaver and Burk. The other linear plots described here were also discussed by Haldane and Stern.
abscissa are functions of the dependent variable, any error in measurement will cause a displacement of points along both axes. In further criticism, they state that, since there is no scale of substrate concentration, it is "difficult to identify the different points".

The method of choice should, of course, be dictated by the most feasible manner of collecting data for the circumstances of an individual study. Since all three methods are derived from the same equation, they should give identical values for $K_m$ and $V_{max}$ within experimental error. Any discrepancies among the kinetic parameters obtained by the different graphical methods may be an indication that the system under study is more complex than one described by the Michaelis-Menten equation.

These equations can be used to interpret the effects of inhibitors provided the rate function conforms to Michaelis-Menten kinetics. The graphical treatments give $V'_{max}$ and $K'_m$, from which it can be determined whether the inhibition is competitive or noncompetitive. An apparent $K_i$ for complete inhibition can then be calculated using values of $V_{max}$ and $K_m$ obtained in the absence of inhibitor.

Dixon (1953) introduced a fourth method for the analysis of the kinetic effects of an inhibitor. The inhibition equation for partially competitive inhibition can be rearranged to give:

$$\frac{1}{v_i} = \frac{1}{V_{max}} \cdot \left[ 1 + \frac{K_m}{S} \cdot \frac{K_i + I}{K_i + \frac{I}{\alpha}} \right]$$

A plot of $1/v_i$ against $I$, at constant substrate concentration, will be linear only if $\alpha$ is infinite, that is to say, only if the inhibition is completely competitive, in which case the equation reduces to:
\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{1}{V_{\text{max}}} \cdot \left[ \frac{K_m}{S} + \frac{K_m}{S} \frac{I}{K_i} \right]
\] (6)

\(K_i\) is given by the negative I value corresponding to an ordinate value of \(1/V_{\text{max}}\), and can be determined either by calculation using a previously determined value of \(V_{\text{max}}\), or as the point of intersection of two curves run at different substrate concentrations.

Similarly, partially and completely non-competitive inhibition can be distinguished. The plot of \(1/v_i\) against I will be non-linear if the inhibition is partially non-competitive, and will reduce to linearity only when the inhibition is completely non-competitive. It is possible to distinguish competitive and non-competitive inhibition if plots of experiments run at two different substrate concentrations are compared. For both cases, the curves will intersect at values corresponding to \(-K_i\); however, for non-competitive inhibition, this intersection also corresponds to the intersection with the abscissa.

Webb\(^3\) (1963, p. 57) has introduced the term fractional inhibition, \(i\), for dealing with inhibition kinetics. The fractional inhibition is equivalent to the percentage inhibition, normalized to a scale of 0-1.0:

\[
i = 1 - \frac{V_i}{V} = \frac{V - V_i}{V} = \frac{\text{percentage inhibition}}{100}
\]

For the generalized inhibition equation, the reciprocal of the fractional inhibition is:

\(^3\)Webb (pp. 150-164) has presented graphical examples of each of the five methods described here for the various types of inhibition of enzymic reactions displaying Michaelis-Menten kinetics.
\frac{1}{I} = \frac{S + K_m}{S(1-\beta) + K_m(\alpha-\beta)} + \frac{K_i(S + K_m)}{S(1-\beta) + K_m(\alpha-\beta)} \cdot \frac{1}{I}

Hence, a plot of 1/i against 1/I is linear for all types of inhibitions. The equation for completely competitive inhibition reduces to:

\frac{1}{I} = 1 + \frac{K_i}{I} \cdot \frac{S + K_m}{K_m}

A plot of 1/i against 1/I intersects the ordinate at a value of 1/i = 1.0 (one hundred percent inhibition) for both completely competitive and completely non-competitive inhibitions. For partial inhibitions, the ordinate intercept is larger than unity, as would be expected since the inhibitor only partially reduces substrate affinity or the catalytic rate.

Inhibition Equations Based on the Carrier Model.

When the rate limiting step in membrane transport is the transit of the carrier-permeant complex across the membrane, the kinetics are of the Michaelis-Menten type. A generalized inhibition equation for this situation can be derived, provided it is assumed that the specific concentration\textsuperscript{4} of inhibitor, I', at face B of the membrane is negligible.

If q is the ratio of the rate of movement of the carrier-inhibitor complex to that of the carrier-substrate complex, the inhibition equation for unidirectional flux is:

\textsuperscript{4} The specific concentration of substrate, S', or of inhibitor, I', is the ratio of the concentration of substrate or inhibitor to its thermodynamic dissociation constant for binding to the carrier. This definition differs from that of "relative substrate concentration", \(\sigma\), proposed by Dixon and Webb (1964, p. 65), where the relative substrate concentration is the ratio of the substrate concentration to the Michaelis constant for an enzyme reaction (see Webb, 1963, p. 65).
\[ J_{i}^{\text{i} A \rightarrow B} = \frac{k_{2} C \cdot \frac{(K_{B} + rS_{B}) S_{A} (\alpha K_{B} + \beta I)}{(K_{B} + rS_{B})(\alpha K_{B} + \beta I) + r(K_{B} + S_{B})(\alpha K_{B} + \beta I)}}{\frac{K_{A} S_{B}}{(K_{B} + rS_{B})(K_{i} + \frac{I}{\alpha})} + r(K_{B} + S_{B})(K_{i} + \beta \frac{I}{\alpha}) + S_{A}} \]  

(7)

For competitive inhibition, \( \beta = 1 \), and the equation reduces to the form

\[ v_{i} = \frac{V_{\text{max}} S_{A}}{K_{t} + S_{A}} \]

where

\[ K_{t}^{' \prime} = K_{t} \cdot \frac{K_{i}}{K_{i} + \frac{I}{\alpha}} + \frac{(1+rq)K_{B} + r(1+q)S_{B}}{(r+1)K_{B} + 2rS_{B}} \cdot \frac{K_{A} I}{K_{i} + \frac{I}{\alpha}} \]

If the rates of transit of the carrier-inhibitor complex and the carrier-substrate complex are equal, \( q=1 \) and the equation for \( K_{t}^{' \prime} \) becomes:

\[ K_{t}^{' \prime} = \frac{K_{t} K_{i} + K_{A} I}{K_{i} + \frac{I}{\alpha}} \]

When the inhibition is completely competitive,

\[ K_{t}^{' \prime} = K_{t} + K_{A} \cdot \frac{I}{K_{i}} = K_{t} \cdot \left[ 1 + \frac{I}{K_{i} K_{t}} \right] \]  

(8)

If \( r = 1 \), \( K_{t} = K_{A} \), and equation (8) is exactly equivalent to equation (4) for inhibition based on the Michaelis-Menten equation for enzyme catalysis. If \( r \neq 1 \), the values of \( K_{i} \) determined for inhibitors of mediated absorption will actually be \( K_{i}(K_{t}/K_{A}) \). Furthermore, from equation (3), \( K_{t} < K_{A} \) if \( r \) exceeds unity. When the value of \( K_{i} \) for a substrate acting as an inhibitor of its own absorption is determined assuming the applicability of Michaelis-Menten kinetics, it is assumed
that the value obtained is equivalent to $K_s$; however, according to equation (8), the value calculated using this assumption will be $K_t$.

If it is not assumed that the transit of the carrier-permeant complex is rate limiting, the kinetic expression for mediated absorption, even when $r=1$ (equation 2) is not of the Michaelis-Menten format. If $K_1 = k_{-5}/k_5$, the steady state equation for completely competitive inhibition of the facilitated diffusion system described by equation (2a) ($r=1$; $K_A = K_B$; $I'_B = 0$) is:

$$J_{A-B} = \frac{k_2}{2} \cdot \frac{k_2 S_A\left(K_A + \frac{k_2}{k_1} + S_B\right) + k_2 S_B\left(1 + \frac{k_5 I}{2k_2 + k_5}\right)}{K_A(K_A + 2k_2) + S_B\left(1 + \frac{1}{K_1} \frac{k_2 + k_5}{2k_2 + k_5}\right)} \cdot \frac{k_2 I}{2k_2 + k_5}$$

The reciprocal velocity equation is thus:

$$\frac{1}{v_1} = \frac{2}{k_2C} + \frac{K_A(K_A + \frac{k_2}{k_1} + S_B)\left(1 + \frac{1}{K_1} \frac{k_2 + k_5}{2k_2 + k_5}\right)}{S_A\left(K_A + \frac{k_2}{k_1} + S_B\right) + \frac{k_2}{k_1} \cdot S_B\left(1 + \frac{k_5 I}{2k_2 + k_5}\right)} \cdot \frac{2}{k_2C} \cdot k_2C$$

A Dixon plot of $1/v_1$ against $I$ will be non-linear with respect to $I$ unless $k_1 \gg k_2$, in which case membrane transit is rate limiting and the rate equations are identical to those defined by the Michaelis-Menten equation. Non-linearity in a Dixon plot is presumed to be diagnostic of partially competitive (or non-competitive) inhibition. However, non-linearity will also arise not only from a kinetic expression like that described above, but also from the kinetics observed for the inhibition.
of the Michaelis-Menten type of transport of a permeant by two separate 
and distinct loci if the kinetic constants for the two systems are 
sufficiently different.

It can be shown (see APPENDIX), that the kinetics of a single trans- 
port system described by equation (2) are also similar to those expected 
for absorption which is mediated by two distinct systems in the absence 
of inhibitor. The kinetic profiles of plots of $1/v$ against $1/S$ and of 
$S/v$ against $S$ are convex hyperbolas; those of $v$ against $v/S$ plot are 
concave hyperbolas. These similarities make the differentiation of a 
single transport system in which membrane transit is not rate limiting 
and two transport systems which are qualitatively identical in their 
relative affinities for various substrates and inhibitors exceedingly 
difficult. It should be emphasized however, that unless it can be 
eunequivocally demonstrated that the kinetics of a single transport system 
in the absence of inhibitor are indeed linear for all three forms of 
graphical analysis, non-linearity of a Dixon plot should not be construed 
as "partially competitive inhibition" in the sense that a strict defini- 
tion demands.
MATERIALS AND METHODS

Maintenance of *Moniliformis dubius*

Cystacanths collected from cockroaches infected at least ten weeks previously were washed thoroughly in deionized water. Groups of 25 cystacanths were administered to male Sprague Dawley rats (Holtzman Co.) weighing 80-100 grams via a pipette inserted through the mouth into the esophagus. The 18-25 rats infected for each experiment were housed in two large wire cages in air-conditioned animal quarters and were given Purina Laboratory Chow (Ralston Purina Co.) and filtered tap water *ad libitum*. Rats not sacrificed for experiments were transferred to less densely populated cages and maintained as stock sources of mature worms.

Colonies of *Periplaneta americana* were maintained in blackened 10, 15, or 25 gallon aquaria covered with lids made of stainless steel or nylon mesh mounted in a wooden frame. The cockroach diet comprised pulverized Purina Laboratory Chow, supplemented occasionally with apples, and tap water. The colonies were replenished periodically with cockroaches trapped on the roof of the Rice University biology building. One week prior to infection with acanthors, 50-100 *Periplaneta* in all stages of development were transferred to a clean ten gallon aquarium and deprived of food and water.

Rats harboring 9-18 week old infections were sacrificed by cervical dislocation. The female parasites were immediately removed from the gut to ice-cold KRT. After each worm had been washed free of intestinal

5 Krebs-Ringer saline buffered with Tris (hydroxy-methyl) amino-methane-maleic acid buffer, pH 7.4. The saline contained: 120 mM NaCl, 4.8 mM KCl, 2.6 mM CaCl₂, and 1.2 mM MgSO₄. Buffer was 25 mM in Tris and in maleic acid, and contained 28.5 mM NaOH. Saline and buffer were made separately in ten-fold concentrated stocks and mixed and diluted for use.
debris, the posterior end was cut off, and the "body fluids" were "milked" into a centrifuge tube. The remaining contents of the pseudocoelomic cavity were flushed into the centrifuge tube with two milliliters of KRT administered through a syringe and 25-gauge needle inserted into the anterior end of the cavity. The carcasses were transferred to chilled tared beakers for subsequent use in the preparation of soluble enzyme fractions.

The acanthors, rinsed at least 5 times in deionized water, were transferred to coarse filter paper, which effectively removed most of the excess water. Commercial applesauce, similarly reduced in moisture content with filter paper, was mixed with the acanthors in approximately equal proportions. The applesauce-acanthor mixture was presented to the cockroaches on small pieces of filter paper.

Absorption Experiments: Kinetics

Experimental Procedure.

Unless otherwise specified, test sugars at appropriate concentrations were labelled with radiocarbon at specific activities between 0.002 and 0.1 mC/m mole, depending on the individual experiment. In inhibition experiments employing labelled substrate at a single concentration, the specific activity was generally 0.02 mC/m mole. All incubations were aerobic.

Incubation media were made up in KRT. In those experiments where individual salts were substituted for Krebs-Ringer saline, the osmolarity of saline-buffer stocks made to 2x concentration was adjusted to that of 2x KRT with the aid of an Advanced Osmometer 89-90 (Advanced Instruments,
Inc., Newton Highlands, Mass.). Procedural modifications for individual experiments are described in context.

Parasites were removed from normally fed rats 32 days post-infection by gently tearing the intestine at 2 to 3 inch intervals. Female worms were immediately transferred to KRT at 36.5°C, and held at that temperature throughout the course of the experiment. Worms were washed in four 100 ml changes of KRT and placed in 50 ml beakers containing 10 ml KRT, two worms per beaker, in a shaking water bath (Research Specialties Co.) in which the experimental incubations were to be performed.

After 30 minutes preincubation, the worms were transferred with a hook to 30 ml beakers containing 4 milliliters of the incubation medium. Following incubations of exactly 4 minutes duration, worms were rinsed in two changes of KRT, blotted on coarse filter paper, and extracted overnight in 2.5 ml of 70% ethanol.

The extracted worms were placed in aluminum tares and dried for 24 hours at 60°C. Dry weights were determined to the nearest 0.1 mg using a Sartorius balance. Aliquots of the ethanol extracts (0.5 ml) and of the incubation media (0.1 ml) were pipetted into 5 ml of toluene-PPO-POPOP scintillation cocktail ("Liquifluor", New England Nuclear Corporation), containing 20% by volume BBS-3 solubilizer (Beckman Instruments), and counted for 2x10 minutes in a Packard 3000 series liquid scintillation spectrophotometer.

Quantification of Results.

Counting efficiencies were determined by the method of channels ratios (Herberg, 1965). Window settings of 50-1000 in the counting
channel and 100-1000 in the monitor channel, with gains of 7.85% in both channels, indicated counting efficiencies of 70-80% for $^{14}$C.

The amount of substrate absorbed was calculated from the specific activity of the medium, in disintegrations per minute (DPM) per micromole of substrate, and from the total DPM in the ethanol extract. Data were expressed as micromoles of substrate absorbed per milligram ethanol extracted dry weight per four minutes. Most determinations were the average of 5 individual replicates.

Absorption Experiments: Metabolism

In experiments where the metabolic fate of absorbed compounds was to be determined, samples of 5 worms each were incubated in medium of specific activity between 0.2 and 1.25 mC/mmole, depending on the substrate and the nature of the experiment. Incubations were of 1, 2, or 4 minutes duration. The worms were extracted in 10 ml 70% ethanol overnight, and then re-extracted in 5 ml 70% ethanol for 8 hours. Aliquots (0.05 ml) were taken from the first extraction for the determination of total uptake; the extracts were combined and evaporated to dryness in vacuo at 42°C.

In most cases, the extracts were treated with chloroform-water (4:1) in order to remove some of the abundant lipids. Extracts were transferred to 250 ml bottles and shaken vigorously for one hour with 100 ml of the chloroform-water mixture. Following a 30 minute centrifugation at 750xg, the aqueous layer was removed to a second bottle and re-extracted with 75 ml chloroform. The second aqueous phase was evaporated to dryness in vacuo at 42°C and stored at -20°C. Samples were subsequently resuspended in 0.2 ml 10% isopropanol, and 25 or 50 microliter aliquots were
subjected to analysis by paper chromatography or high voltage paper electrophoresis.

Paper Chromatography and High Voltage Paper Electrophoresis

**Solvent and Buffer Systems.**

One-dimensional descending chromatography on Whatman #1 paper was conducted in metal chromatography cabinets (Research Specialties Co.) equipped with 10 inch glass solvent troughs and glass anti-siphon rods. Two solvent systems were employed:

Solvent #1  n-Butanol:ethanol:acetone:water = 50:40:30:20  
(Gray and Fraenkel, 1954)

Solvent #2  n-Butanol:propionic acid:water = 63:31:4
(Benson et al., 1950, cited and described by Crowley et al., 1963)

High voltage paper electrophoresis on Whatman #4 paper was carried out in two buffer systems: 0.03 M sodium borate, pH 9.2, and 0.2 M ammonium formate, pH 3.6. Electrophoretograms were run at 1500 volts for 1-1/2 hours using a Savant 3000 volt power supply and flat aluminum plate cooled initially to 5°C by circulating refrigerant.

For quantitative chromatography, solvent #1 was employed almost exclusively because of its relative insensitivity to interference by salts and charged polymers. Solvent #2 and paper electrophoresis were used to verify the nature of compounds identified by their relative mobilities in solvent #1.
**Qualitative Analysis of Chromatograms.**

Radioactive standards and samples were treated similarly. Chromatograms and electrophoretograms were cut into 1 - 1\(\frac{1}{2}\) inch wide longitudinal strips. The radioactive areas were localized using either a Vanguard Autoscan 880 or a Packard Model 7201 gas-flow radiochromatogram scanner. Non-radioactive carbohydrate standards were visualized chemically using the silver nitrate method of Trevelyan et al. (1950). Standards for the chromatography of worm extracts were mixed with comparable extracts to compensate for the interference of ever-present lipids as well as water soluble components.

**Quantitative Analysis of Chromatograms.**

The quantitative distribution of radioactivity on chromatograms was determined by liquid scintillation counting (Davidson, 1962). 1 x 3-1/2 cm strips were inserted into vials containing 10 ml Liquifluor scintillation cocktail. For radiocarbon, up to 8 such strips could be counted in a single scintillation vial without noticeable reduction in counting efficiency; for tritium label, single strips were cut into 4 pieces and placed into vials so that all pieces rested flat on the bottom of the vial.

Radiocarbon samples were counted and corrected for quenching as described for liquid samples. In dual label counting, each vial was counted at least 4 x 5 minutes, and quench corrections were made on each counting cycle separately. Carbon overflow into the tritium channel and efficiency of corrected tritium counts were calculated on the basis of carbon counting efficiency determined by the channels.
ratios method. Activity of the various radioactive regions was expressed as a percentage of the total DPM recovered.

**Elution of Compounds for Verification of Identity.**

Following scintillation counting, strips of high activity were removed from the scintillation cocktail and washed 3 times in toluene to remove most of the fluor. The washed strips were cut into small pieces and added to glass columns containing sufficient glass distilled water to just cover the packed paper bed. Elution was effected by exhaustively washing with glass distilled water at a flow rate of approximately 0.5 ml per minute. The eluates were dried in vacuo at 42°C and redissolved in 0.1 ml 10% isopropanol for paper chromatography and electrophoresis.

**Quantification of the Trehalose Region.**

Eluates of the regions of chromatograms run in solvent #1 corresponding in mobility to trehalose were dissolved in glass distilled water. Aliquots were passed through tandem columns containing cation and anion exchange resins (Dowex-50Wx4, H⁺, and AG-3x4, OH⁻, Bio-Rad Laboratories). The neutral effluents were analyzed by paper chromatography and paper electrophoresis to insure that trehalose was the only uncharged component. The minimum proportion of the "trehalose region" corresponding to authentic trehalose was calculated on the basis of the percentage of the total radioactivity applied to the columns recovered in the neutral effluent.

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6 At standard window settings for C¹⁴, paper samples counted at 53% efficiency. Settings for dual label counting (tritium channel: 50-1000, 45.5% gain; carbon channels: 100-1000 counting, 120-1000 monitoring, 2.9% gains) gave efficiencies of 6-8% and 10-19%, respectively, for samples on paper.
Chemical Determination of Carbohydrates

The levels of total ethanol soluble carbohydrates in extracts of worms, expressed as micromoles of glucose equivalents, were determined using the phenol-sulfuric acid method of Dubois et al. (1956). Non-reducing sugars (predominately trehalose) were determined by phenol-sulfuric assay of diluted aliquots following 30 minute hydrolysis at 98°C in 30% (w/v) KOH. The levels of reducing sugars were calculated by difference.

Glucose in the incubation medium was measured by the glucose oxidase method ("Glucostat", Worthington Biochemicals Corp.). Glucose in worm extracts was determined with "Glucostat-Special" reagent.

Glycogen was extracted from both fresh worms and dried, ethanol extracted carcasses by digestion for one hour at 98°C in 5 volumes of 30% KOH and precipitated with 1.5 volumes of 95% ethanol. The samples were allowed to stand in the refrigerator for at least one hour to ensure adequate flocculation. The glycogen was centrifuged at 500xg for 15 minutes, and the precipitate redissolved in 2.5 ml distilled water. The dissolved glycogen was then reprecipitated with 4 ml 95% ethanol, and the pellet was washed at least four times in 70% ethanol to remove residual KOH and trehalose. The washed pellet was dissolved in 5 ml glass distilled water, and aliquots of appropriate dilutions were assayed for glucose equivalents by the phenol-sulfuric method. When the incorporation of absorbed radioactivity was determined, one milliliter of the redissolved washed pellet was added to 15 ml Liqui-fluor containing BBS-3, counted, and corrected for quenching by the channels ratios method.
The absorbance of all colorimetric assays was determined with a Zeiss PMQ II spectrophotometer. Phenol-sulfuric assays were read at a wavelength of 490 nanometers; glucose oxidase assays at 420 nanometers.

Kinase Assays

Preparation of the Enzyme Fraction.

"Body walls" of female worms, obtained during the isolation of acanthors, were homogenized in 3 volumes of cold KRT using a motor driven all-glass Ten Broeck homogenizer. The homogenate was cleared of cellular debris by two 10-minute centrifugations at 500xg in the SS-34 rotor of a Sorvall RC2-B refrigerated centrifuge. The cloudy preparation was then centrifuged for one hour at 12,000xg, and the supernate was filtered through glass wool to remove some of the abundant lipid. The filtrate was centrifuged at 37,500xg for 2-1/2 hours, and the soluble enzyme fraction, again filtered through glass wool, was lyophilized and stored at -20°C.

The resuspended lyophilizate was partially purified on a column of Sephadex G-25 (50-150μ) equilibrated in 1/5 strength KRT. The jacketed column, cooled to 4°C by circulating refrigerant, had a bed volume of 250 ml. Because of the gross quantities of lipid in the preparation, it was impossible to achieve satisfactory results with a single column passage. The crude fraction was therefore chromatographed in large quantities, and four excluded volumes were collected after the passage of the void volume. The effluent was reduced in volume by lyophilization, rechromatographed, and collected in one excluded volume. The resultant preparation, free of low molecular weight organic compounds, was transferred to individual flasks in quantities sufficient for a single assay, lyophilized, and stored at -20°C.
Assay Conditions.

Kinase activity was assayed using $^{14}$C labelled substrates. All assay media contained 0.05 M MgCl$_2$, 0.1 M Tris-maleate, pH 7.4, 0.5 microcuries of labelled substrate at the appropriate concentrations, and saturating levels of disodium ATP. Glucokinase assay tubes contained approximately 1 mg protein in a total volume of 0.2 ml, and the incubations were of 30 minutes or 1 hour duration. Fructokinase was assayed in a total volume of 0.8 ml containing less than 0.1 mg protein incubated for 20 minutes. All incubations were conducted in shaking water bath at 36.5°C. Reactions were stopped by immersion in a boiling water bath for 10 minutes.

Protein Determinations.

Aliquots of individual enzyme preparations were transferred to test tubes and stored at -20°C. Protein was subsequently determined with the Folin-Coicalteu reagent (Lowry et al., 1951), using crystalline bovine serum albumin as a standard. Absorbance was read at a wavelength of 660 nanometers in a Zeiss PMQ II spectrophotometer.

Analysis of Product Formation.

Individual assay tubes were centrifuged at 500xg for ten minutes to precipitate the denatured protein. Aliquots of the reaction mixture (25 microliters) were spotted onto Whatman #1 chromatography paper, and the hexose substrates were separated from the phosphorylated products by descending chromatography in solvent #1. The amount of product formed was calculated from the percentage of radioactivity recovered in regions corresponding to phosphorylated compounds and the total amount
of substrate present in the medium initially. Since phosphorylated compounds remain at or near the origin in this chromatography system, corrections were made for non-specific residual activity determined by chromatography of incubation media containing heat-killed enzyme.

The reaction velocity was calculated as micromoles of substrate phosphorylated per mg protein per hour. For purposes of kinetic analysis, the substrate concentration was calculated as the geometric mean of the initial and final concentrations in those cases where the amount converted to product exceeded 10% of the total radioactivity recovered.

Miscellaneous

Statistical calculations were made using a Hewlett-Packard model 9100-B programmable calculator. Graphical determinations of kinetic relationships were effected using least squares regression analysis where applicable. The kinetic parameters were determined as the average of values derived from the three methods of graphical analysis. Analysis of variance employed the Student's "t" test for the difference between means.

Radioisotopes were obtained from New England Nuclear Corporation. Reagent grade chemicals were purchased primarily from Fisher Scientific Company. Maleic acid was obtained from Mallinckrodt. Unlabelled carbohydrates and nucleotide triphosphates were purchased from Sigma Chemical Company. Glucose-free 3-O-methylglucose was obtained from Calbiochem.

With the exceptions of L-sorbose, L-fucose, and L-rhamnose, all sugars used were the D-enantiomorphs. $^{14}$C substrates were uniformly
labelled in all carbon atoms. Tritiated glucose was labelled at the C-6 position.
RESULTS

Kinetic Studies of Carbohydrate Absorption in *Moniliformis*

Absorption of Glucose.

Preliminary experiments were conducted to determine suitable conditions for extended kinetic studies. Consideration of various oppositely directed parameters resulted in the selection of 32 day old female worms as the standard experimental material: worms older than five weeks were highly variable in size, and consequently in their absorption properties; worms younger than four weeks, although much less variable, were too small to be manipulated readily. Female worms were chosen because their larger size minimized the time lag between infection of the hosts and recovery of parasites of sufficient size for experimental manipulation. The rate of absorption of radioglucose from a 2 mM solution was found to be constant over a period of ten minutes (Figure 1). Four minutes was therefore chosen as a convenient incubation period for all kinetic studies.

The rate of absorption of radioglucose is a non-linear function of the external glucose concentration (Figure 2). Although there was a considerable variation in absorption rates from experiment to experiment, as the examples in Table I demonstrate, the composite kinetic profile obtained when data from five individual experiments were pooled may be considered representative of the kinetic properties of the glucose absorptive system.


TABLE I

Variation in Experimentally Determined
Glucose Absorption Velocities

<table>
<thead>
<tr>
<th>Glucose Concentration (mM)</th>
<th>v ± S.E.</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>0.5</td>
<td>---</td>
<td>3.23 ± .11</td>
</tr>
<tr>
<td>1.0</td>
<td>4.27 ± .14</td>
<td>5.01 ± .20</td>
</tr>
<tr>
<td>2.0</td>
<td>7.87 ± .29</td>
<td>9.50 ± .54</td>
</tr>
<tr>
<td>3.0</td>
<td>10.29 ± .36</td>
<td>11.77 ± .46</td>
</tr>
<tr>
<td>4.0</td>
<td>12.49 ± .28</td>
<td>12.61 ± .87</td>
</tr>
<tr>
<td>5.0</td>
<td>13.07 ± .66</td>
<td>14.30 ±1.18</td>
</tr>
<tr>
<td>10.0</td>
<td>16.13 ± .41</td>
<td>17.76 ±1.69</td>
</tr>
</tbody>
</table>

When the combined data for glucose uptake were examined graphically, all three types of plots showed excellent correlations with linearity (Figures 3-5), indicating that the glucose absorptive system can be described by Michaelis-Menten kinetics. The average value for the maximal absorption velocity under standard experimental conditions was calculated using the extrapolated values obtained from each of the three plots of the composite data and found to be 20.66 ± 0.02 micromoles per gram ethanol extracted dry weight per four minutes. The calculated glucose concentration yielding half-maximal absorption velocity (Kₐ) was 2.77 ± 0.01 mM. It should be emphasized that the small standard errors for these calculated kinetic constants reflect
only the tightness of fit of the data points to the linear extrapolation for each of the plots of the composite profiles. The variation between kinetic constants calculated from individual experiments is quite large.

The effects of the nutritional state of the parasites on the kinetics of sugar absorption were examined using worms from rats which had been deprived of food for 24 hours prior to the experiment. The rates of absorption of radioglucose at several substrate concentrations were considerably higher for worms taken from fasted hosts than were those for control parasites from rats which had been allowed to feed ad libitum. When the absorption data for starved worms were multiplied by the ratio of the average dry weights of the fasted and control worms (0.711) in an attempt to normalize the results to comparable surface areas, the calculated uptake velocities of the experimental groups were still significantly greater than those of the controls (Figure 6).

Absorption of Other Hexoses

The absorption kinetics of mannose, 2-deoxyglucose, and N-acetylglucosamine were found to become saturated with increasing substrate concentration (Figure 7). Within the range of substrate concentrations examined, the uptake kinetics for each of these C-2 analogs of glucopyranose appeared to conform to those predicted by the Michaelis-Menten equation. Figure 8 demonstrates the linear relationship observed when the data for the absorption of each of these hexoses were examined using a plot of $v$ against $v/s$, which is the most sensitive of the three analytical plots to deviations from Michaelis-Menten kinetics. The
calculated kinetic constants for the absorption of the C-2 glucopyranose analogs examined in this study are presented in table II.

TABLE II

Kinetic Constants for Uptake of C-2 Glucopyranose Analogs

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$V_{\text{max}} \pm \text{S.E.}$ (µmoles/gram/4 min.)</th>
<th>$K_t \pm \text{S.E.}$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20.66 ± .02</td>
<td>2.77 ± .01</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>10.99 ± .12</td>
<td>2.52 ± .13</td>
</tr>
<tr>
<td>Mannose</td>
<td>24.70 ± .18</td>
<td>4.08 ± .06</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>10.47 ± .06</td>
<td>3.89 ± .04</td>
</tr>
</tbody>
</table>

When the absorption kinetics of fructose, 3-O-methylglucose and galactose were examined, they, too, were found to be non-linear with respect to substrate concentration (Figure 9). However, graphical analysis revealed that the absorption of these hexoses does not conform to Michaelis-Menten kinetics. The plots of $v$ against $v/s$ for fructose and 3-O-methylglucose (Figure 10) and for galactose (Figure 11) are hyperbolic in form. A double reciprocal plot of the data for galactose uptake (Figure 12), illustrates the danger of using this method for the analysis of absorption kinetics; the dramatic non-linearity observed in Figure 11 might easily go undetected in a Lineweaver-Burk analysis of the same data.

The kinetics observed for the absorption of fructose, galactose, and 3-O-methylglucose can be accounted for by either of two hypotheses: 1) absorption mediated through a single locus for which
transit of the carrier-permeant complex is not the rate limiting step, or 2) absorption mediated through two qualitatively distinct transport loci. For either case, $V_{\text{max}} = V_{1\text{max}} + V_{2\text{max}}$ for two sites) is the limit of the slope of a plot of $S/v$ against $S$ as the substrate concentration becomes large (Figures 13 and 14). If absorption is mediated through a single locus, a value for $K_i$ can be estimated from the limit of the slope of a $v$ versus $v/S$ plot at high substrate concentrations (see APPENDIX). The estimated values for $V_{\text{max}}$ and for $K_i$ (assuming a single transport locus) for those hexoses whose uptake does not follow Michaelis-Menten kinetics are, respectively: fructose, 38.5, 27 mM; 3-O-methylglucose, 16.3, 18 mM; and galactose, 33.2, 77 mM.

**Inhibition of Hexose Absorption.**

A variety of glucose analogs and potential metabolic inhibitors were examined for their effects on the absorption of radioglucose from a 2 mM solution. Sugars and sugar alcohols were tested at concentrations of 40 mM. The concentrations of general metabolic inhibitors were 1 mM, except for p-chloromercuribenzoic acid, which was at a concentration of 0.5 mM. The results of this survey are presented in Table III.

Analysis by paper chromatography and enzymatic assay of the sources of $\alpha$- and $\beta$-methylglucosides used in this survey revealed that each contained glucose contaminants of approximately 0.6%. This level of free glucose is not quite sufficient to account for the 20% inhibitions observed, but it does cast doubt on the significance of the residual effects of these glycosides as inhibitors of hexose uptake.
### TABLE III

**Effects of Potential Inhibitors on the Uptake of 2⁻ mM Glucose**

<table>
<thead>
<tr>
<th>Inhibitory</th>
<th>Not Inhibitory</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetylglucosamine</td>
<td>Arabinose</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>Gluconic acid</td>
</tr>
<tr>
<td>Fructose</td>
<td>Glucuronolactone</td>
</tr>
<tr>
<td>Galactose</td>
<td>Lactose</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>Lyxose</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>Mannitol</td>
</tr>
<tr>
<td>Maltose</td>
<td>Rhamnose</td>
</tr>
<tr>
<td>Mannose</td>
<td>Ribose</td>
</tr>
<tr>
<td>α-Methylglucoside</td>
<td>Sorbitol</td>
</tr>
<tr>
<td>β-Methylglucoside</td>
<td>Sodium Fluoride</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td></td>
</tr>
<tr>
<td>Salicin</td>
<td></td>
</tr>
<tr>
<td>Tagatose</td>
<td></td>
</tr>
<tr>
<td>p-Chloromercuribenzoic acid</td>
<td></td>
</tr>
</tbody>
</table>

Tagatose, the C-4 epimer of fructose, produced a 13% inhibition of glucose absorption at a substrate to inhibitor ratio of 1:20. This level of inhibition is comparable to that produced by the C-4 epimer of glucose, galactose, under the same conditions.

When 5 mM concentrations of 2-deoxyglucose, mannose, N-acetylglucosamine, and fructose were tested for their kinetic effects on the absorption of glucose, all were found to be competitive inhibitors (Figures
15 and 16). Similar results were obtained with glucosamine, 3-O-methylglucose, and galactose as inhibitors of glucose uptake (Figure 17).

To determine whether these inhibitions were partially or completely competitive, the effects of several different concentrations of glucose, 2-deoxyglucose, mannose, 3-O-methylglucose, and fructose on the uptake of radioglucose at 2 mM concentration were examined. The linear relationship between the reciprocal of the inhibited velocities and the concentrations of inhibitor (Dixon plot) indicated that these hexoses are completely competitive inhibitors of glucose absorption (Figures 18 and 19, upper curves). Completely competitive inhibition demands that the intercept with the ordinate of a Webb plot (1/i versus 1/I) be unity. Although the 1/i intercept of the Webb plot of the inhibition of glucose uptake by fructose (Figure 19, lower graph) is 1.29, the low levels of inhibition observed at the four substrate to inhibitor ratios tested, as well as the relatively large standard errors (10%) in the velocities determined in the presence of 30 and 40 mM fructose, make this value not significantly different from unity.

Similar relationships were observed for the inhibitions of 2-deoxyglucose and mannose absorption. Glucose, fructose, and N-acetylglucosamine competitively inhibit the uptake of 2-deoxyglucose (Figures 20 and 21). The diagnostic plots of the effects of 2-deoxyglucose, glucose, and fructose at several concentrations revealed that these hexoses are completely competitive inhibitors of mannose uptake (Figures 22 and 23).
When the effects of increasing concentrations of glucose-6-phosphate as an inhibitor of glucose uptake were examined, the plots of both $1/v$ versus $1$ and $1/i$ versus $1/i$ displayed dramatic deviations from linearity (Figure 24). Non-linearity in a Webb plot of inhibition kinetics indicates that the phenomenon is not a first order function of inhibitor concentration. The equations presented in the THEORETICAL section of this dissertation predict that the relationship between the reciprocal of the fractional inhibition and the reciprocal of the inhibitor concentration will be strictly linear, whether the absorption being examined displays simple Michaelis-Menten kinetics, non-Michaelis-Menten kinetics, or kinetics diagnostic of two distinct transport loci, regardless of the type of inhibition involved (partial or complete, competitive or non-competitive). The secondary relationship between inhibitor concentration and the degree of inhibition of glucose uptake for glucose-6-phosphate and maltose becomes evident when the fractional inhibition is plotted as a function of inhibitor concentration. Comparison of the kinetics for glucose-6-phosphate and maltose as inhibitors of glucose uptake to those for the truly first order inhibitors, glucose and 2-deoxyglucose (Figure 25), suggests that the inhibitory effects observed in the presence of the former may be due to free glucose liberated into the medium as a result of their hydrolysis.

The necessity of using methods other than the Lineweaver-Burk plot for the analysis of kinetic relationships first became strikingly evident when the effect of glucose on the absorption of fructose was examined. Regression analysis of double reciprocal plots of two separate determinations generated kinetic constants which indicated
that glucose is a non-competitive inhibitor of fructose uptake (Figure 26). When the data were re-examined using a plot of $v$ against $v/S$, however, the kinetic profiles for fructose absorption in the absence of inhibitor and in the presence of glucose or 2-deoxyglucose were, of course, non-linear (Figure 27). Although the values of $V_{max}$ extrapolated from the limited number of data points in these inhibition curves differ from that calculated for fructose absorption in the absence of inhibitor, the fact that the apparent transport constants estimated from the slopes of the linear portions of the $v$ versus $v/S$ plots are markedly larger than the $K_v$ for fructose uptake suggests that these hexoses exert their effects by interfering with the binding of fructose to the carrier site (or sites). More detailed analyses are necessary before accurate estimates of kinetic parameters can be made, but it is reasonable to assume, in the light of the other inhibition data, that these hexoses act as competitive inhibitors of fructose uptake.

The $v$ versus $v/S$ plots of the data obtained when the inhibition of $N$-acetylglucosamine absorption by other hexoses was examined are presented in Figure 28. The deviations from linearity observed when glucose and 2-deoxyglucose were tested as inhibitors over a range of substrate concentrations extending below that within which the uninhibited kinetics were examined suggests that the anomaly observed in the absorption kinetics for fructose, galactose, and 3-O-methylglucose is also characteristic of $N$-acetylglucosamine uptake. It is expected that similar deviations from linearity would be found for the uptake of $N$-acetylglucosamine in the absence of inhibitor at sufficiently low substrate concentrations.
The Dixon plot of the effect of increasing concentrations of glucose on the absorption of 2 mM fructose is a convex curve typical of "partially competitive (or non-competitive) inhibition" (Figure 29). Again, this relationship is suggestive either of absorption mediated through a single transport locus for which both the substrate-carrier dissociation constant and the rate of transit of the carrier-substrate complex are kinetically important, or of absorption mediated by two separate systems having different relative affinities for glucose and fructose.

In an attempt to differentiate between these two possibilities, the effects of paired hexoses at equal concentrations as inhibitors of fructose absorption were compared to the effects of the individual hexoses at two-fold concentrations. The results of the examination of mannose and galactose as inhibitors of 2 mM fructose uptake and of N-acetylglucosamine and 2-deoxyglucose as inhibitors of 5 mM fructose uptake are presented in Table IV.

The results of these examinations are inconclusive. Had the percentage inhibition effected by the two inhibitors in concert exceeded that caused by the more effective of the inhibitors at a two-fold concentration, two distinct transport loci would most definitely be implicated. However, such results would be expected only if the relative affinities of the two hexoses for the separate transport loci were different, that is, if each of the hexoses in the pair preferentially inhibited one of the transport sites.
TABLE IV

Effects of Pairs of Inhibitors on Fructose Absorption

<table>
<thead>
<tr>
<th>Inhibitor A</th>
<th>Inhibitor B</th>
<th>Percentage Inhibition ± 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mM Mannose</td>
<td>2.5 mM Galactose</td>
<td>52</td>
</tr>
<tr>
<td>5.0 mM Mannose</td>
<td>5.0 mM Galactose</td>
<td>63</td>
</tr>
<tr>
<td>10.0 mM Mannose</td>
<td>10.0 mM Galactose</td>
<td>73</td>
</tr>
</tbody>
</table>

I.  S = 2 mM Fructose

<table>
<thead>
<tr>
<th>Inhibitor A</th>
<th>Inhibitor B</th>
<th>Percentage Inhibition ± 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mM 2-Deoxyglucose</td>
<td>2.5 mM N-Acetylglicosamine</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

II. S = 5 mM Fructose

<table>
<thead>
<tr>
<th>Inhibitor A</th>
<th>Inhibitor B</th>
<th>Percentage Inhibition ± 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

Table V is a compilation of the data for the effects of 40 mM concentrations of hexoses as inhibitors of the absorption of glucose and mannose at 2 mM concentrations and of fructose and galactose at 8 mM concentrations. Under the conditions of these determinations, the relative order of efficacy of the various hexoses as inhibitors is the same for all four substrates: glucose, 2-deoxyglucose > mannose > 3-O-methylglucose > fructose > galactose > sorbose = 0.
TABLE V
Effects of Glucose Analogs on the Uptake of Metabolizable Hexoses

<table>
<thead>
<tr>
<th>INHIBITOR (40 mM)</th>
<th>SUBSTRATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose (2 mM)</td>
</tr>
<tr>
<td>Glucose</td>
<td>90</td>
</tr>
<tr>
<td>Mannose</td>
<td>85</td>
</tr>
<tr>
<td>Fructose</td>
<td>47</td>
</tr>
<tr>
<td>Galactose</td>
<td>13</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>88</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td>63</td>
</tr>
<tr>
<td>Sorbose</td>
<td>0</td>
</tr>
</tbody>
</table>

Percentage Inhibition ± 5

The Qualitative Nature of the Glucose Absorptive System

Carbohydrate Levels in Female Moniliformis dubius.

In order to ascertain whether the absorption of glucose occurs against a concentration difference, the levels of glucose and other carbohydrates in 70% ethanol extracts of worms were determined as described in the MATERIALS AND METHODS. The results of representative determinations are presented in Table VI. The levels of carbohydrate are expressed as micromoles of glucose equivalents per gram ethanol extracted dry weight of worms ± the standard error of the mean. Values in parentheses represent the percentage of the dry weight accounted for by each fraction.
### TABLE VI

Carbohydrate Levels in 32 Day Old *Moniliformis dubius* Females

<table>
<thead>
<tr>
<th>ETHANOL SOLUBLE CARBOHYDRATES</th>
<th>GLUCOSE CONCENTRATION (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>Alkali-Stable</strong></td>
</tr>
<tr>
<td>349.1</td>
<td>229.4</td>
</tr>
<tr>
<td>± 10.2</td>
<td>± 3.1</td>
</tr>
<tr>
<td>(6.3)</td>
<td>(4.1)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>372.6</td>
<td>269.2</td>
</tr>
<tr>
<td>± 9.5</td>
<td>± 6.6</td>
</tr>
<tr>
<td>(6.7)</td>
<td>(4.9)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>357.1</td>
<td>283.2</td>
</tr>
<tr>
<td>± 9.9</td>
<td>± 6.6</td>
</tr>
<tr>
<td>(6.4)</td>
<td>(5.1)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>350.3</td>
<td>246.5</td>
</tr>
<tr>
<td>± 6.0</td>
<td>± 2.3</td>
</tr>
<tr>
<td>(6.3)</td>
<td>(4.4)</td>
</tr>
</tbody>
</table>

The overall concentration of glucose, in micromoles per milliliter of worm water, was calculated on the basis of a wet weight to ethanol extracted dry weight ratio of 4.44 ± .09 for female worms of this age. For physiological conditions comparable to those under which the kinetic examinations of hexose uptake were conducted, the glucose concentration in 32 day old female *Moniliformis dubius* appears to be approximately 27 mM.

When the amount of radioactivity recovered from ethanol extracts of worms which had been incubated for ten minutes in 1 mM radioglucose...
was compared to the disappearance of enzymatically determined glucose from the incubation media, the calculated absorption velocities were virtually identical. The average velocity determined from measurements of ethanol soluble radioactivity in ten samples was $12.88 \pm .29$ micro-moles of glucose absorbed per gram ethanol extracted dry weight per ten minutes; that determined from enzymatic assay of glucose depletion in the incubation media for the same ten samples was $13.54 \pm .94$. Hence, the tracer determinations of unidirectional flux of glucose appear to represent the net flux of glucose into worms against a sizable concentration difference.

In as much as the body of acanthocephalans consists essentially of two distinct compartments, the body wall and the pseudocoelom, the possibility that carbohydrates are partitioned disproportionately between these compartments was investigated. Female worms recovered from hosts 32 days post infection were washed free of intestinal debris and divided into four groups of six worms each. Two groups were immediately blotted on coarse filter paper, weighed, and transferred to tubes containing 70% ethanol. Worms in the other two groups were weighed as a group and placed in KRT at $36.5^\circ$C. As rapidly as possible, individual worms were removed from the KRT, blotted, and carefully cut at the anterior end so that none of the worm tissue was lost. The pseudocoelomic contents were gently "milked" into graduated centrifuge tubes, and the cavities were "flushed" with two milliliters of KRT. The body walls were recombined into the original groups of six, weighed, and placed in 70% ethanol. The volumes of the pseudocoelomic extracts were adjusted to 15 ml with KRT. After centrifugation at low
speed, the insoluble residue of the pseudocoelomic contents was extracted in 70% ethanol.

Aliquots of the ethanol extracts of worm bodies, the water soluble pseudocoelomic contents, the ethanol soluble extracts of the pseudocoelomic residue, the ethanol insoluble residues, and KOH digests of the extracted worm bodies were assayed for total carbohydrates and for alkali-stable carbohydrates by the phenol-sulfuric acid method as described in the MATERIALS AND METHODS. The values for the levels of carbohydrates in these fractions, expressed as micromoles of glucose equivalents per gram initial wet weight, are summarized in Table VII.

**TABLE VII**

Distribution of Carbohydrates Between the Body Wall and Pseudocoelom of Female *Moniliformis dubius*

<table>
<thead>
<tr>
<th></th>
<th>ETHANOL SOLUBLE CARBOHYDRATES</th>
<th>ALKALI-STABLE, ETHANOL</th>
<th>ALKALI-REDUCING (E BY diff.)</th>
<th>INSOLUBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. Whole worms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracted bodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>83.4</td>
<td>54.8</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td><strong>B. Partitioned worms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracted body walls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol extracts of body walls</td>
<td>68.7</td>
<td>44.8</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>Pseudocoelom:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRT soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>16.3</td>
<td>13.7</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25.9</td>
<td>21.8</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Total in partitioned worms</td>
<td>94.6</td>
<td>66.6</td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td>Percent in pseudocoelom</td>
<td>27</td>
<td>33</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>
The wet weights of the body walls after the removal of the pseudo-coelomic contents revealed that this fraction constituted approximately 17% of the total wet weight of the parasites. The high proportion of alkali-stable, ethanol soluble carbohydrate (trehalose) in the pseudo-coelom is compatible with the maturation of ovarian balls into young acanthors, in which high levels of trehalose are stored (McAlister and Fisher, 1972). There is no apparent disproportionality in the distribution of reducing sugars between the body wall and the pseudocoelom. Hence, the estimated glucose concentration of 27 mM appears to be applicable to the body wall as a whole.

Effect of Temperature on the Absorption of Glucose.

The influence of temperature on the velocity of glucose uptake was examined over a range of 22°C at two substrate concentrations. Worms were equilibrated at the test temperature for 20 minutes prior to the experimental incubation. The absorption velocities observed when worms were incubated for four minutes in 1.25 or .20 mM solutions of glucose are presented in Table VIII.

Although uptake rates were dramatically influenced by temperature below the physiological range, the thermal parameters for absorption at 1.25 mM, a concentration less than half the calculated $K_t$, and at 20 mM, a nearly saturating concentration, were almost identical. The "activation energy" for glucose uptake calculated from the slopes of Arrhenius plots of these data (Fig. 30) was 37,450 calories/mole. Chemical analysis of the ethanol extracts of worms revealed no differences in the levels of ethanol soluble carbohydrates between any treatments.
TABLE VIII
Effect of Temperature on Glucose Uptake

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>$V^2$ ± S.E. mean (μmoles/gm/4 min.)</th>
<th>$Q_{10}$</th>
<th>$H^O$ (Kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.5</td>
<td>.290 ± .038</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>29.5</td>
<td>2.112 ± .215</td>
<td>4.9</td>
<td>36.6</td>
</tr>
<tr>
<td>36.5</td>
<td>6.379 ± .250</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>43.5</td>
<td>9.043 ± .585</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$S = 1.25$ mM glucose

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>$V^2$ ± S.E. mean (μmoles/gm/4 min.)</th>
<th>$Q_{10}$</th>
<th>$H^O$ (Kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.5</td>
<td>.682 ± .055</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>29.5</td>
<td>5.052 ± .451</td>
<td>5.9</td>
<td>38.3</td>
</tr>
<tr>
<td>36.5</td>
<td>17.504 ± .424</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>43.5</td>
<td>28.395 ± 1.060</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$S = 20$ mM glucose

Effects of Cation Deletion on Glucose Absorption.

The observation that glucose absorption occurs against an apparent concentration difference at the organismal level suggested that the transport mechanism might involve sodium-dependent cotransport. A series of experiments were conducted to examine the influence of the deletion of sodium and other cations from the incubation medium on the absorption of glucose. Media were prepared in which either the sodium or all the ionic species of Krebs-Ringer saline were replaced by a single salt species. The buffer systems were 25 mM Tris-Cl, pH 7.4
or 25 mM Tris buffered to pH 7.4 with maleic acid. The osmolarities of 2x stocks of incubation media were adjusted to that of 2x stocks of KRT.

Worms were preincubated in the test medium for 20 minutes, or preincubated in KRT and rinsed thoroughly in four changes of test medium, prior to the determination of uptake velocities. The absorption of glucose was found to be unaffected by the total substitution of Tris-Cl, LiCl, KCl, NaCl, MgCl₂, choline Cl, or Na₂SO₄ for the ionic constituents of normal saline in the presence of 25 mM Tris-maleate or Tris-Cl buffer. Preincubation for 20 minutes in solutions of KRT containing the cardiac glycoside ouabain at a concentration of 0.5 mM had no effect on the subsequent absorption of radioglucose at a concentration of 2 mM.

Attempts to examine the effects of total ion deletion were confounded by the dramatic influence of pH on the rate of glucose absorption (Figure 31). A test medium isosmotic to KRT was prepared using mannitol to replace the ionic species. Worms were preincubated in KRT, rinsed briefly in four changes of the test medium, and then incubated in 2 mM glucose in the test medium for 60 seconds. The amount of glucose absorbed from the mannitol medium was 60% less than that absorbed from the control KRT medium. However, the velocity of glucose absorption from KRT at a pH corresponding to that of the salt-free mannitol (pH 5.4) is approximately 50% less than that observed at a pH of 7.4. Hence the reduction of glucose uptake in the mannitol solution is wholly attributable to the lower pH of that medium.
Metabolism of Absorbed Glucose.

The results of several preliminary experiments suggested that the metabolism of absorbed glucose is intimately linked with the actual uptake process. The dramatic increase in absorption velocity in worms taken from starved hosts has already been described. Preincubation of worms in 5 or 10 millimolar solutions of glucose for 30 minutes was found to cause a significant reduction in the subsequently determined rates of radioglucose uptake. Chemical analysis of ethanol extracts of worms from these preloading experiments (Table IX) revealed that the levels of total ethanol soluble carbohydrates were significantly higher in experimental worms, suggesting that the reduced uptake rates observed might be due to the saturation of systems involved in the metabolism of absorbed glucose. Although this hypothesis at first seemed incompatible with the high endogenous levels of glucose in Moniliformis, the possibility that absorbed glucose was rapidly being metabolized was investigated.

Worms were incubated in 2.7 or 10 mM $^{14}$C-glucose at high specific activity for periods of 1-4 minutes. Some samples were further incubated in unlabelled glucose for up to 40 minutes. The worms were extracted in 70% ethanol, and the ethanol extracts were treated with chloroform to remove lipids as described in the MATERIALS AND METHODS. A summary of the results obtained when extracts were analyzed by paper chromatography in solvent #1 and by high voltage paper electrophoresis in formate and borate buffers is presented in Table X. The percentages in each fraction are based on the total radioactivity recovered on the chromatograms.
### TABLE IX

**Effect of 30 Minutes Preincubation in Glucose on the Subsequent Uptake of C<sup>14</sup> Glucose**

<table>
<thead>
<tr>
<th>Pre-incubation (mM glu.)</th>
<th>Incubation (mM glu.)</th>
<th>% Inhibition</th>
<th>Ethanol Soluble Carbohydrates&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P (Carbohydrates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>2.0 mM</td>
<td>9.78</td>
<td>370.7</td>
<td>+ 4.5</td>
</tr>
<tr>
<td></td>
<td>+ .13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>2.0 mM</td>
<td>6.59</td>
<td>402.1</td>
<td>&lt; .05</td>
</tr>
<tr>
<td></td>
<td>+ .41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>1.25 mM</td>
<td>8.58</td>
<td>372.8</td>
<td>+ 8.7</td>
</tr>
<tr>
<td></td>
<td>+ .42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>1.25 mM</td>
<td>6.60</td>
<td>409.4</td>
<td>&lt; .01</td>
</tr>
<tr>
<td></td>
<td>+ .28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>10 mM</td>
<td>18.77</td>
<td>377.2</td>
<td>+ 10.6</td>
</tr>
<tr>
<td></td>
<td>+ .36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>10 mM</td>
<td>12.45</td>
<td>446.3</td>
<td>&lt; .01</td>
</tr>
<tr>
<td></td>
<td>+ .55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> micromoles glucose absorbed per gram ethanol extracted dry weight per four minutes

<sup>b</sup> micromoles glucose equivalents per gram ethanol extracted dry weight
TABLE X
Chemical Distribution of Radioactivity Recovered from Ethanol Extracts of Worms Incubated in Uniformly Labelled Radioglucose

<table>
<thead>
<tr>
<th>Glucose Conc. (mM)</th>
<th>Incubation (min.)</th>
<th>Post-incubation (min.)</th>
<th>% Recovered Radioactivitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>2.7</td>
<td>1</td>
<td>--</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>10.0</td>
<td>1</td>
<td>--</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

a I = sugar-phosphates + sugar nucleotides + pyruvic acid
II = trehalose + malic acid + fumaric acid
III = alanine + lactic acid + succinic acid
IV = glucose
At the end of a one minute incubation in either 2.7 or 10 mM glucose, only 8% of the total radioactivity was recovered as glucose. As much as 23% of the activity chromatographed as early intermediates of glucose metabolism (phosphorylated sugars), while over 50% had properties characteristic of the disaccharide trehalose. Significant amounts of radioactivity were incorporated into glycogen only form the 10 mM incubation medium. Upon extended incubation, the percentage of the radioactivity in the "trehalose" region increased at the expense of phosphorylated intermediates, and stabilized at a level of approximately 70%.

The trehalose region of chromatograms was eluted for further identification. Of the radioactivity applied to tandem columns of cation and anion exchange resins, 68.3% was recovered in the neutral effluent. This radioactivity chromatographed as a single peak corresponding in mobility to authentic trehalose in all four solvent systems. The value of 68% is to be considered a minimum for the trehalose content of the region. Borate electrophoresis of extracts from four minute incubations indicated that trehalose accounted for approximately 80% of the radioactivity chromatographing in the disaccharide region in solvent #1.

At most 32% of the radioactivity absorbed in a four minute incubation enters the pseudocoelomic cavity. Eleven worms were incubated individually in 2 mM glucose for four minutes, and, after rinsing in KRT, their pseudocoelomic fluids were rapidly "milked" into 95% ethanol with 2 ml KRT. The percentages of the total radioactivity
absorbed recovered from the pseudocoelomic fluids ranged from 2.0 to
4.8 and averaged 3.25 ± .43. Hence, the chemical distribution of radio-
activity in worm extracts, at least for incubations of no longer than
four minutes duration, can be assumed to represent the distribution
within the body wall.

**Kinase Assays**

Because virtually all of the absorbed glucose appeared to be meta-
bolized within the time span required for manipulation of the worms,
the remote possibility that the observed saturation kinetics of hexose
uptake were really a manifestation of the metabolism of passively
absorbed substrate was examined. Since phosphorylation is a necessary
first step in the metabolism of hexoses, and since the mutual competi-
tions among hexoses observed in uptake experiments would require that
all interact at an initial step in the metabolic pathway, some of the
kinetic properties of hexose kinases obtained from high speed super-
nates of body wall homogenates were examined.

The enzyme preparation was dialysed by gel filtration on a column
of Sephadex G-25 as described in the MATERIALS AND METHODS. All
reaction mixtures contained 50 mM MgCl₂ and 100 mM Tris-maleate buffer,
pH 7.4. In assays of glucokinase, 20 mM ATP was used to insure that
the trehalose synthesizing system present in this preparation was com-
pletely inhibited (McAlister and Fisher, 1972). Concentrations of
ATP in fructokinase assays were 5 mM.

The existence of at least two distinct kinases was suggested by
a survey of the phosphorylative capacity of the enzyme preparation
toward a variety of hexoses at a single substrate concentration. Media containing 20 mM ATP, 0.92 mg protein and 12.5 mM hexose in a total volume of 0.2 ml were incubated for one hour at 36.5°C and analyzed by paper chromatography in solvent #1. The amounts of substrate phosphorylated, normalized to a value of 1.0 for glucose, were: mannose, 1.73; fructose, 2.67; galactose, 0.48; 2-deoxyglucose, 0.48; 3-O-methylglucose, 0.40; and glucosamine, 0.84. When these hexoses were examined at concentrations of 12.5 mM for their ability to inhibit the phosphorylation of 5 mM glucose, the following inhibitions were observed: glucose, 63%; mannose, 52%; fructose, 35%; galactose, 14%, 2-deoxyglucose, 26%; 3-O-methylglucose, 23%; and N-acetylglucosamine (substituted for glucosamine), 25%.

The kinetic effects of 5 mM 2-deoxyglucose, 5 mM mannose, and 5 mM glucose on the phosphorylation of radioglucose were examined in one hour incubations of media containing 1.47 mg protein in a total volume of 0.2 ml. Although the large amounts of initial substrate converted to product (as much as 65% for low substrate concentrations) make the calculation of meaningful kinetic constants impracticable, the relative inhibitory effects of the different hexoses can be determined. The plot of the reciprocal of the reaction velocity against the reciprocal of the geometric mean of the initial and final substrate concentrations presented in Figure 32 illustrates that glucose and mannose are potent inhibitors of the soluble glucokinase of Moniliformis. However, 2-deoxyglucose, which is a more potent inhibitor of glucose absorption than is mannose, has little effect on the glucokinase under the conditions of this examination.
Preliminary studies indicated that the activity of the fructokinase in this preparation was greater than that of glucokinase. In order to avoid the pitfalls of substrate depletion, the conditions for the assay of the inhibition of fructokinase were altered: the volume of the incubation medium was increased to 0.8 ml, the amount of protein in each assay tube was reduced to 0.057 mg, and the assay was run for only 20 minutes. When the effects of 2.5 mM concentrations of fructose, glucose, 2-deoxyglucose, 3-O-methylglucose, and N-acetylglucosamine on the phosphorylation of fructose at various concentrations were examined under these conditions, the maximum amount of substrate depleted was 6.7%. When the data were examined using a double reciprocal plot (Figure 33), fructokinase was found to be totally insensitive to any of the hexoses tested other than fructose itself.

The possibility that other kinases, having affinities paralleling those observed for the mutual interactions of hexoses in absorption studies, were lost, either through denaturation or because they are associated with membrane components, cannot be completely ruled out. However, the disparity between the affinities of hexoses for the soluble kinases examined and the efficacy of their interactions in absorption strongly suggests that the kinetic interactions determined in absorption studies truly represent competitions for stereospecific sites at the parasite-medium interface.

**Metabolism of Other Absorbed Substrates**

The results obtained when the metabolic fates of absorbed substrates other than glucose were determined are summarized in Table XI.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incubation (min.)</th>
<th>Post-incubation (min.)</th>
<th>Uptake&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Recovered Radioactivity&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose (2 mM)</td>
<td>4</td>
<td>--</td>
<td>10.5</td>
<td>14 66 13 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose (10 mM)</td>
<td>4</td>
<td>--</td>
<td>11.1</td>
<td>15 68 10 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose (22 mM)</td>
<td>2</td>
<td>--</td>
<td>3.9</td>
<td>13 56 22 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose (20 mM)</td>
<td>4</td>
<td>--</td>
<td>2.7</td>
<td>12 69 9 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose-6-phosphate (2.3 mM)</td>
<td>1</td>
<td>--</td>
<td>0.6</td>
<td>24 53 12 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Deoxyglucose (2 mM)</td>
<td>4</td>
<td>--</td>
<td>2.1</td>
<td>11 78 8 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-O-Methylglucose (2 mM)</td>
<td>2</td>
<td>20</td>
<td>2.8</td>
<td>18 1 26 40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>4</td>
<td>--</td>
<td>1.4</td>
<td>2.4 3.2 94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>1.6</td>
<td>13 21 68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>4.3</td>
<td>4.7 3 43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Uptake in micromoles of substrate per gram ethanol extracted dry weight.

<sup>b</sup> I = sugar-phosphates + sugar nucleotides + pyruvic acid
II = trehalose + malic acid + fumaric acid
III = succinic acid + lactic acid + alanine
IV = hexose
The chemical distribution of radiocarbon from absorbed fructose and mannose is virtually identical to that observed for glucose. The chromatography solvent employed does not permit the differentiation of the phosphorylated derivatives of glucose, mannose, and fructose, nor of the hexoses themselves. However, since fructose and mannose are capable of supporting fermentation and glycogenesis in *Moniliformis* (Laurie, 1957, 1959), the presence of phospho-hexose isomerasases for both mannose-phosphate and fructose-phosphate must be assumed. Hence, after mannose and fructose are absorbed, phosphorylation ultimately leads to their entry into the pathways of glucose metabolism.

The metabolism of absorbed galactose appears to be limited by the capacity of *Moniliformis* to phosphorylate this hexose. Although radiocarbon from galactose appears in the disaccharide regions of chromatograms, the levels of radioactivity in phosphorylated sugars are much lower than those found for other fermented sugars. The relatively large percentage of radioactivity in the hexose region was recovered as galactose.

The data for the chemical distribution of radioactivity absorbed from solutions of glucose-6-phosphate and maltose support the hypothesis that the inhibitory effects of these substrates on glucose absorption are due to free glucose liberated by their hydrolysis. The radioactive tracers used in these incubations were found to be free of glucose contaminants when examined by paper chromatography, yet the distribution of radioactivity is essentially identical to that observed when glucose was used as a substrate. No enrichment of the regions of phosphorylated sugars or disaccharides was found. Although the low
rates at which these substrates were absorbed can be interpreted as a manifestation of a very low affinity for the transport site, they can also be attributed to a second order relationship between the appearance of free glucose as a result of substrate hydrolysis and its subsequent absorption.

Absorbed 2-deoxyglucose, N-acetylglucosamine, and 3-O-methylglucose were found to be metabolized only sparingly. The low rates of metabolism of these hexoses were exploited in experiments designed to determine whether Moniliformis females are capable of accumulating absorbed hexose against a concentration difference.

**Accumulation of Poorly Metabolized Hexoses.**

Worms were incubated for 4 minutes in 2 mM solutions of 3-O-methylglucose or N-acetylglucosamine at high specific activity, and then transferred to 10 milliliters of KRT to allow equilibration of the contents of the worms with the external medium over a period of 10 minutes postincubation. The worms were extracted in 70% ethanol, and the extracts were treated with chloroform and chromatographed in solvent #1.

Radioactivity in the postincubation media was determined by liquid scintillation counting of 2x2 cm pieces of chromatography onto which 50 microliter aliquots of the solutions had been spotted. Radioactivity in the worms was determined both from dried aliquots of the ethanol extract and from the recovery of activity on paper chromatograms. The concentration of unmetabolized substrate was calculated by correcting the total "concentration" of radioactivity for the percentage of the activity remaining in unaltered substrate.
The ratio of the concentration of substrate in the worms to that in the postincubation media was calculated assuming that all of the activity in the media was in a form chemically identical with substrate. Although drying the aliquots to be counted on paper eliminated any errors in the estimation of the hexose concentration in the media due to the presence of volatile metabolic products (ethanol), the estimates do include any non-volatile metabolic products which might have been extruded from the worms. The high salt content of the postincubation solutions made analysis of the chemical distribution of radioactivity by paper chromatography impracticable. Hence, the calculated concentration ratios are minimal. The experimental determination of 2-deoxyglucose accumulation was achieved in the same manner. Incubations in the case of this hexose were of 2 minutes duration, and post-incubations were for 20 minutes. The results of these experiments are summarized in Table XII.

TABLE XII

Accumulation of Poorly Metabolized Hexoses

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Maximum Conc. of Postincubation Medium (mM)</th>
<th>Substrate Conc. in Worms (μmoles/ml worm water)</th>
<th>Minimum Accumulation Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-O-Methylglucose</td>
<td>0.0112</td>
<td>0.0197</td>
<td>1.64</td>
</tr>
<tr>
<td>(2 mM)</td>
<td>0.0110</td>
<td>0.0168</td>
<td></td>
</tr>
<tr>
<td>N-Acetylgalcosamine</td>
<td>0.0198</td>
<td>0.0607</td>
<td>2.69</td>
</tr>
<tr>
<td>(2 mM)</td>
<td>0.0204</td>
<td>0.0472</td>
<td></td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>0.0194</td>
<td>0.0808</td>
<td>4.26</td>
</tr>
<tr>
<td>(2 mM)</td>
<td>0.0193</td>
<td>0.0836</td>
<td></td>
</tr>
</tbody>
</table>
Compartmentation of Endogenous Glucose.

The evidence presented thus far concerning the nature of glucose absorption and accumulation would appear, upon superficial examination, to be somewhat contradictory. It has been shown that there is a net absorption of glucose from 1 mM external solutions into worms having an average concentration of glucose in the body wall of approximately 27 mM. The accumulation of N-acetylglucosamine, 3-O-methylglucosyl, and 2-deoxyglucose to concentrations higher than those in the external medium has been demonstrated directly. These observations are consistent with an "active transport" mechanism for hexose accumulation.

However, no evidence has been found for the involvement of sodium in the absorption of exogenous glucose. Furthermore, those hexoses which are capable of supporting fermentation in Moniliformis are phosphorylated almost immediately upon their absorption. The reduced absorption velocities observed following preincubation in glucose appear to result from 10-15% increases in the concentration of internal ethanol soluble carbohydrates.

The inconsistencies of these observations are resolved if it is assumed that the substantial pool of endogenous glucose is compartmentalized in some region of the body wall physically removed from the absorptive surface. Although some form of "active transport" system, perhaps sodium dependent in nature, might be involved in maintaining the glucose pool within this compartment, the experiments designed to examine the requirement for sodium in the absorption of glucose from the external environment would not reflect the intra-organismal involvement of cations in accumulation processes.
Some suggestions as to the mode through which the rapid metabolism of absorbed glucose is coupled with the accumulation of high levels of glucose reserves within the body wall of the worm can be gleaned from the studies of the chemical distribution of absorbed radiocarbon over prolonged periods of incubation summarized in Table X. The question was examined more extensively using dual isotope labelling in chase experiments.

Two samples of worms (Group A) were incubated for two minutes in 4 ml of uniformly labelled C$^{14}$-glucose (1 mM, 6.25 mC/mmole), transferred to 10 ml of unlabelled glucose (1 mM) for 10 minutes, and then incubated for two minutes in C-6-tritium labelled glucose (1 mM, 125 mC/mmole). One sample of the pair was immediately extracted in 70% ethanol (treatment 1); the other was postincubated for ten minutes in unlabelled glucose (treatment 2). In order to compensate for the effects of the non-random distribution of tritium label among the glucose carbons, a second pair of samples (Group B) was treated identically, except that the order of incubation in the radioactive isotopes was reversed. As a further control for disparities arising from isotope effects, a third pair of samples was preincubated for twelve minutes in unlabelled glucose, and then incubated for two minutes in a 1 mM mixture of the two isotopes; one sample was transferred immediately to ethanol, while the other was postincubated for ten minutes in unlabelled glucose.

Aliquots of the chloroform-treated extracts were analysed by descending paper chromatography in solvent #1. Radioactivity in the
various postincubation media was determined both for aqueous samples and for aliquots dried on 2x2 cm pieces of chromatography paper. The methods employed for determining the activities of each isotope are described in the MATERIALS AND METHODS. In no case was more than ten percent of the absorbed radioactivity liberated into the medium in subsequent postincubations.

The distribution of each isotope in the various chemical fractions and the ratios of the activities of isotopes in each fraction are presented in Table XIII

TABLE XIII

Chemical Distribution of Radioactivity Recovered from Ethanol Extracts of Worms Following Sequential Incubation in Dual Isotopes.

<table>
<thead>
<tr>
<th>% Recovered Radioactivity</th>
<th>Ia</th>
<th>Ib</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>1.0</td>
<td>4.4</td>
<td>69.7</td>
<td>14.0</td>
<td>5.1</td>
</tr>
<tr>
<td>$^{3}$H</td>
<td>11.6</td>
<td>7.5</td>
<td>68.1</td>
<td>9.8</td>
<td>6.0</td>
</tr>
<tr>
<td>ratio $^{3}$H/$^{14}$C</td>
<td>12.05</td>
<td>1.72</td>
<td>0.98</td>
<td>0.70</td>
<td>1.18</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>0.9</td>
<td>2.6</td>
<td>65.0</td>
<td>14.9</td>
<td>5.4</td>
</tr>
<tr>
<td>$^{3}$H</td>
<td>1.2</td>
<td>1.6</td>
<td>80.3</td>
<td>6.0</td>
<td>3.6</td>
</tr>
<tr>
<td>ratio $^{3}$H/$^{14}$C</td>
<td>1.29</td>
<td>0.61</td>
<td>1.24</td>
<td>0.40</td>
<td>0.66</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{3}$H</td>
<td>0.9</td>
<td>1.3</td>
<td>82.7</td>
<td>5.8</td>
<td>4.0</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>13.2</td>
<td>8.2</td>
<td>57.2</td>
<td>11.2</td>
<td>6.4</td>
</tr>
<tr>
<td>ratio $^{14}$C/$^{3}$H</td>
<td>15.12</td>
<td>6.24</td>
<td>0.69</td>
<td>1.93</td>
<td>1.60</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{3}$H</td>
<td>0.9</td>
<td>1.2</td>
<td>78.8</td>
<td>6.0</td>
<td>5.6</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>1.1</td>
<td>3.4</td>
<td>67.1</td>
<td>16.2</td>
<td>4.4</td>
</tr>
<tr>
<td>ratio $^{14}$C/$^{3}$H</td>
<td>1.21</td>
<td>2.90</td>
<td>0.85</td>
<td>2.72</td>
<td>0.78</td>
</tr>
<tr>
<td>Group A + Group B - Average ratios, second isotope to first isotope:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>13.59</td>
<td>3.98</td>
<td>0.84</td>
<td>1.32</td>
<td>1.39</td>
</tr>
<tr>
<td>2.</td>
<td>1.25</td>
<td>1.76</td>
<td>1.05</td>
<td>1.56</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Fraction Ia corresponds to sugar-phosphates; Ib contains sugar nucleotides and possibly pyruvic acid; fractions II, III, and IV correspond to the same fractions described in Table X. The combined isotope ratios, calculated as the average of the ratios for each order of isotope presentation, do not differ significantly from those obtained when each group was corrected separately for isotope effects using the ratios observed in extracts of worms which had been incubated in both isotopes simultaneously.

As was the case for the experiments summarized in Table X, the percentage of radioactivity chromatographing in the "trehalose" region accounted for well over 60% of the total activity recovered from the worms. Although postincubation in unlabelled glucose "chased" most of the isotope from the phosphorylated sugar region to the trehalose region, the percentage of radioactivity in glucose did not decrease proportionately. Immediately after incubation in the second isotope, the ratio of isotopes in the phosphorylated sugars was approximately 13:1, while that for glucose was only slightly greater than unity. After ten minutes postincubation, the isotope ratio in fraction Ia had fallen to 1.25:1, while that in the glucose region was 0.72:1.

Immediately after incubation in the second isotope, the glucose region appeared slightly enriched in that isotope, regardless of the order of isotope presentation. Following the ten minute postincubation, the percentage of radioactivity from the first isotope recovered as glucose was greater than that for the second isotope! The apparent absolute increase in the percentage of the first isotope in the glucose
region following the postincubation in unlabelled glucose must arise from the resynthesis of glucose from other carbohydrate components, just as the absolute decrease in the percentage of the second isotope in the glucose region must be a manifestation of the conversion of part of that radioisotope of glucose to trehalose. This reversal of isotope enrichment suggests that while recently absorbed glucose is rapidly incorporated into the trehalose pool of the body wall of Moniliformis, that trehalose pool is in biochemical equilibrium with a glucose pool which is physically separated from the absorptive regions of the tegument!
DISCUSSION

The studies described in this dissertation approach the definition of the mechanisms of hexose absorption in *Moniliformis dubius* from two aspects. Kinetic examinations of the uptake of several hexoses and of the mutual inhibitory effects among these monosaccharides have been directed toward defining the structural specificities of the absorptive system or systems. Examinations of the influences of different physical, chemical, and physiological parameters on the absorption of glucose were conducted to determine the qualitative mechanism of carbohydrate acquisition as it relates to the survival needs of the parasite. The conclusions reached by each of these approaches are not, however, mutually independent. Indeed, the information gained concerning the effect of the physiological state of the organism on its absorptive potential has been critical in clarifying questions arising in some of the kinetic analyses.

Glucose, mannose, fructose, and galactose are capable of supporting fermentation in *Moniliformis* (Laurie, 1957). The absorption of each of these hexoses, as well as that of the non-fermentable analogs 3-O-methylglucose, 2-deoxyglucose, and N-acetylglucosamine, is a mediated process. The mutually inhibitory effects of these monosaccharides upon the absorption of one another strongly suggest that, at physiological concentrations, their entry into the parasite is mediated through a single common transport locus.

The hyperbolic form of the $v$ versus $v/S$ plot of the uptake kinetics for fructose, galactose, and 3-O-methylglucose, and for
N-acetylglucosamine in the presence of glucose and 2-deoxyglucose acting as competitive inhibitors, is a manifestation either of the participation of two distinct transport loci or of absorption mediated through a single locus for which the membrane transit of the carrier-permeant complex is not the only important kinetic parameter. Although the data do not permit the unequivocal exclusion of the former possibility, they strongly suggest the latter.

The differences in the calculated maximal uptake velocities among the hexoses examined might be considered evidence in support of the two site hypothesis. The maximal unidirectional fluxes of various substrates whose uptake is mediated by a single system would be expected to be identical. It must be remembered, however, that more than 90% of the glucose absorbed during a four minute incubation is recovered in a chemically altered form. Furthermore, the apparent maximal absorption velocity for glucose is much greater for worms from fasted hosts than is that for worms from rats which have been allowed to feed ad libitum. Hence, the apparent \( V_{\text{max}} \) is a function of the metabolism of absorbed substrate.

The low maximal absorption velocities obtained for 2-deoxyglucose, N-acetylglucosamine, and 3-O-methylglucose can be attributed to the fact that these analogs are metabolized only sparingly during a four minute incubation. Since the initial internal concentrations of these analogs are zero, there can be no dilution of the activity of absorbed tracer. With the influx of substrate in the absence of metabolism or isotopic dilution, a substantial internal localized concentration of radioactive substrate can develop during the course
of a 4-minute incubation, resulting in an oppositely directed efflux. The observed net flux would then be lower than the true unidirectional flux, and, as a consequence, the apparent maximal unidirectional flux would be low.

The absorption velocities have been corrected neither for passive diffusion of permeant nor for the incorporation of radioactivity into the ethanol insoluble fraction of the worms (glycogen). cursory inspection of the absorption profiles for 2-deoxyglucose and N-acetylgalactosamine suggests that any diffusion component is negligible.

The uptake of galactose is not saturated at 50 mM substrate concentrations. However, the maximum diffusion component for both galactose and fructose uptake, calculated on the basis of a greater than 90% inhibition of their uptake at 8 mM concentrations by 40 mM glucose, is 0.03 micromoles per millimolar substrate concentration per 4 minutes. Since the 1:5 substrate to inhibitor ratio is not saturating, the maximal diffusion component, if any, is even smaller. Attempts to determine plateau values for inhibition of absorption using hexose concentrations greater than 40 mM were not made because of the possible osmotic effects of the high concentrations of sugar. Studies of galactose absorption at concentrations above 50 mM were not attempted for the same reason.

If the absorption data for fructose and galactose are corrected for diffusion using the value of 0.03 micromoles per millimolar substrate concentration per four minutes, the hyperbolic v versus v/S plots do not become linear. Instead, they take on a "sigmoid" form.
At low substrate concentrations, where the deviation from linearity is most extreme, any diffusion component is minimal. Correction of the data at higher substrate concentrations changes the nearly linear asymptotic arm of the hyperbola to a convex curve. This behavior is not predicted by the kinetic model for two Michaelis-Menten-type absorption sites. Although the model for a single absorption site for which membrane transit is not rate limiting predicts a sigmoid v versus v/S plot under special circumstances (see APPENDIX), passive diffusion can probably be considered a negligible component of the absorption of hexoses by Moniliformis.

That the absorption data were not corrected for incorporation of radioactivity into glycogen may not, however, be negligible. The differential incorporation into glycogen of activity absorbed from 2.7 and 10 mM glucose solutions indicates that the appearance of absorbed substrate in the ethanol insoluble fraction of the worms is not a linear function of the total uptake. Correction for the insignificant amounts of radioactivity incorporated into glycogen at low external substrate concentrations has little effect on the calculated uptake velocities; at higher substrate concentrations, however, correction for the radioactivity in the ethanol insoluble fraction of the worms, which may be as much as 6% of the total recovered activity under the conditions of the kinetic studies reported here, significantly increases the calculated absorption velocities. Inclusion of the radioactivity in the ethanol insoluble fractions in the calculation of uptake velocities accentuates the non-linearity of the diagnostic kinetic plots. Since it was not feasible to determine incorporation
into the ethanol insoluble fraction for each experiment, the kinetic relationships reported in this dissertation are based entirely on the amount of radioactivity recovered in the ethanol soluble fractions of extracts.

Because of the differential incorporation of absorbed substrate into glycogen, the calculated maximal absorption velocities for metabolized hexoses may be lower than the true values for unidirectional flux, all other parameters being equal. However, other metabolic parameters are not necessarily equal. The greater percentage of radioactivity incorporated into the glycogen fraction when worms are incubated in high concentrations of radioglucose reflects a change in the balance of metabolic pathways within the worms. This alteration may be a direct mass action effect due to a partial saturation of some of the metabolic steps, or it may involve allosteric effects due to localized changes in the concentrations of endogenous sugars and sugar-phosphates.

At least two, and possibly more, hexokinases are present in high-speed supernates of female body wall homogenates. Besides a glucokinase, which appears to interact with mannose, fructose, and galactose as well as glucose, a highly active fructokinase has been demonstrated. The data suggest that the phosphorylation of mannose and galactose is effected not only by the glucokinase, but also by one or more additional kinases. Whether the fructokinase is functional in galactose and mannose phosphorylation has not been determined.

The phosphorylation of fructose, mannose, and galactose by additional enzymes distinct from the glucokinase suggests the possibility
that the parameters governing the metabolism of these hexoses following their absorption may differ from those affecting the metabolism of absorbed glucose. The only direct evidence available concerning the control of carbohydrate metabolism in *Moniliformis* comes from the studies by McAlister and Fisher (1972) on the biosynthesis of trehalose in vitro. These authors found a marked inhibition of trehalose synthesis by high levels of ATP. This finding is compatible with the expectation that under favorable environmental conditions the metabolic capacities of the parasite be directed toward glycogenesis. It must be assumed, however, that the balance of metabolism is controlled by other factors as well. Until these factors are examined, it is impossible to determine to what extent the differences in metabolism of absorbed glucose, mannose, fructose, and galactose contribute to the higher apparent maximal uptake velocities observed for the latter two hexoses.

The relative efficacies of glucose, mannose, fructose, galactose, 3-O-methylglucose, and 2-deoxyglucose as inhibitors of the absorption of the four metabolizable monosaccharides are identical for each substrate. Therefore, if absorption of some or all of these hexoses is mediated through two distinct loci, it must be the case that the relative order of affinities of each of the examined hexoses for the two sites is the same. This is equivalent to saying that if indeed there be two loci, they are sterically similar but kinetically different.

The linearity of the plots of the absorption kinetics for glucose, mannose, and 2-deoxyglucose over the range of concentrations examined does not permit differentiation between the one-site and the two-site
hypotheses. Given the results of the mutual inhibition experiments, it is probable that the diagnostic plots of the absorption of these C-2 analogs will approach a hyperbolic form at sufficiently low substrate concentrations, regardless of the number of transport sites involved.

The extrapolated kinetic constants for the substrates examined in this study, assuming a single transport locus, are summarized in Table XIV.

**TABLE XIV**

Kinetic Constants for the Absorption of Hexoses by *Moniliformis dubius*

(Assuming a Single Transport Locus)

<table>
<thead>
<tr>
<th>Hexose</th>
<th>$V_{\text{max}} \pm \text{S.E.}$ (µmoles/gram/4 minutes)</th>
<th>$K_t \pm \text{S.E.}$ (mM)</th>
<th>$K_i \pm \text{S.E.}$ (mM)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (a)</td>
<td>20.66 ± .03</td>
<td>2.77 ± .01</td>
<td>2.40 ± .09</td>
<td>7</td>
</tr>
<tr>
<td>(b)</td>
<td>19.07 ± .67</td>
<td>2.29 ± .17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>10.99 ± .12</td>
<td>2.52 ± .13</td>
<td>2.95 ± .12</td>
<td>6</td>
</tr>
<tr>
<td>Mannose</td>
<td>24.70 ± .18</td>
<td>4.08 ± .06</td>
<td>4.16 ± .16</td>
<td>5</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td>$\sim$ 16.3</td>
<td>$\sim$ 18</td>
<td>15.90 ± .72</td>
<td>4</td>
</tr>
<tr>
<td>Fructose</td>
<td>$\sim$ 38.5</td>
<td>$\sim$ 27</td>
<td>24.41 ± 1.49</td>
<td>5</td>
</tr>
<tr>
<td>Galactose</td>
<td>$\sim$ 33.2</td>
<td>$\sim$ 77</td>
<td><em>(52)</em></td>
<td><em>(1)</em></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>10.47 ± .06</td>
<td>3.89 ± .04</td>
<td>7.20 ± .65</td>
<td>2</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>--</td>
<td>--</td>
<td>6.68 ± .78</td>
<td>2</td>
</tr>
</tbody>
</table>

The two sets of kinetic constants for glucose uptake illustrate the variability in the absorption velocities determined in different experiments. The first set of values (a) was calculated from the pooled data of five experiments; the second (b) represents the kinetics
observed under conditions most comparable to those prevailing when the inhibition studies were conducted (light regimen in the animal room, time of day at which rats were sacrificed, etc.).

Inhibition constants ($K_i$) were calculated from extrapolated values for $K_i'$ according to the Michaelis-Menten relationship $K_i' = K_i (1 + I/K_i)$. If the kinetics of hexose absorption are described by equation 2a of the THEORETICAL section, this assumption gives a value for $K_i'$ which is greater than or equal to the true carrier-inhibitor dissociation constant (see APPENDIX). The apparent inhibitor constants for each hexose acting as an inhibitor of the uptake of various substrates are not significantly different from one another within the limitations of the experimental conditions. The $K_i$ values presented in Table XIV are the averages of values calculated for (n) individual determinations for the hexose as an inhibitor of the uptake of various C-2 analogs of glucose.

It was not technically possible to run control curves for each experiment in which the effect of an inhibitor at a single concentration on the absorption of a substrate at several concentrations was examined. The day to day variation in observed absorption velocities and apparent kinetic constants therefore introduced a certain degree of uncertainty in the calculation of the $K_i$ values. The observed $K_i$s for glucose, 2-deoxyglucose, and mannose cannot be considered different from their transport constants. Although the $K_t$ values for galactose, fructose, and 3-O-methylglucose are only approximations, there is a reasonable correlation between these values and the apparent $K_i$s for their inhibition of glucose, 2-deoxyglucose, and mannose uptake.
The disparity between the $K_t$ for N-acetylglucosamine uptake and
the $K_i$ for its inhibition of glucose and 2-deoxyglucose absorption
might be interpreted as evidence for the existence of two transport
sites. However, the transport constant for N-acetylglucosamine uptake
was calculated on the basis of the results of a single experiment, and
therefore may not be reliable. Furthermore, the interaction of the
acetylated nitrogen with the transport site may introduce an addi-
tional chemical variable such that the kinetic properties of the
system are altered in some manner which is not representative of its
interactions with unsubstituted hexoses.

If the absorption of fructose and N-acetylglucosamine were
mediated by two absorption loci, the combined effects of 2-deoxyglucose
and N-acetylglucosamine as inhibitors of fructose uptake might be
expected to be synergistic. However, increased inhibition of fructose
absorption by these two hexoses in concert, or by mannose and
galactose in concert, could not be demonstrated.

The cumulative evidence strongly supports the hypothesis that
the absorption of hexoses across the tegumentary surface of Monili-
formis dubius is mediated by a single species of transport locus.
The observed values for the apparent transport constants of absorbed
hexoses and for the apparent inhibitor-carrier dissociation constants
are compatible with a single transport locus whose kinetics are
described by equation 2a (equation A-9 of APPENDIX for radioisotope
uptake), notwithstanding the values for N-acetylglucosamine. For
those substrates having relatively low transport constants (glucose,
2-deoxyglucose, and mannose), the kinetic contribution of the carrier-
substrate association-dissociation equilibrium is negligible ($k_2 \ll k_1$), and the observed kinetics appear to be described by the Michaelis-Menten equation. The high $K_i$s for 3-O-methylglucose, fructose, and galactose reflect their low affinities for the absorption locus; the kinetic contribution of the binding of these hexoses is not negligible ($k_2$ is not negligible with respect to $k_1$), the calculated transport constants and carrier-inhibitor dissociation constants are functions of the kinetic properties of the absorption site, and the observed uptake profiles are not described by the Michaelis-Menten equation.

A number of studies indicate that cestodes and acanthocephalans have at least limited hydrolytic activities at their tegumentary interfaces with the environment. The rabbit tapeworm *Cittotaenia* appears to have a surface disaccharide hydrolase which potentiates the absorption of the hexose moieties (Read and Rothman, 1958b). Using isolated tegumentary preparations from *Hymenolepis diminuta*, Lumsden et al. (1968) found histochemical evidence for the presence of phosphohydrolases. Arme and Read (1970) and Dike and Read (1971a) confirmed the external orientation of the tegumentary phosphohydrolases in this species by demonstrating the release of hydrolysis products in the medium when whole worms were incubated in the appropriate substrates. Dike and Read (1971b) examined the kinetics of glucose and glucose-6-phosphate absorption in the presence of a number of transport and/or phosphohydrolase inhibitors. They concluded that glucosephosphate is hydrolyzed before its absorption, but that the proximity or orientations of the glucose transport system and the tegumentary phosphohydrolase within a "surface compartment" confers a kinetic advantage to the uptake of the products of hydrolysis.
Histochemical studies at the level of the light microscope (Crompton, 1963) indicated the presence of leucine aminopeptidase activity in the striped and radial layers of the tegument of the duck acanthocephalan Polymorphus minutus. Uglem (1972) has found externally oriented leucine- and alanine aminopeptidase activities in intact Neoechinorhynchus cristatus as demonstrated by the liberation of fluorescent hydrolysis products of the respective L-aminoacyl-β-naphthylamides into the incubation medium in vitro. Uglem has further reported that preliminary studies with Moniliformis suggest that the intrinsic tegumentary aminopeptidases in this parasite offer a kinetic advantage for the absorption of liberated amino acid hydrolysis products.

The kinetics of the inhibition of glucose absorption by maltose and glucose-6-phosphate and the chemical distribution of the low levels of radioactivity absorbed by worms incubated in $^{14}C$ maltose or $^{14}C$ glucose-6-phosphate suggest that, in Moniliformis, free glucose is liberated from these compounds as a result of their hydrolysis in the external medium or at the parasite-medium interface. The ultrastructural studies by Rothman (1967) and Pyram (1971) indicate that the intrinsic surface phosphohydrolases of Moniliformis are restricted to the membranes lining the tegumentary crypts. If these histochemically demonstrated phosphohydrolases are indeed responsible for the liberation of free glucose from glucose-6-phosphate, they do not appear to offer any kinetic advantage to the subsequent absorption of organic hydrolysis products.
Rothman found glucose-6-phosphate to be a potential substrate for the crypt phosphohydrolase(s); however, no biochemical data are available concerning the kinetics of the glucose-6-phosphatase activity. It may be the case that the affinity of the phosphohydrolase(s) for glucose-6-phosphate is quite low, and that the hydrolysis of glucose-6-phosphate at low concentrations is therefore minimal. This is consistent with the relatively low levels of absorption of radioactivity from media containing 2.3 mM glucose-6-phosphate and with the relatively high concentrations of glucose-phosphate necessary for significant inhibition of glucose uptake. On the other hand, if the activity of the crypt phosphohydrolases toward glucose-6-phosphate is high, and if exogenous phosphoglucose has access to the crypt membranes, the data would seem to suggest that the transport system for hexoses in *Moniliformis* is restricted to the plasmalemma exclusive of the membranes lining the crypts.

The possible relationship between the phosphohydrolases on the crypt membranes and the effects of glucose-6-phosphate on glucose absorption deserve further investigation. The localization of any disaccharidase activities present in the tegument of *Moniliformis* must await the development of histochemical techniques for the demonstration of the highly diffusible hydrolysis products.

The importance of exogenous carbohydrate to the well-being of intestinal helminths, in particular cestodes and acanthocephalans, is well documented. The levels of glycogen in cestodes and in *Moniliformis* are dramatically reduced upon starvation of the host (reviewed in Read and Simmons, 1963, and in von Brand, 1966). Using concurrent infections
of *Hymenolepis* and *Moniliformis*, Holmes (1961) found a "crowding effect" exerted not only by the population density within a single species, but also between species. Read and Rothman (1957a, b) and Roberts (1961, 1966) have demonstrated a correlation between the manifestation of the "crowding effect" and the quality and quantity of carbohydrates available to *Hymenolepis* in the rat intestine. The studies by Read and Rothman (1958) described in the INTRODUCTION established not only that the levels of glycogen in *Moniliformis* are depleted when the host is deprived of dietary polysaccharide, to the point that the prolonged absence of exogenous carbohydrate causes cessation of growth in males and death in females, but also that the levels of stored polysaccharide in these parasites are subject to a large diurnal fluctuation, presumably correlated with the feeding habits of the rat. These observations make obvious the necessity that cestodes and acanthocephalans possess efficient mechanisms for obtaining and storing carbohydrates when these compounds are available to them in their environments.

The levels of storage polysaccharide in *Moniliformis* fall within the range of values reported for *Hymenolepis* and a spectrum of other cestodes (see von Brand, 1966). However, *Moniliformis* contains, in addition, significant levels of trehalose and glucose, and the concentrations of these sugars also appear to fluctuate with the nutritional state of the parasite.

In the studies presented in this dissertation, 32 day old female worms recovered from rats at ten A.M. were found to have endogenous trehalose and glucose levels which constitute, respectively, 4.4 and 1.9% of the ethanol extracted dry weight. McAlister (1970) found
comparable trehalose levels in 6-14 week old females recovered at approximately the same time of day. His data suggest that the percentage of the wet weight attributable to trehalose decreases in older worms. Fairbairn (1958) reported values of 2.3 and 3.0% of the tissue solids for trehalose and glucose, but he did not specify the age, sex, or nutritional state of the parasites on which his determinations were made. Laurie (1959) found trehalose levels (1.74-2.48% of the dry weight) comparable to those reported by Fairbairn in six week old male and female worms taken from rats which had been starved for 24 hours. He reported that starvation reduced the endogenous levels not only of glycogen, but also of glucose, and that in vitro incubation of fasted worms for 2 or 4 hours in metabolizable carbohydrates caused dramatic increases in the levels of glycogen and glucose, but not trehalose.

The reported fluctuations in levels of glucose and glycogen, and possibly trehalose, suggest that the day to day variation in observed uptake kinetics, as well as the dramatic increase in the apparent rates of glucose absorption in worms from starved hosts, are due to the influence of the levels of endogenous carbohydrates on the rate of metabolism of absorbed substrates. The inhibitory effects of preincubation in glucose on the subsequent absorption of radioglucose and the concomitant rise in the levels of total carbohydrate are consistent with this hypothesis.

Read (1967a) suggested that the inhibitory effects of preincubation in galactose on the subsequent sodium-dependent transport of cycloleucine in the dogfish spiral valve were the result of localized increases in the intracellular sodium concentration due to the cotransport
of sodium and galactose. The absorption of hexoses in *Moniliformis*, however, does not appear to be dependent on the external concentration of sodium. No direct evidence for the involvement of any monovalent cation in the transtegumentary absorption of glucose has been found. Furthermore, hexose absorption is insensitive to the glycoside phlorizin. All sodium-dependent carbohydrate transport systems thus far examined have been shown to be extremely sensitive to phlorizin (cestodes reviewed in the INTRODUCTION, vertebrate tissues discussed in Schultz and Curran, 1970).

The observation that absorbed hexoses are rapidly metabolized in *Moniliformis* and the failure to demonstrate a sodium dependence for glucose absorption are, on superficial examination, disconcerting when one attempts to draw general correlations between adaptations of parasitic organisms for the acquisition of nutrients and the availability of those nutrients in the environment within the host. Since the biochemical environment of extracellular parasites resembles that of the host tissues in the same "ecological niche", a similarity in the overall mechanisms for the acquisition of required nutrients in the host tissues and in the parasites might be predicted. In a paper presented at the fourth annual meeting of the Southwestern Association of parasitologists, Southworth (1971b) pointed out that such similarities do exist between the hexose absorption systems of *Hymenolepis* and of the blood-stream forms of trypanosomes and the host tissues in their respective environments.

In vitro, the blood-stream forms of the African trypanosomes die within minutes in the absence of exogenous sugars (see von Brand, 1966).
They have no polysaccharide reserves, and their carbohydrate metabolism does not involve the mitochondrial citric acid cycle. Hexose absorption in *Trypanosoma gambiense* is not sodium dependent, nor is it concentrative. In fact, it appears that the rate limiting step in carbohydrate metabolism in these protozoans is the actual absorption process (Southworth, 1971a). Trypanosomes are thus remarkably similar to mammalian erythrocytes in both the efficiency of their carbohydrate metabolism and the thermodynamic nature of their hexose absorptive systems (literature for erythrocyte systems cited in Stein, 1967, pp. 164-165). As Southworth remarked, there is no teleological need for any concentrative hexose absorptive system in either of these cellular forms. The homeostatic mechanisms of the mammal maintain the levels of blood glucose at concentrations which are nearly saturating for the facilitated diffusion systems of both erythrocytes and *Trypanosoma gambiense*.

In contrast to the relatively stable glucose levels in the blood, the levels of carbohydrate in the vertebrate intestine fluctuate as a function of the ingestion of dietary polysaccharide. In a broad spectrum of vertebrates, the absorption of glucose, and of several analogs of glucose which have similar configurations at the 2-carbon, across the apical surface of the intestinal epithelium is mediated by a sodium-dependent cotransport system (see Stein, 1967, p. 186, and Schultz and Curran, 1970). The studies of glucose absorption in cestodes discussed in the INTRODUCTION indicate that these lumen-dwelling helminths also possess sodium-dependent membrane transport
systems which are capable of effecting the accumulation of glucose against a concentration difference.

Although the endogenous levels of glucose reported for *Hymenolepis* and other cestodes are relatively low (Fairbairn, 1958), the thermodynamic asymmetry of the sodium-dependent hexose transport systems in these organisms undoubtedly potentiates the quantitative efficiency of carbohydrate acquisition and storage; it ensures the biochemical flow of glucose toward glycogenesis even when the levels of glucose in the fluids of the intestinal lumen fall below the levels within the cestodes. The reduced probability for efflux of absorbed substrates thus maximizes the amount of potential metabolic energy which can be stored as polysaccharide during the periods when glucose is available to the parasite.

*Hymenolepis diminuta* and *Moniliformis dubius*, as adults, share a common habitat; they therefore face the same physiological stresses in terms of the availability of nutrients in their environment. The adaptations by which these helminths cope with the problem of maintaining adequate carbohydrate reserves, however, are mechanistically dissimilar. Of the radioactivity absorbed by *Hymenolepis* during a one minute incubation, 95% remains in the form of glucose (Phifer, 1960c). Less than 10% of the glucose absorbed by *Moniliformis* in one minute can be recovered as chemically unaltered glucose. The entry of exogenous hexoses across the tegumentary plasmalemma in this acanthocephalan is mediated by a facilitated diffusion system. In contrast to the cestodes, the energy dependent transducing systems which must be involved in establishing and maintaining the substantial levels of
free glucose in Moniliformis are quite distinct from the tegumentary absorptive system.

The results of the experiments in which the chemical fate of absorbed glucose was determined, in particular, those employing dual isotope labelling, strongly suggest that the accumulation of carbohydrates in Moniliformis dubius occurs through the following sequence of events:

Absorbed glucose is rapidly phosphorylated upon its entry. Some of the phosphoglucose is metabolized in the pathways leading directly to energy production. If the levels of phosphorylated sugars, free sugars, and/or other biochemical effectors in the outer regions of the tegument are sufficiently high, some of the phosphoglucose is incorporated into glycogen. Most, however, is converted, either as glucose-6-phosphate or as UDPG, to the disaccharide trehalose.

As trehalose, the previously absorbed hexose carbons are no longer in a chemical form capable of interacting with the surface absorptive system. The concentration of glucose in the region of the absorptive surface is thus maintained at a low level, so that the further downhill entry of glucose through facilitated diffusion is possible.

The trehalose in the body wall appears to be in dynamic biochemical equilibrium with a glucose pool which is physically separated from the absorptive regions of the tegument. The morphology of the body wall of Moniliformis dubius makes the sub-tegumentary muscle layers a likely candidate for the region of compartmentalization of the substantial pool of free glucose in the body wall of this acanthocephalan.
It is postulated that the accumulation of free glucose within the body wall involves the transfer of glucose moieties from trehalose. Since the distribution of trehalose within the body wall is unknown, the precise mechanism by which the hexose moieties, whose efflux is prevented by virtue of their incorporation into this disaccharide, enter the compartmentalized glucose pool cannot be determined. It is possible, though highly unlikely, that the boundary of the glucose compartment is freely permeable to trehalose, but impermeable to glucose, and that the accumulation of glucose within the pool arises as a result of the hydrolysis of permeating trehalose. Since the accumulation of the non-fermentable hexoses 2-deoxyglucose, 3-O-methyl-glucose, and N-acetylgalactosamine does not appear to involve the synthesis and breakdown of a trehalose-like disaccharide, however, it must be the case that some system for the translocation of hexoses across the boundary of the glucose pool is present. Whether the accumulation of these poorly metabolized hexoses is a manifestation of their "active transport" into a compartment within the body wall or of counterflow driven by exchange with the glucose in the compartment cannot be determined from the data. However, regardless of the mediating mechanism, the apparent accumulation of these hexoses against a concentration difference is indicative of a membrane transport system within the body wall of Moniliformis dubius females which is capable of mediating (or maintaining) the accumulation of a sizeable concentration of glucose.

The levels of total carbohydrates in Hymenolepis and Moniliformis suggest that the efficiencies of the mechanisms for carbohydrate
accumulation in these helminths are quantitatively comparable. Nothing is known of the trehalose degradative enzymes in *Moniliformis*. Until the energy requirements for the mobilization of the hexose moieties of trehalose into glycogen and into the glucose pool are understood, the energetic efficiency of the carbohydrate assimilation systems in this organism are difficult to evaluate.

The "activation energy" for glucose uptake in *Moniliformis* (37.5 kcal/mole) is almost twice that reported by Phifer (1960a) for the concentrative absorption of glucose in *Hymenolepis* (22 kcal/mole). In fact, it is approximately equal to the sum of the "activation energies" for the sodium-dependent cotransport of glucose in *Hymenolepis* and the passive diffusion of ionic solutes across a variety of biological membranes (see Stein, 1967, p. 104). This large thermodynamic parameter may reflect the energetics of the combined processes of facilitated diffusion across the tegumentary plasmalemma and the subsequent metabolism of absorbed glucose, or it may be indicative of the intimate relationship of the two activities. However, the interpretation of "activation energies" calculated from the thermal dependence of membrane absorption rates is complex, and may involve parameters, such as generalized alterations in the structural integrity of the highly dynamic membrane, which are totally independent of the properties of the transport system. Any attempt to draw conclusions concerning the energetic efficiencies of the glucose accumulation systems in *Moniliformis* in comparison to that in *Hymenolepis* on the basis of apparent "activation energies" for hexose absorption is not warranted.
The existence of two distinct permeability barriers to the accumulation of carbohydrates in the body wall of *Moniliformis* has interesting evolutionary implications. It would appear that the boundary of the compartmentalized glucose pool possesses a concentrative system which may be mechanistically similar to those of the cestode tegument and the apical surface of the vertebrate intestinal epithelium.

The facilitated diffusion system in the tegumentary surface may be a remnant of the physiological requirements of morphological structures carried over from embryonic or larval stages of the life cycle of the organism. The presence of high levels of trehalose in the maturing acanthors (McAlister, 1970; McAlister and Fisher, 1972) and in the hemolymph of the intermediate host (Treherne, 1960) is consistent with a metabolism in juvenile stages which is geared toward the utilization of trehalose as a major source of carbohydrate. On the other hand, although the absorption of trehalose was not examined in this study, the presence of exogenous trehalose was found to have no effect on the absorption of glucose by adult worms *in vitro*. The integration of a non-concentrative mediated absorption system and the rapid phosphorylation and incorporation into trehalose of absorbed hexose may be an alternative evolutionary approach to increasing the efficiency of carbohydrate acquisition and storage under favorable conditions in order to meet the stresses of the diurnal variations in carbohydrate availability in the vertebrate intestine. The survival of the acanthocephala as a group and of *Moniliformis dubius* in particular attests to the success of the adaptations which these organisms have adopted to meet the challenges of their parasitic life style.
REFERENCES


APPENDIX

The reciprocal of the equation describing the velocity of absorption mediated by two distinct systems having transport constants $K_1$ and $K_2$ and maximal velocities $V_1$ and $V_2$, each of which separately can be described by Michaelis-Menten kinetics, is:

$$\frac{1}{v} = \frac{K_1 + K_2}{S} + \frac{K_1 K_2}{S^2} \left( \frac{V_1 K_2 + V_2 K_1}{V_1 + V_2 + \frac{V_1 K_2 + V_2 K_1}{S}} \right)$$

The slope of a Lineweaver-Burk plot is given by:

$$m = \frac{1}{V_1 + V_2 + \frac{V_1 K_2 + V_2 K_1}{S}} \left[ K_1 + K_2 + \frac{K_1 K_2}{S} - \frac{1 + \frac{K_1 + K_2}{S}}{\frac{V_1 + V_2}{V_1 K_2 + V_2 K_1} + \frac{1}{S}} \right]$$ (A-1)

And the derivative of the slope is:

$$\frac{dm}{dt} = \frac{2 (K_1 K_2 - m)}{V_1 + V_2 + \frac{V_1 K_2 + V_2 K_1}{S}}$$ (A-2)

These equations describe a convex hyperbola which intersects the ordinate at $1/V_{\text{max}} = 1/(V_1 + V_2)$. Assuming that $K_2 > K_1$, the extension of the real arm of the hyperbola intersects the abscissa at $-1/K_2$; the intersection of the imaginary arm of the hyperbola is $-1/K_1$. The Dixon and Webb, (1964, pp. 89-90) have presented examples of the shapes of double reciprocal curves expected for different combinations of values for $K_1$, $K_2$, $V_1$, and $V_2$. 
The reciprocal of the flux equation for a facilitated diffusion system for which \( r = 1 \) and membrane transit is not the rate limiting step (equation 2a) is:

\[
\frac{1}{v} = \frac{2}{k_2 C} + \frac{2}{k_2 C} \cdot \frac{K_A (K_A + S_B + 2 \frac{k_2}{k_1})}{S_A (K_A + \frac{k_2}{k_1} + S_B) + \frac{k_2}{k_1} \cdot S_B}
\]

The slope of a Lineweaver-Burk plot is given by:

\[
m = \frac{2 K_A}{k_2 C} \cdot \frac{K_A + \frac{2}{k_1} \cdot S_B}{\frac{K_A}{k_1} + \frac{2}{k_1} \cdot S_B + \frac{2}{k_1} \cdot \frac{S_B}{S_A}} \left[ 1 - \frac{\frac{k_2}{k_1} \cdot S_B}{S_A (K_A + S_B + \frac{k_2}{k_1}) + \frac{k_2}{k_1} \cdot S_B} \right]
\]

(A-3)

The derivative of the slope is:

\[
\frac{d m}{d(1/S)} = -\frac{2 m \frac{k_2}{k_1} \cdot S_B}{K_A \cdot \frac{K_A}{k_1} + \frac{2}{k_1} \cdot S_B + \frac{2}{k_1} \cdot \frac{S_B}{S_A}}
\]

(A-4)

These equations also describe a convex hyperbola for the Lineweaver-Burk plot of permeant absorption. The limit of the slope as \( 1/S_A \) becomes very large is zero: the asymptotic arm of the hyperbola approaches a horizontal line. The intercept of the hyperbola with the ordinate is

\[
2/k_2 C = 1/V_{\text{max}}
\]

Kinetic constants for mediated absorption systems described by equation 2a can be extrapolated from \( V \) versus \( V/S \) and \( S/V \) versus \( S \) plots of the data. The slope of a plot of \( S/V \) against \( V \) is given by:
\[ m = \frac{2}{k_2 C} + \frac{2}{k_2 C} \cdot \frac{K_A(K_A + 2 \frac{k_2}{k_1} + S)}{S_A(K_A + \frac{k_2}{k_1} + S_B) + \frac{k_2}{k_1} S_B} \left(1 - \frac{K_A + 2 \frac{k_2}{k_1} + S_B}{K_A + \frac{k_2}{k_1} + S_B + \frac{k_2}{k_1} \cdot \frac{S_B}{S_A}} \right) \] \hspace{1cm} (A-5)

When \( S_A \) is large, the convex hyperbola approaches its asymptote, and the limiting value of the slope is \( 2/k_2 C = 1/V_{\text{max}} \), which can be determined with greater accuracy than can the intercept of a Lineweaver-Burk plot.

The slope of a \( v \) versus \( V/S \) plot is:

\[ m = \left[ \frac{k_2}{k_1} S_B (v - \frac{k_2 C}{2}) + v K_A (K_A + 2 \frac{k_2}{k_1} + S_B) \right]^2 \left(1 - \frac{k_2}{k_1} \cdot \frac{S_B}{v^2 K_A (K_A + 2 \frac{k_2}{k_1} + S_B)} \right) \] \hspace{1cm} (A-6)

As \( S \) becomes very large, \( v \) approaches \( V_{\text{max}} = k_2 C/2 \), and the limiting value of the slope is:

\[ \lim_{S_A \to \infty} (m) = -K_A \cdot \frac{K_A + 2 \frac{k_2}{k_1} + S_B}{K_A + \frac{k_2}{k_1} + S_B} = -K_{\text{est}} \] \hspace{1cm} (A-7)

According to equation 2a, the concentration of substrate yielding half-maximal velocity is:

\[ K_A + \frac{k_2}{k_1} \cdot \frac{(K_A - S_B)}{K_A + S_B + \frac{k_2}{k_1}} = K_{\text{est}} = \frac{k_2 \cdot S_B}{K_A + \frac{k_2}{k_1} \cdot S_B} \] \hspace{1cm} (A-8)
Hence, the value estimated from a $v$ versus $v/S$ plot gives a reasonable estimate for $K_t$, albeit somewhat large.

The formulation describing completely competitive inhibition of a system whose kinetics are described by equation 2a is equation 9 of the THEORETICAL section. It can be shown that the limiting value of the slope of a $V$ versus $V/S$ plot is:

$$\lim_{{S_A \to \infty}} m = -K_{test} \left( 1 + \frac{1}{K_i} \cdot \frac{k_2 + k_{-5}}{2k_2 + k_{-5}} \right)$$

where the carrier-inhibitor dissociation constant, $K_i$, is $k_{-5}/k_5$.

Equation 2a is applicable to measurements of absorption kinetics based upon chemical determinations of absorbed substrate. When permeant uptake is measured using a radioactive substrate, the endogenous concentration of that substrate is not described by the term $S_B$. $S_B$ refers to the internal concentration of substrate which is identical to $S_A$ in all respects; hence, for experiments using radioactive tracers, $S_B$ refers only to the internal concentration of radioisotope. If the endogenous concentration of unlabelled substrate (or substrate analog) is $Q$, and its dissociation constant, $K_Q$, equals $k_{-5}/k_5$, then the equation for unidirectional flux of radioactive tracer equivalent to equation 2a is:
\[ v = \frac{k_2 C}{2} \left\{ S_A \cdot \left[ (K_S + \frac{k_2}{k_1})(1 + \frac{k_2 Q}{2k_2 + k_{-5}}) + S_B \right] + \frac{k_2}{k_1} S_B \right\} + \frac{k_2}{k_1} S_B + K_S \cdot \left[ (K_S + 2 \frac{k_2}{k_1})(1 + \frac{Q}{K_Q} \cdot \frac{k_2 + k_{-5}}{2k_2 + k_{-5}}) + S \right] \]  

If the following substitutions are made:

\[(K_S + \frac{k_2}{k_1})(1 + \frac{k_2 Q}{2k_2 + k_{-5}}) = f\]

\[\frac{K_S}{K_Q} \cdot \frac{k_2 Q}{2k_2 + k_{-5}} = i\]

\[(K_S + 2 \frac{k_2}{k_1})(1 + \frac{Q}{K_Q} \cdot \frac{k_2 + k_{-5}}{2k_2 + k_{-5}}) = q\]

then the rate equation can be written:

\[ v = \frac{k_2 C}{2} \cdot \left\{ \frac{S_A(f + S_B)}{S_A(f + S_B + 1)} + \frac{k_2}{k_1} S_B + K_S(q + S_B) \right\} \]

If it is assumed that \( S_B \) remains constant (which it does not), the slope of a \( 1/v \) versus \( 1/S \) plot is:

\[ m = \frac{2}{k_2 C} \cdot \frac{K_S(q + S_B)(f + S_B) - i \frac{k_2}{k_1} \cdot S_B}{\left[ f + S_B + \frac{k_2}{k_1} \cdot \frac{S_B}{S_A} \right]^2} \]  

(A-10)
and the derivative of the slope is:

\[
\frac{d(m)}{d(1/S)} = - \frac{2 \frac{k_2}{k_1} \cdot S_B \cdot m}{f + S + \frac{k_2}{k_1} \cdot \frac{S_B}{S_A}}
\]

The slope of a plot of S/v against S is:

\[
m = 2 \frac{k_2}{k_2 \cdot C} + \frac{2K_S (q + S_B) \cdot k_2 \cdot S_B}{k_2 \cdot C \cdot (S_A \cdot (f + S_B) + \frac{k_2}{k_1} \cdot S_B)^2} + \frac{2 \frac{k}{k_2} \cdot \frac{k_2}{k_1} \cdot S_B}{(f + S_B)^2 + \frac{k_2}{k_1} \cdot S_B^2} \quad (A-11)
\]

When S becomes large, the slope of a S/v versus S plot reduces to:

\[
\lim_{S \to \infty} (m) = 2 \frac{k_2}{k_2 \cdot C} \cdot (1 + \frac{i}{f + S_B}) = \frac{1}{v_{\text{max}}}
\]

The transport constant can still be estimated from the slope of a v versus v/S plot,

\[
m = - \left[ \frac{(K_S \cdot (q + S_B) + \frac{k_2}{k_1} \cdot S_B) \cdot v - \frac{k_2}{k_1} \cdot \frac{k_2}{k_2} \cdot S_B}{(f + S_B + i) \left[ v^2 \left( K_S \cdot (q + S_B) + \frac{k_2}{k_1} \cdot S_B \right) \right] + \frac{k_2}{k_1} \cdot \frac{k_2}{k_2} \cdot \frac{f + S_B}{f + S_B + i} \cdot (2v)} \right]^2 \quad (A-12)
\]

whose limit as v approaches \(v_{\text{max}}\) is:
\[
\lim_{v \to v_{\text{max}}} = \frac{K_S(q + S_B) - \frac{k_2}{k_1}S_B (1 + \frac{1}{f + S_B})}{f + S_B + 1} + \frac{\frac{k_2}{k_1}S_B \cdot i}{(f + S_B)^2 (K_S(q + S_B) + \frac{k_2}{k_1}S_B)}
\]

\[
\Rightarrow \frac{K_S(q + S_B) - \frac{k_2}{k_1}S_B (1 + \frac{1}{f + S_B})}{f + S_B + 1} = K_t
\]

The assumption that \(S_B\) remains constant leads to equations which predict that the \(v\) versus \(v/S\) plot has sigmoid characteristics. The rate of change of the slope given by equation (A-12) has an inflection at

\[
v = \frac{k_2 C}{2} \cdot \frac{k_2 S_B}{K_S(q + S_B) + \frac{k_2}{k_1}S_B}
\]

However, \(S_B\) is not constant. By definition, the concentration of internal radioactive tracer at time zero is zero. If \(S_B\) were to remain zero, the slopes of each of the plots derived above would be constant, that is, all would be straight lines, as is the case when the affinity of the carrier for substrate is large, so that \(k_1 \gg k_2\). In either case, the observed absorption kinetics would then conform to those predicted by the Michaelis-Menten equation.
Absorption of 2 mM $^{14}$C glucose by 32 day old \textit{Moniliformis dubius} females.

Abscissa = time, in minutes;
Ordinate = micromoles of glucose absorbed per gram ethanol extracted dry weight.
Absorption of $^{14}$C glucose as a function of glucose concentration.

Abscissa = mM glucose;
Ordinate = micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 3

Lineweaver-Burk plot of $^{14}$C glucose absorption.

$S = \text{mM glucose;}$

$V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.}$
FIGURE 4

V versus V/S plot of C$^{14}$ glucose absorption.

$S = \text{mM glucose;}$

$V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.}$
FIGURE 5

$S/V$ versus $S$ plot of $^{14}C$ glucose absorption.

$S = \text{mM glucose;}$

$V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.}$
FIGURE 6

Effect of host starvation on the velocity of $^3$H glucose absorption.

Upper curves, Lineweaver-Burk plots;
Lower curves, V versus S plots.

$S = $ mM glucose;
$V = $ micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.

Fed = control worms;
Starved = worms from rats deprived of food for 24 hours prior to experiment;
Starved, corrected for weight difference = data for experimental worms normalized to control worms by multiplication by the ratio of the average weights of experimental and control parasites.
FIGURE 7

Absorption of $^{14}C$ mannose, 2-deoxyglucose, and N-acetylglucosamine as a function of substrate concentration.

Abscissa = mM substrate;
Ordinate = micromoles substrate absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 8

V versus V/S plots of $^{14}$C mannose, 2-deoxyglucose,
and N-acetylglucose absorption.

$S = \text{mM substrate;}$

$V = \text{micromoles substrate absorbed per gram ethanol extracted dry weight per 4 minutes.}$
FIGURE 9

Absorption of $^{14}$C fructose, 3-O-methylglucose, and galactose as a function of substrate concentration.

Abscissa = mM substrate;
Ordinate = micromoles substrate absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 10

V versus V/S plots of C\textsuperscript{14} fructose and 3-O-methylglucose absorption.

\[ S = \text{mM substrate}; \]
\[ V = \text{micromoles substrate absorbed per gram ethanol extracted dry weight per 15 minutes}. \]
FIGURE 11

V versus V/S plot of C$^{14}$ galactose absorption.

S = mM galactose;

V = micromoles galactose absorbed per gram ethanol extracted dry weight per 1/4 minutes.
FIGURE 12

Lineweaver-Burk plot of $^{14}$C galactose absorption.

Inset: Lineweaver-Burk plot for concentration range 5-50 mM.

$S =$ mM galactose;

$V =$ micromoles galactose absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 13

S/V versus S plots for \( ^{14}C \) fructose and 3-O-methylglucose absorption.

\[ S = \text{mM substrate}; \]
\[ V = \text{micromoles substrate absorbed per gram ethanol extracted dry weight per 4 minutes}. \]
FIGURE 14

\[ S/V \] versus \[ S \] plot of C\textsuperscript{14} galactose absorption.

\[ S = \text{mM galactose}; \]
\[ V = \text{micromoles galactose absorbed per gram ethanol extracted dry weight per 4 minutes}. \]
FIGURE 15

Lineweaver-Burk plots of $^{14}C$ glucose absorption
in the presence of N-acetylglucosamine,
2-deoxyglucose, fructose, mannose,
and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = \text{mM glucose};$

$V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes}.$
INHIBITOR:

- △ N-ACETYL GLUCOSAMINE
- ■ 2-DEOXY GLUCOSE
- ○ o FRUCTOSE
- △ MANNOSE
- ● ● NONE

\[
\frac{1}{v} \text{ vs } \frac{1}{s}
\]
FIGURE 16

V versus V/S plots of $^{14}C$ glucose absorption in the presence of N-acetylglucosamine, 2-deoxyglucose, fructose, mannose, and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = mM$ glucose;

$V =$ micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 17

$V$ versus $V/S$ plots of $^{14}$C glucose absorption in the presence of 3-O-methylglucose, glucosamine, galactose, and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = \text{mM glucose;}$

$V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.}$
FIGURE 18

Effects of increasing concentrations of 2-deoxyglucose and glucose on absorption of C\textsuperscript{14} glucose.

Upper curves, Dixon plots;
Lower curves, Webb plots.

Glucose concentration = 2 mM;
I = mM inhibitor;
V = micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes;
i = fractional inhibition = 1-\(v_i/v\)
FIGURE 19

Effects of increasing concentrations of 3-O-methylglucose, fructose, and mannose on absorption of 2 mM C\(^{14}\) glucose.

I = mM inhibitor;

V = micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes;

i = fractional inhibition = 1 - v\(_f\)/v.
FIGURE 20

Lineweaver-Burk plots of $^{14}$C 2-deoxyglucose absorption
in the presence of glucose, N-acetylglucosamine,
fructose, and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = $mM 2-deoxyglucose;

$V =$ micromoles 2-deoxyglucose absorbed per gram ethanol extracted dry
weight per 4 minutes.
FIGURE 21

V versus V/S plots of C$^{14}$ 2-deoxyglucose absorption in the presence of glucose, N-acetylglucosamine, fructose, and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = \text{mM 2-deoxyglucose}$;

$V = \text{micromoles 2-deoxyglucose absorbed per gram ethanol extracted dry weight per 4 minutes.}$
Effects of increasing concentrations of 2-deoxyglucose, glucose, and mannose on the absorption of 2 mM C^{14} mannose.

Upper curves, Dixon plots;
Lower curves, Webb plots.

I = mM inhibitor;
V = micromoles mannose absorbed per gram ethanol extracted dry weight per 4 minutes;
i = fractional inhibition = 1 - v_1/v.
FIGURE 23

Effects of increasing concentrations of fructose on absorption of 2 mM C\textsubscript{14} mannose.

Upper curve, Dixon plot;
Lower curve, Webb plot.

I = mM fructose;
V = micromoles mannose absorbed per gram ethanol extracted dry weight per 4 minutes;
i = fractional inhibition = 1 - v\textsubscript{i}/v.
FIGURE 24

Effects of increasing concentrations of
glucose-6-phosphate on absorption
of 2 mM C14 glucose: Webb plot.

Inset, Dixon plot.

I = mM glucose-6-phosphate;

V = micromoles apparent glucose-6-phosphate absorbed per gram ethanol
extracted dry weight per 4 minutes;

\[ i = \text{fractional inhibition} = 1 - \frac{v_i}{v}. \]
FIGURE 25

I versus I plots for inhibition of absorption of 
2 mM C\textsuperscript{14} glucose by glucose, 2-deoxyglucose, 
glucose-6-phosphate, and maltose.

I = mM inhibitor;

i = fractional inhibition = 1 - v_i/v.
FIGURE 26

Lineweaver-Burk plot of $^{14}$C fructose absorption in the presence of 5 mM glucose and in the absence of inhibitor.

$S = \text{mM fructose};$

$V = \text{micromoles fructose absorbed per gram ethanol extracted dry weight per 4 minutes}.$
FIGURE 27

V versus V/S plots of $^{14}$C fructose absorption in the presence of glucose and 2-deoxyglucose and in the absence of inhibitor.

Inhibitors at 5 mM concentrations.

$S =$ mM fructose;

$V =$ micromoles fructose absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 28

V versus V/S plots of C$^{14}$ N-acetylglucosamine absorption in the presence of glucose, 2-deoxyglucose, fructose, and no inhibitor.

Inhibitors at 5 mM concentrations.

$S = \text{mM } N$-acetylglucosamine;

$V = \text{micromoles } N$-acetylglucosamine absorbed per gram ethanol extracted dry weight per 4 minutes.
FIGURE 29

Effect of increasing concentrations of glucose on absorption of 2 mM C\textsuperscript{14} fructose.

Upper curve, Dixon plot;
Lower curve, Webb plot.

I = mM glucose;
V = micromoles fructose absorbed per gram ethanol extracted dry weight per 4 minutes;
i = fractional inhibition = 1 - v_1/v.
FIGURE 30

Effect of temperature on C\text{14} glucose absorption.

Upper curves, Arrhenius plots;
Lower curves, velocity versus temperature plots.

\[ V = \text{micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.} \]

Closed circles = 1.25 mM glucose;
Open circles = 20 mM glucose.
FIGURE 31

Effect of pH on absorption of 2 mM C^{14} glucose.

Abscissa = pH of incubation medium;
Ordinate = micromoles glucose absorbed per gram ethanol extracted dry weight per 4 minutes.
Lineweaver-Burk plots of phosphorylation of C$^{14}$ glucose by high speed supernate of body wall homogenates in the presence of glucose, mannose, 2-deoxyglucose, or no inhibitor.

Each reaction mixture contained:

- 10 µmoles MgCl$_2$
- 20 µmoles Tris-Maleate buffer, pH 7.4
- 4 µmoles Disodium ATP
- 0.5 µcurie C$^{14}$ glucose
- 1.47 mg protein

Total volume of reaction mixture = 0.2 ml

Concentrations of inhibitors = 5 mM.

Incubated for 60 minutes at 36.5°C.

$S =$ mM glucose = geometric mean of the initial and final glucose concentrations;

$V =$ micromoles of glucose phosphorylated per milligram protein per hour.
INHIBITOR:

- □ 2-DEOXY GLUCOSE
- ■ MANNOSE
- ○ GLUCOSE
- ● NONE
FIGURE 33

Lineweaver-Burk plots of phosphorylation of $^{14}C$ fructose by high speed supernates of body wall homogenates in the presence of fructose, glucose, 2-deoxyglucose, or no inhibitor.

Each reaction mixture contained:

40 µmoles MgCl$_2$
80 µmoles Tris-Maleate buffer, pH 7.4
4 µmoles Disodium ATP
0.5 µcurie $^{14}C$ fructose
0.057 mg protein

Total volume of reaction mixture = 0.8 ml.

Concentration of inhibitors = 2.5 mM.

Incubated for 20 minutes at 36.5°C.

$S =$ initial mM fructose;

$V =$ micromoles fructose phosphorylated per milligram protein per hour.
INHIBITOR:

- □ 2-DEOXY GLUCOSE
- ▲ FRUCTOSE
- ● GLUCOSE
- ▲ ▲ NONE

$\frac{1}{V}$ vs. $\frac{1}{S}$