LIST OF PUBLICATIONS


Effect of Thiabendazole and Fenbendazole on Glucose Uptake and Carbohydrate Metabolism in *Trichuris globulosa*

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**ABSTRACT**


14C-glucose uptake by adult *Trichuris globulosa* was found to be a non-linear function of time and limiting substrate concentration. The uptake was a two component process, an initial rapid burst, followed by a lower steady state. Linear transformation of the substrate saturation kinetics by Eadie plot gave a $K_t$ value of $5 \times 10^3$ M and $J_{\text{max}}$ of 5 $\mu$mol mg$^{-1}$ dry weight min$^{-1}$. Presence of the benzimidazole drugs, thiabendazole and fenbendazole, markedly inhibited the uptake process at concentrations in which the motility of the parasites was not affected. *T. globulosa* was found to possess the full complement of enzyme systems of glycolysis, Krebs cycle, phosphatases, transaminases and other NAD- and NADP-dependent enzymes. Thiabendazole and fenbendazole lowered the $V_{\text{max}}$ of most of these enzymes and affected the substrate-affinity constant ($K_m$) in some of them. The mode of action of these drugs was discussed in the light of the data obtained.

**INTRODUCTION**

Fenbendazole (methyl 5-phenylthio-1H-benzimidazole-2-yl-carbamate) and thiabendazole (2-(4-thiazolyl)-1H-benzimidazole) are effective drugs against parasitic nematodes. Benzimidazoles and specifically benzimidazole carbamates are thought to induce the disappearance of cytoplasmic microtubules in the nematodes intestinal or tegumental cells. This is followed by a block in the transport of secretory vesicles, leading to impaired coating of the membranes, accompanied by decreased digestion and absorption of nutrients, such as glucose and amino acids (Borgers et al., 1975a). Polymerization of purified tubulin (microtubular protein) is inhibited in the presence of these drugs (Kohler and Bachmann, 1980; Laclette et al., 1980). Further, these drugs cause cytoplasmic autolysis, degenerative changes at the brush-border area, cellular necrosis and finally death of the parasite. However, no information is available...
uptake of glucose (V) as a function of substrate concentrations were examined using plots of each of three linear transformations of the Michaelis-Menten equation; the double reciprocal S/V plot of Lineweaver-Burk, the S/V vs. S plot of Woolf and V vs. V/S plot of Eadie (Siegel, 1976). Apparent \( J_{\text{max}} \) (maximum of uptake activity) and \( K_s \) (substrate-affinity constant) values were calculated from the unweighted linear transformations. However the data were more appropriate, as an Eadie plot, rather than as the more widely used Lineweaver-Burk plot, since this was least sensitive to random experimental variation, yet most sensitive to systematic deviation from Michaelis-Menten kinetics (Starling and Fisher, 1975).

**Enzyme assay**

The nematodes were homogenized in the respective buffer system of the enzyme assay, sonicated briefly in ice-water with a microprobe (B. Braun Instruments), centrifuged at 6000 \( g \) for 15 min at 4 °C and the supernatant was used as the source of enzyme activity. The effects of thiabendazole and fenbendazole on various enzymes were determined by the direct addition of these drugs (100 \( \mu \)mol each) to the enzyme assay systems.

The following methods were used for enzyme assays: glycogen phosphorylase (+ AMP) by Niemeyer et al. (1961); hexokinase by Crane and Sols (1955); glucose-6-phosphatase by Swanson (1955); fructose-1, 6-diphosphatase by McGilvery (1955); glucose-6-phosphate isomerase by Slein (1954); lactate dehydrogenase by King (1959); succinate dehydrogenase by Kun and Aboud (1949); malate dehydrogenase by Davidson and Cortner (1967); glucose-6-phosphate dehydrogenase by Julian and Riethal (1975); acid and alkaline phosphatases by Natelson (1963); and alanineoxaloacetate and aspartate-oxaloacetate transaminases by Reitman and Frankel (1957). Phosphorus and proteins were estimated by the methods of Fiske and Subbarow (1925) and Lowry et al. (1951), respectively. All enzyme activities were expressed in \( \mu \)mol product formed mg⁻¹ protein h⁻¹ at 37 °C.

**RESULTS**

The results of a time-course study of \( ^{14} \)C-glucose uptake by \( T. \) globulosa are shown in Fig. 1. An initial rapid uptake for the first 2 min was followed by a second lower rate of uptake for the next 15 min achieving steady state. From this study, experimental incubations of 2 min were chosen as optimum for further studies. During prolonged incubations from 1 to 5 h, although the parasites were visibly alive and showed movement, they did not accumulate further radioactivity, instead the uptake rate was inhibited. This inhibition was greater in the presence of thiabendazole and fenbendazole. Glucose absorption was also found to be a non-linear function of limiting substrate concentration.
Results in Table 1 show that the enzymes of glycolysis, phosphatases, transaminases and Krebs cycle were present in *T. globulosa*. They also followed typical Michaelis-Menten substrate saturation kinetics. Thiabendazole and fenbendazole lowered the V_max of glycogen phosphorylase (+ AMP), hexokinase,

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control</th>
<th>Thiabendazole</th>
<th>Fenbendazole</th>
<th>Control</th>
<th>Thiabendazole</th>
<th>Fenbendazole</th>
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<tr>
<td><strong>Glycolysis:</strong></td>
<td></td>
<td></td>
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<tr>
<td>Glycogen phosphorylase (+ AMP)</td>
<td>1.56</td>
<td>1.56</td>
<td>1.56</td>
<td>100.00</td>
<td>83.33</td>
<td>63.50</td>
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<td>Hexokinase</td>
<td>7.14</td>
<td>7.14</td>
<td>7.14</td>
<td>666.60</td>
<td>500.00</td>
<td>333.33</td>
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<tr>
<td>Glucose-6-phosphatase</td>
<td>0.02</td>
<td>1.00</td>
<td>0.55</td>
<td>100.00</td>
<td>100.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Fructose-1, 6- diphosphatase</td>
<td>0.76</td>
<td>0.62</td>
<td>0.35</td>
<td>4.54</td>
<td>4.54</td>
<td>4.54</td>
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<td>Glucose-6-phosphate isomerase</td>
<td>1.42</td>
<td>0.52</td>
<td>0.52</td>
<td>25.00</td>
<td>8.33</td>
<td>5.00</td>
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<tr>
<td>Lactate dehydrogenase</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>31.25</td>
<td>20.83</td>
<td>17.85</td>
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<td><strong>Krebs Cycle and other NAD- and NADP- dependent enzymes:</strong></td>
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<tr>
<td>Succinate dehydrogenase</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>3.33</td>
<td>2.50</td>
<td>5.00</td>
</tr>
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<td>Malate dehydrogenase</td>
<td>0.37</td>
<td>0.21</td>
<td>0.21</td>
<td>0.31</td>
<td>0.31</td>
<td>0.41</td>
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<td>Glucose-6-phosphate dehydrogenase</td>
<td>0.06</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
<td>0.25</td>
<td>0.31</td>
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<td><strong>Phosphatase:</strong></td>
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<td>ATPase (Mg²⁺- dependent)</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>29.41</td>
<td>22.22</td>
<td>29.41</td>
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<td>Acid phosphatase</td>
<td>0.90</td>
<td>0.60</td>
<td>0.90</td>
<td>35.71</td>
<td>23.80</td>
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<td>Alkaline phosphatase</td>
<td>1.49</td>
<td>1.01</td>
<td>0.53</td>
<td>22.72</td>
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<td><strong>Transaminase:</strong></td>
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<tr>
<td>Alanine oxoglutarate</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>50.00</td>
<td>50.00</td>
<td>38.46</td>
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<tr>
<td>Aspartate oxoglutarate</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>71.42</td>
<td>50.00</td>
<td>62.50</td>
</tr>
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</table>

Thiabendazole and fenbendazole have been added to the standard enzyme assay medium at the concentration of 100 μmol in incubation medium dissolved in 0.1% DMSO. Control incubations received equal amounts of DMSO. The enzyme activities were determined in triplicate which agreed within 5% limit. The mean values were plotted according to Lineweaver-Burk (Siegel, 1976), and K_m and V_max here directly determined from the plot.
the substrate utilization is reduced, while in others the maximum apparent initial velocity of the enzyme is affected, probably because of a reduced number of available molecular proteins (enzymes). The immediate, and possibly the only, fate of absorbed glucose is phosphorylation, catalysed by hexokinase, to be utilized in the glycolytic pathway. Glucose uptake is also known to be rapid, selective and not dependent on the diffusion rate, which is thought to be accomplished by phosphorylation at the place of absorption (Weatherly et al., 1963; Uglem et al., 1978). As both the processes of glucose uptake and hexokinase activity are slowed down by the benzimidazole drugs, this may indicate a common or similar mode of action. These results, however, strongly suggest that these drugs may actually bring about real inhibition in the process of glucose phosphorylation as one of the causes of parasitic death (Van den Bossche, 1972).

Inhibition of glucose-6-phosphatase and also of fructose-1, 6-diphosphatase may, therefore, be caused by reduced substrate availability, as a consequence of inhibited glucose phosphorylation. Inhibition of these enzymes and the transaminases indicates the breakdown of the process of gluconeogenesis as these are the rate-limiting enzymes in the formation of glucose from amino acids and other sources. Inhibition of lactate dehydrogenase and Krebs cycle enzyme systems, such as succinate and malate dehydrogenases, strongly suggests the arrest of carbon flux in the glycolytic pathway and generation of the necessary energy through oxidative phosphorylation. Glucose-6-phosphate dehydrogenase inhibition will result also in the blockade of supply of reducing equivalents to the nucleotide and fatty acid biosynthesis. Among these enzymes, glucose-6-phosphatase occupies a centrally located key position in the process of glycogenolysis, glycogenesis, glycolysis and gluconeogenesis. Inhibition of this enzyme may ultimately be the reason for the decreased generation of ATP which proves fatal to the parasites.

In conclusion, evidence is provided in *T. globulosa*, thiabendazole and fenbendazole impair the process of glucose uptake, inhibit the enzymes of carbohydrate and intermediary metabolism and may cause death to the parasites by a decreased generation of energy. However, the mode of action of these drugs in the processes remains inconclusive as no single enzyme system is affected. They seem to block the binding and utilization of the substrates by the enzymes and transporter proteins.

REFERENCES


Glucose uptake by *Trichuris globulosa* of Read et al., 1974) except that maleate was replaced by HCl since maleate was shown to be toxic in metabolic studies (Webb, 1966). In order to minimize the effects of diurnal variations, care was taken to commence experiments at the same time each day (10 a.m.). The worms were also randomly put into groups of 3–4, each group constituting a single sample. Following a 2 min incubation, the incubation medium was rapidly removed by aspiration. Worms were rinsed thoroughly in 3×10 ml changes of cold KRT, blotted on coarse filter paper and extracted overnight in 2 ml of 70% ethanol. The extracted carcasses were dried overnight at 80°C in pre-weighed aluminium tares and ethanol extracted dry weights were determined. Aliquots of incubation medium and worm ethanol extracts were counted in a liquid scintillation spectrometer (Beckman Instruments) using methanolic toluene and phosphors (4 g PPO+500 mg POPOP/700 ml toluene+300 ml methanol) as the scintillant. Absorption velocities were calculated from specific activity of the media and total activity in worm extracts and expressed as μmole substrate absorbed/mg ethanol extracted dry weight/2 min. For these, the CPM/sample were converted to specific activity by comparison with standards and employing appropriate calculations. All determinations were expressed as the means of 3–4 replicates with standard deviations.

**RESULTS**

The absorption of 14C-glucose by adult *T. globulosa* was found to be a two component process, an initial rapid uptake for the first 2 min was followed by a second lower rate of uptake for the next 10 min, finally achieving a steady state (Fig. 1). 14C-glucose uptake was also found to be specifically dependent on Na+ ions as replacing NaCl in the assay system with KCl and LiCl resulted in reduced uptake. Uptake in the presence of choline chloride represented the non-mediated portion of the process (passive influx). The effect of pH on 14C-glucose absorption was studied using three buffer systems ranging from pH 5 to 9 (Fig. 2). The uptake was found to be dependent on the changes in pH of the incubation medium, the maximum absorption being at pH 7.4.

![Graph showing glucose uptake over time](image)

**FIG. 1.** Time course of 14C-glucose uptake in *T. globulosa* in the presence of Na+, K+, Li+ and choline+. Each point is the mean of three determinations. Longitudinal bars indicate S.D. of the mean.
Glucose uptake by *Trichuria globulosa*

**Inhibitor** = Unlabelled glucose [mM]

(A) 

Unlabelled glucose [mM] vs. slope (μmoles glucose uptake/mg dry wt. of T. globulosa/min)

(B) 

Phlorizin [mM] vs. slope (μmoles glucose uptake/mg dry wt. of T. globulosa/min)
uptake occurred optimally at pH 7.4 in vitro. The data clearly indicates that the glucose influx is mediated, since influx is saturable and inhibited by monosaccharides (i.e. the influx is stereospecific). The first indication that glucose uptake by nematodes involved a mediated process was provided by Bueding et al. (1961). These authors also demonstrated that dithiazanine inhibited glucose uptake by *T. vulpis* and caused decreased levels of adenosine transport, free glucose and storage carbohydrates. Subsequent studies showed that glucose uptake by *Ascaris suum* was inhibited by dithiazanine and mebendazole. Rutherford & Webster (1974) demonstrated that glucose uptake by *Mermis nigrescens* was non-linear with respect to concentration and the uptake was inhibited by phlorizin and 2,4-dinitrophenol, suggestive of a mediated glucose transport system.

The completely competitive inhibition of uptake of 14C-glucose by unlabelled glucose (replot of slope versus 1/[I]) suggests that glucose does not enter the body by non-specific, non-saturable mechanism, i.e. simple diffusion. Also, the fact that absorption of glucose within the physiological hexose concentration range is mediated by a single transport locus, is based upon the criteria that completely competitive inhibition of 14C-glucose uptake is effected by unlabelled glucose. Dixon plots of phlorizin and ouabain suggest that these are partially competitive inhibitors or relatively poor inhibitors of 14C-glucose uptake, hence there is a sizeable component of glucose absorption which is not sensitive to phlorizin or ouabain. Phlorizin appears to be a partially competitive inhibitor of glucose influx in other parasites (Fischer & Read, 1971; Pappas et al., 1973). It is probable that the inhibition of uptake of 14C-glucose in the presence of disaccharide sugars and sugar phosphates is mediated by free glucose released by hydrolysis (Dike & Read, 1971).

Results of the present study suggest that glucose transport is Na+-dependent. Little mediated glucose influx in *T. globulosa* in the absence of Na+ suggests that Na+ may be an obligatory co-substrate for the influx process. A short exposure to Na+-free media would not be expected to deplete the environment of Na+ immediately proximal to the worm surface, since the efflux of Na+ during that time from underlying worm tissue could provide the requisite Na+ for glucose transport. An analogous situation has been presented by Semenza (1971) for Na+ coupled glucose transport across the brush border of mammalian enterocytes. It is Semenza's view that Na+ exiting the cells, goes first into an unstirred layer, adjacent and external to the plasmalemma, before appearing in the lumen and would be available in the unstirred layer to activate the glucose permeability of the membrane. Read et al. (1974) considered it possible that the small mediated influx of 14C-glucose by *Hymenolepis diminuta* (Cestoda) in ostensibly Na+ free media may also be due to the presence of effluxed Na+ in an unstirred layer.

In *T. globulosa* the influx of 14C-glucose is affected by replacement of Na+ ions but not considerably. Starling (1975) reported that K+ is a non-specific antagonist of Na+ in activating glucose transport but as this antagonism is weak, Read et al. (1974) did not observe K+ inhibition.

It would be interesting to compare the specificity of the transport system and the enzymes involved in the metabolism of absorbed molecules. The principal natural substrate of the hexose transport system is glucose which has only one immediate fate: phosphorylation by hexokinase. The presence and characterization of this enzyme in *T. globulosa* has been established (Jasra et al., 1990); the processes of glucose absorption and maximum of apparent enzyme velocity of hexokinase were strongly inhibited by the benzimidazole group of drugs.