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Uptake of 3-o-Methylglucose by Healthy and Hypomyces-infected Squash Hypocotyls

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Abstract. Rates of uptake of 3-*o*-methylglucose (MeG) by squash (*Cucurbita maxima*) hypocotyl sections from above lesions caused by *Hypomyces solani* f. sp. *cucurbitae*, race 1, are 2-fold greater than uptake by comparable tissues from healthy plants. Kinetic analyses indicate (i) that a single (constitutive) carrier system, with a Michaelis constant (*Km*) of 25 to 30 mM, mediates the transport of MeG into healthy hypocotyl cells and (ii) that an additional (inducible) system with a much lower *Km* (*ca.* 2 mM) is present in diseased hypocotyls. In both systems MeG uptake is inhibited competitively by glucose. The inducible transport system(s) in diseased tissues has a higher temperature coefficient, greater sensitivity to metabolic inhibitors and larger accumulation capacity than the one in healthy plants. While the nature of the constitutive system is ambiguous, the inducible carrier mechanism is a typical active transport system. These results indicate that increased rates of uptake and accumulation of metabolites by diseased tissues can be caused by new transport systems.

Changes in host membrane permeability during pathogenesis have been noted in many plant diseases (13). In a recent study of permeability changes in *Hypomyces*-infected squash hypocotyls, it was concluded that the permeability of host cells in tissues above lesions did not differ from that of comparable cells in healthy plants (6). In fact, the functioning of plasma membranes as barriers to the movement of water and low molecular weight nonelectrolytes was not altered in individual cells until the fungus grew within their vicinity (1–3 cell diameters). Then, the permeability change was sudden, and the plasmolyzability of host cells was destroyed.

Thatcher (11) reported that the permeation of glucose into plant cells was higher in infected tissues than in healthy ones. He used the plasmolytic method, which indirectly measures the rate of movement of an osmotically active substance into cells. Thatcher suggested that the increased rate of movement of solutes across membranes was due to the partial destruction of membrane components by phospholipolytic or proteolytic enzymes (12). More recently, studies of the movement of metabolites into and out of many types of cells of animals, higher plants, and microorganisms indicate that many substances (*e.g.*, glucose and closely related sugars) are transported by special carrier systems (10). Thus, an increase in the rate of movement of metabolites into host cells in infected plants could be caused by the activation or biosynthesis of a new transport system rather than the modification or destruction of normal membrane components.

In this study, transport kinetics of 3-*o*-methylglucose are compared in healthy squash hypocotyls and hypocotyl tissues above lesions caused by *Hypomyces* (*Fusarium*) *solani* f. sp. *cucurbitae*, race 1. With the use of a glucose derivative, 3-*o*-methylglucose, uptake by glucose transport systems can be investigated while complications from metabolic processes are avoided (8).

Materials and Methods

Inoculation Procedures. Cultures of the pathogen (*Hypomyces solani* f. sp. *cucurbitae* Snyd. and Hans., race 1) were maintained as described previously (5). Squash (*Cucurbita maxima* Dcne "Pink Banana") were grown in the greenhouse in U. C. mix (1). Three- to 6-day-old seedlings were inoculated with 1 ml suspensions of macroconidia (6×10^5/ml) at the base of hypocotyls. Uninoculated seedlings of identical plantings were maintained as controls. Lesions developed at the base of hypocotyls 5 to 7 days after inoculation. As in a previous study (6), seedlings with lesions 1 to 2 cm in length were used as experimental materials.

Uptake of 3-o-methylglucose. Methods for studying uptake are modified from those described by Reinhold and Eshbar (8). Batches of 20 hypocotyl cross sections (*ca.* 1.5 mm thick) were rinsed briefly (30 sec), blotted dry, and weighed (400–500 mg fresh wt). Sections were taken from uninoculated hypocotyls (healthy tissues) or from inoculated ones 1 to 20 mm above lesions (diseased tissues) from

an appropriate number of seedlings. Each batch was made up of sections at random from a number of plants. Sections were placed in 1 ml labeled 3-*o*-methylglucose solution in test tubes and shaken continuously for 3 hr at 25°. At the end of the incubation period, the sections were rinsed with distilled water for 30 min to remove the labeled sugar derivative from the disc surfaces and the apparent free space. Finally, each batch was extracted with 1 ml 95 % ethanol for 18 to 24 hr at −20°. Portions of the ethanol extracts were taken for radioactivity measurements.

Reinhold and Eshbar (8) found no evidence of metabolism of 3-*o*-methylglucose by carrot discs. Using similar methods, no evidence of metabolism by squash hypocotyl tissues was found.

Chemicals. 3-*o*-methylglucose was supplied by Calbiochem, Los Angeles, California. The 3-*o*-methyl-^{14}C-glucose was obtained from New England Nuclear Corporation, Boston, Massachusetts, and diluted with unlabeled 3-*o*-methylglucose to adjust the specific activity to 0.01 μc/μmole.

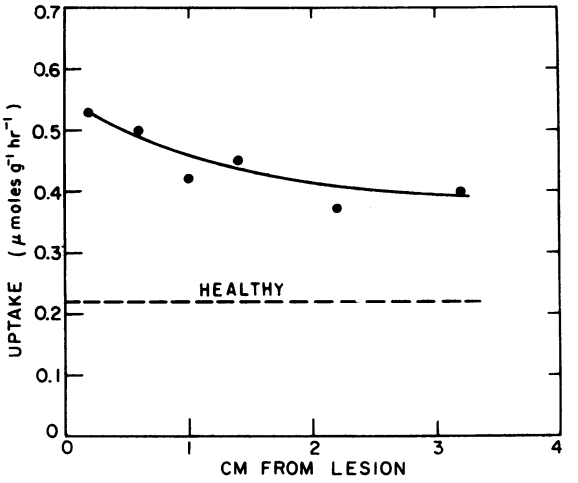

FIG. 1. Rate of uptake of 3-*o*-methylglucose (5 mM) as a function of distance above upper margin of lesions.

Results

Kinetics. Rates of 3-*o*-methylglucose (MeG) uptake in diseased tissues 0 to 3 cm above the upper margin of the lesion are 2-fold higher than rates from corresponding tissues of healthy plants (Fig. 1).

Hyperbolic relationships were evident when rates of uptake of MeG by healthy hypocotyl sections were plotted as a function of substrate concentration (Fig. 2A). Even though similar patterns were observed in tissues above lesions, the plots did not yield rectangular hyperbolas. MeG uptake data were plotted by the Woolf-Hofstee procedure (2), with rate of uptake (V) as a function of V/S, where S is the external MeG concentration. A rectangular hyperbola should give a straight line when plotted in this way.

With healthy tissue, Woolf-Hofstee plots are interpreted as linear as shown in Fig. 2C. However, single straight lines could not be fitted to data from diseased plants. As shown in Fig. 2B, it appears that 2 straight lines describe plots for diseased materials. Although different combinations of MeG concentrations were used, identical relationships were obtained with plots of data for uptake by healthy and diseased tissues in 4 separate experiments. With these plots, the maximum rates of uptake (Vm) and Michaelis constants (K*m*) were estimated (2).

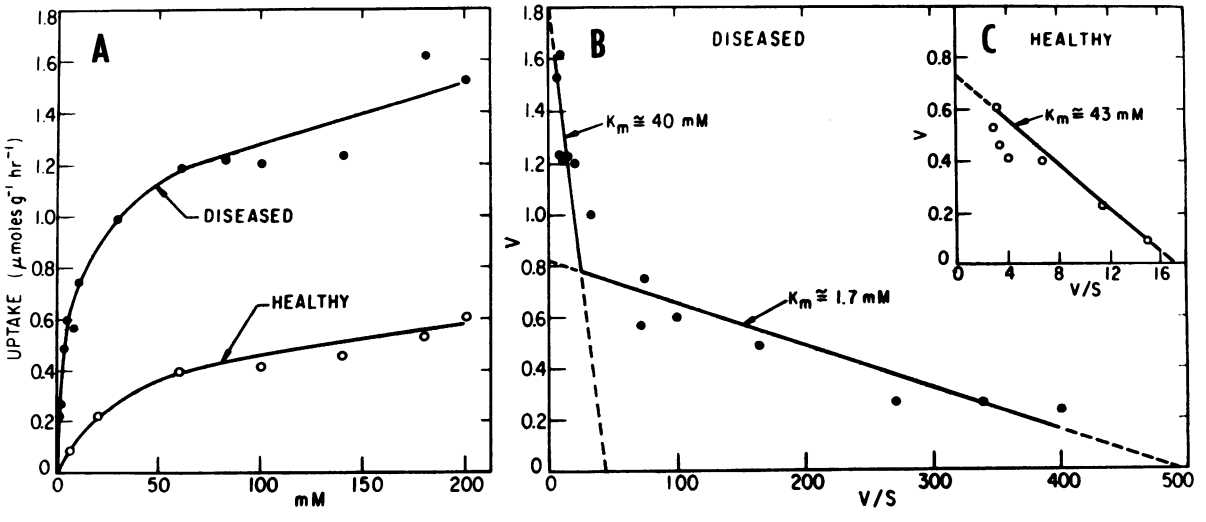

FIG. 2. A) Rate of uptake (V) as a function of external 3-*o*-methylglucose (MeG) concentrations (S). B) V as a function of V/S with diseased squash hypocotyl tissues. C) Same as B except hypocotyl tissues from healthy plants.

Table I. *Michaelis Constants (Km) for 3-o-Methylglucose Transport by Healthy and Diseased Squash Hypocotyls*

Expt. No.	Km (mM) Healthy	Diseased[1] A	Diseased[1] B
1	18.1	27.7	2.3
2	21.5	18.1	2.7
3	43.0	40.0	1.7
4	18.0	37.5	2.0
Mean ± SD	25.2±10.2	30.8±8.7	2.2±0.3

[1] A refers to *Km* values derived from straight lines in Woolf-Hofstee plots (2) fitted to uptake data at highest 3-*o*-methylglucose (MeG) concentrations (50–200 mM) while B refers to *Km* values derived from a second line for the lowest MeG concentrations (0.6–10 mM)

Michaelis constants for MeG uptake by hypocotyl sections from healthy and diseased plants are given in table I. For each experiment with diseased tissues, 2 *Km* values were estimated. Straight lines fitted through points at the higher MeG concentrations yielded mean (± SD) *Km* values (30.8 ± 8.7 mM) that did not differ statistically ($t = 0.8$, $df = 6$) from those for healthy tissues (25.2 ± 10.2 mM). Lines fitted through points at lower MeG concentrations with diseased tissues yielded much lower *Km* values (2.2 ± 0.3 mM).

Methods were not sufficiently sensitive to detect uptake accurately when MeG concentrations in bathing solutions containing healthy materials were below 1 mM. However, several uptake studies with healthy tissues at low substrate concentrations (1–5 mM) failed to reveal a second straight line when data were plotted by the Woolf-Hofstee procedure.

Temperature Coefficients. In the range 20 to 30°, temperature coefficients (Q_{10}) for MeG uptake were determined for healthy and diseased hypocotyl tissues. When the external concentration was 100 mM, the Q_{10} values were 2.1 and 2.7 for healthy and diseased tissues, respectively. When the substrate concentration was 5 mM, the Q_{10} values were 2.0 and 4.1 for healthy and diseased materials.

Accumulation of 3-o-Methylglucose. Sections (1.3 g) from healthy or diseased plants were bathed in 2.5 ml 50 mM MeG at 25° for 2.5 hr, rinsed briefly (30 sec) with 5 ml water, and reincubated in 4 ml water for 3.5 hr. One hr after the start of the final incubation, the bathing solutions with healthy and diseased sections contained 1.2 and 1.0 mM MeG, respectively. After 3.5 hr incubation, the same bathing solutions contained 1.0 and 0.6 mM MeG while the MeG concentrations in healthy and diseased sections were 1.6 and 2.4 mM, respectively, assuming 1 g tissue equals 1 ml.

Based on the progressive depletion of MeG from bathing solutions (<0.1 mM), uptake was still proceeding in another experiment where the internal:external ratios of MeG in healthy and diseased materials were 7:1 and 60:1, respectively.

Table II. *Effect of Respiratory Inhibitors on Apparent Osmotic Volume and Rate of Uptake of 3-o-Methylglucose*

The apparent osmotic volume (AOV) is the fraction of the tissue volume (TV) remaining when the tissue fraction measured as apparent free space (AFS) is subtracted from the tissue volume, *i.e.* TV - AFS = AOV. The TV is estimated from weight, *i.e.*, 1 mg fresh wt = 1 μl, and the AFS is determined by the mannitol procedure (6).

Inhibitor	Concn. M	Control (%) Apparent osmotic volume	Control (%) 3-*o*-Methylglucose uptake Healthy	Control (%) 3-*o*-Methylglucose uptake Diseased
KCN	10^{-3}	78	83	56
NaN_3	5×10^{-4}	75	80	53
2,4-dinitrophenol	10^{-4}	61	67	20
	5×10^{-5}	62	81	47
	10^{-5}	95	85	92

Effect of Respiratory Inhibitors on Uptake. Metabolic inhibitors appeared to inhibit uptake of MeG in both healthy and diseased tissues (table II). However, the level of inhibition was greater with diseased tissues than with healthy ones.

Interpretations of effects of metabolic inhibitors may be confused with their direct or indirect effect on membranes (7). Dinitrophenol is reported to increase the apparent free space of sunflower hypocotyls (3) and oat roots (9). As shown in table II, the per cent apparent osmotic volume was decreased by several inhibitors to the same extent as uptake was inhibited in healthy tissues. Because solutes in the apparent free space are lost during the rinsing period, that fraction (cytoplasm?) of the osmotic volume made more permeable by inhibitors will lose MeG taken up previously, and uptake processes will appear to be inhibited.

Uptake of MeG (25 mM) by either healthy or diseased tissues was inhibited by either glucose or galactose (50 mM). Inhibition was 2-fold greater in diseased than in healthy materials, and glucose was more effective than galactose in inhibiting uptake. Sodium chloride (50 or 100 mM) had no effect on uptake. Glucose inhibition of MeG transport was competitive in the higher MeG concentration ranges (20–200 mM) with healthy materials or in the lower MeG concentration ranges (0.5–10 mM) with diseased materials.

Phlorizin, a powerful inhibitor of sugar uptake by many animal tissues (10), was not very effective in inhibiting uptake of MeG by squash hypocotyl sections. At 0.5 mM, phlorizin inhibited uptake of MeG by healthy (100 mM MeG) and diseased (2.5 mM MeG) tissues by 20 and 25 %, respectively.

Table III. *Effect of Aging of Squash Hypocotyl Sections on 3-o-Methylglucose Uptake*

Hypocotyl cross sections (1.5 mm thick) from squash seedlings were incubated in shallow (4 mm) water baths at 25°. At designated time intervals, 20 sections were blotted dry, weighed, and shaken in 1 ml 5 mM 3-*o*-methylglucose (MeG) for 2 hr at 25°.

Incubation time	MeG Uptake	
	Healthy	Diseased
hr	*μmoles g⁻¹ hr⁻¹*	
0	0.23	0.45
1	0.28	...
2	0.46	...
4	0.78	...
16	1.52	...
21	1.45	...
28	1.31	1.35

Effect of Aging of Hypocotyl Sections on Uptake. The rate of MeG uptake was increased when hypocotyl sections from either healthy or diseased hypocotyls were aged. Increases in rates of uptake began after sections were incubated in water for short periods (table III). Although rates of MeG uptake by freshly excised sections from healthy and diseased hypocotyls differed, rates of uptake by aged sections from these sources were similar.

Utilizing uptake data taken at different MeG concentrations with aged sections from healthy or diseased plants, Woolf-Hofstee plots resembled those drawn with data obtained with fresh sections from diseased plants. The K*m* determined from transport data taken at low MeG concentrations (0.5–5 mM) is 1 to 2 mM.

Antibiotics, such as chloramphenicol (50 μg/ml) or combinations of streptomycin sulfate and penicillin G (each at 50 μg/ml) had no effect on the development of the greater uptake capacity of MeG during aging. On the other hand, antimetabolites (actinomycin D or cycloheximide, 10 μg/ml) prevented the development of a greater uptake capacity by aging hypocotyl sections or halted the development of this ability when added in mid-course (Fig. 3).

Discussion

The characteristics of uptake of MeG by squash hypocotyl sections in relation to concentration may be described by Michaelis enzyme kinetics; this suggests that a carrier-mediated transport system is involved in the movement of MeG into squash cells (10). On the basis of kinetic analyses, I propose that a single (constitutive) carrier system is present in healthy squash hypocotyl cells and that at least 2 carrier systems are present in hypocotyl tissues above lesions.

Judging from the Michaelis constant, the constitutive carrier system present in healthy hypocotyls is also present in diseased tissues and possesses a low affinity (1/K*m*) for MeG. The new (inducible) carrier system in diseased tissues has a much greater affinity for MeG. When the average maximum rate of MeG uptake (Vm) of the inducible carrier system is subtracted from the average Vm of all carrier systems in diseased tissues, the rate difference (1.0 μmole g^{-1} hr^{-1}) is equivalent to the average Vm (0.9 μmole g^{-1} hr^{-1}) determined for uptake by healthy hypocotyls. This suggests that the constitutive carrier system in healthy hypocotyls is not converted into a new-type system in response to *Hypomyces*-infection and that both systems can function simultaneously.

Reinhold and Eshbar (8) reported that aged storage roots of *Daucus carota* possess a MeG-carrier system with a K*m* of 2.4 mM. (This K*m* is similar to those determined for the inducible system in *Hypomyces*-infected squash hypocotyls.) The rates of uptake by carrot tissues deviated from those expected for a single carrier system, and Reinhold and Eshbar (8) suggested that the uptake values plotted as a function of V/S at high substrate concentrations "possibly lie on a second straight line."

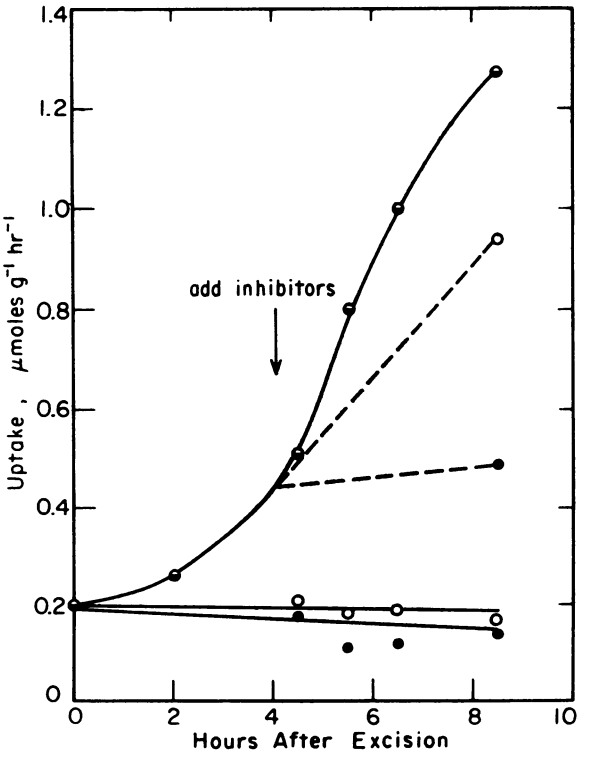

FIG. 3. Effect of antimetabolites on the increase of 3-*o*-methylglucose (MeG) transport capacity of squash hypocotyl sections during aging. Sections incubated in 5 mM MeG at 25°. ◡, control (water); ○, actinomycin D (10 μg/ml); and ●, cycloheximide (10 μg/ml).

The presence of 2 MeG-carrier systems in carrot tissues was not suggested, however.

When healthy or diseased squash hypocotyl sections were aged, MeG transport capacity increased. In the case of diseased sections, however, the effects may be quantitative rather than qualitative. The *Km* values of the MeG carrier systems in aged squash hypocotyl sections are similar to those in *Hypomyces*-infected squash hypocotyls.

Studies with antimetabolites indicate that the increased transport of MeG during aging is dependent upon protein synthesis. Thus, the change in kinetic characteristics of MeG uptake during aging or pathogenesis may be related to the synthesis of a new sugar-carrier system.

During studies on wound respiration of potato slices, Hackett *et al.* (4) noted that as slices aged, the ability to take up $^{32}PO_4^{3-}$ increased greatly. Increased ability to take up metabolites may be a general wound response. Perhaps the sugar-carrier systems with low *Km* values in aged healthy and fresh diseased squash hypocotyl sections are formed in response to similar processes.

Other differences in the carrier systems in healthy and diseased tissues exist. The Q_{10} values of uptake by diseased tissues are higher than those determined with healthy material. Q_{10} values obtained with diseased hypocotyls are probably a measure of the activity of 2 carrier systems. Simple calculations show that the Q_{10} for uptake by the inducible carrier system in diseased material is between 3 and 4. Although other explanations should not be excluded, the greater temperature sensitivity of the inducible carrier system may reflect a greater dependency on metabolic processes. The greater effect of metabolic inhibitors on uptake by diseased hypocotyl tissues supports this contention.

The capacity to accumulate MeG against a concentration gradient is a property of both healthy and infected tissue. However, diseased tissues accumulate the sugar derivative to a greater extent than healthy ones. Increased accumulation in infected tissues is characteristic of many plant diseases (14), and changes in transport systems may partially account for this response.

The inducible carrier system in diseased hypocotyls appears to be a typical active transport system, but the nature of the constitutive system is not clear. A simple facilitated diffusion system cannot account for the accumulation of MeG by healthy hypocotyl tissues. Perhaps intracellular membrane systems (*e.g.* organelles, tonoplast) provide other forms of transport which result in accumulation in cellular compartments while uptake, the rate limiting step, is a form of facilitated diffusion.

Thatcher (11) suggested that increased host cell membrane permeability in infected tissues supplied the parasite with nutrients. However, evidence of permeability increases in infected plant tissues by plasmolytic methods is no assurance that passive permeability properties of plasma membranes are changed or that metabolites will be available in greater abundance to parasites growing in intercellular spaces. In fact, if "new" transport systems (such as the inducible MeG-transport system in *Hypomyces*-infected hypocotyls) are formed, "permeability" for metabolite uptake would be greater, but more nutrients would not necessarily be available to the parasite. Also, "new" carrier systems with greater affinity for their substrates would make host cells better competitors than cells in healthy hypocotyls for extracellular metabolites released during wounding or infection.

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