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Correlation of Growth Inhibition Patterns to Nucleoside Transport Models in *Neurospora crassa*

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Growth of inhibition patterns provide evidence for a common nucleoside transport or utilization system, a separate system or systems for adenine transport, and another adaptable mechanism of adenosine transport.

The transport of adenosine into *Neurospora crassa* conidia has been reported to proceed via two transport systems, one that takes up both purine and pyrimidine nucleosides and another that transports only purine nucleosides (4). This model for competitive transport of related compounds provides an explanation for the growth antagonisms of pyrimidine auxotrophs reported some time ago (2). We have examined the growth of purine and pyrimidine auxotrophs on combinations of purine and pyrimidine bases and nucleosides to determine whether the growth rates can be used to predict the specificity of the transport systems.

The growth rate of strain *ad-8*, which lacks adenylosuccinate synthetase (EC 6.3.4.4) activity (1), was measured by the tube method (3) on Vogel medium (5) containing varying concentrations of adenine and adenosine (Fig. 1). Growth rates were taken from the linear part of the growth curve. To maximize the effects of potential inhibitors, growth rates (Table 1) were measured at adenine and adenosine concentrations that gave (i) ½ maximal growth rate (0.2 and 0.01 mM, respectively) and (ii) near optimal growth rate (0.4 and 0.1 mM, respectively). In all cases, the potential competitor was added in a concentration 10 times that of the purine supplement. Other nucleosides including inosine, guanosine, and uridine, but not xanthosine, prevented the growth of *ad-8* on limiting adenosine. Xanthosine or the purine bases had no effect on growth rate when adenosine was used to satisfy the auxotrophy of *ad-8*, indicating that these compounds are not efficiently transported by the same system or systems. A similar conclusion can be reached from the experiments using adenine to satisfy the growth requirement. With the exceptions of inosine which had a slightly inhibitory effect and xanthine which caused a slight increase, other purine bases and nucleosides did not significantly alter the growth rate of *ad-8*. This would suggested that the adenine transport mechanism is relatively specific.

Further evidence for a general transport system for nucleosides was obtained by growing the pyrimidine auxotroph *pyr-3* on uridine with excess adenosine, which prevented growth, or excess adenine, which did not (Table 2). The growth rate of the wild-type strain SL74A from which the *ad-8* and *pyr-3* mutants were derived was approximately 4 mm/h on minimal medium and on each medium used in this study.

The long lag before growth begins, followed by normal growth when *ad-8* is grown on adenosine plus inosine, guanosine, or uridine (Table 1), is suggestive of enzyme "adaptation" (induction or derepression). This growth pattern does not represent a developmental change in transport when mycelia are produced, since conidial and mycelial inocula gave rise to the same lag period when tested on adenosine plus inosine (Fig. 2). However, conidia, but not mycelia, taken from adapted colonies show a similar lag, indicating that the system is not incorporated into conidia.

In conclusion, the inhibition of growth of adenine or pyrimidine auxotrophs by combinations of nucleosides is compatible with competitive transport of nucleosides by a common carrier. If a separate purine nucleoside system is present, it does not transport sufficient adenosine to allow growth, as shown by the inhibition of growth of *ad-8* by uridine. On the other hand, the lack of inhibition of *ad-8* given adenine and

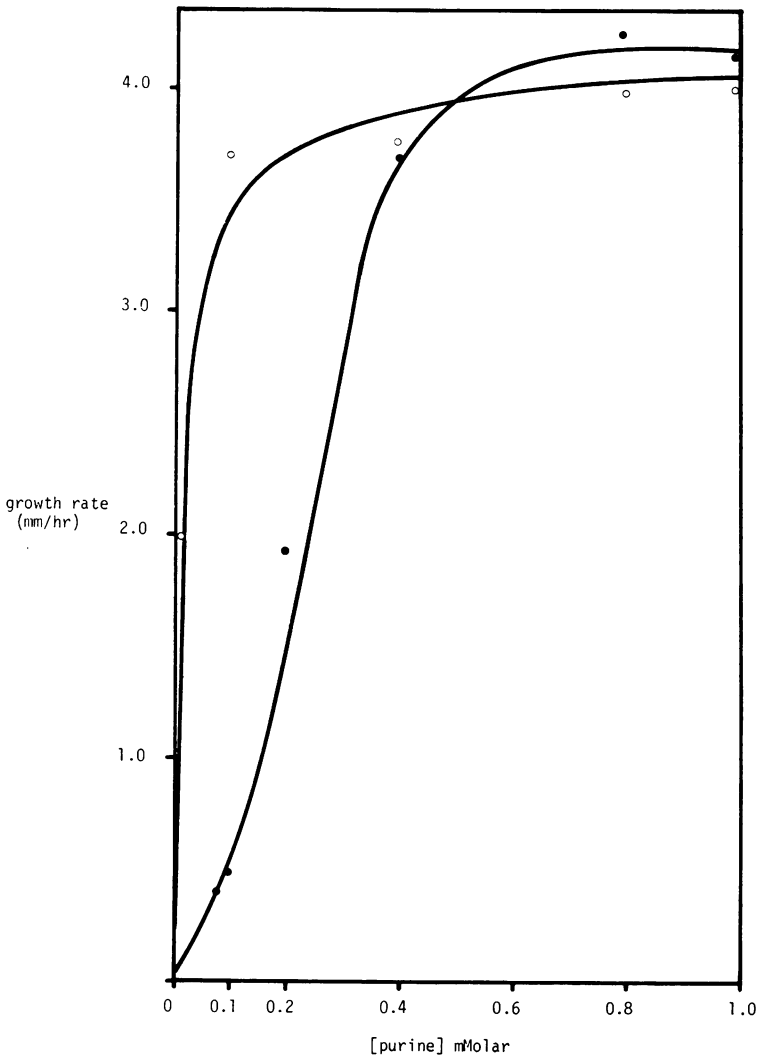

FIG. 1. *Linear growth rates of ad-8 at various adenine (●) or adenosine (○) concentrations.*

TABLE 1. *Rates of growth of ad-8A on minimal media plus adenine or adenosine in the presence and absence of potential inhibitors*[a]

Inhibitor[b]	Growth rate (mm/h)			
	Adenine		Adenosine	
	0.2 mM	0.4 mM	0.01 mM	0.10 mM
None (control)	1.92	3.66	2.20	3.68
Guanine	1.39	3.95	1.68	3.68
Xanthine	1.68	4.01*	2.06	3.56
Hypoxanthine	1.65	3.38	2.27	3.51
Inosine	1.52	3.03**	No growth	[3.55][c]
Guanosine	2.03	3.38	No growth	[3.93][c]
Uridine	2.33	3.49	No growth	[2.91][c]**
Xanthosine	2.25	3.73	2.12	3.50

[a] Growth rates were calculated only on the linear portion of the growth curves, usually between 12 to 84 h after inoculation and incubation at 30 C. In most cases, growth rates were averaged from four replicates, although in some cases three or eight replicates were made. *, Significantly different from the growth rate of the control at 5% probability level, as determined by Dunnett procedure for the comparison of all treatment means with a control. **, Significantly different from the growth rate of the control at the 1% probability level.

[b] Inhibitor concentration was 10 times the concentration of adenine or adenosine in all cases.

[c] Values in parenthesis are growth rates between 40 and 84 h. In these cases no growth was seen for approximately 30 h after inoculation.

TABLE 2. *Rates of growth (mm/h) of pyr-3 on minimal media plus 0.1 mM uridine*

Strain	Growth rate on:		
	0.1 mM uridine	0.1 mM uridine + 1.0 mM adenosine	0.1 mM uridine + 1.0 mM adenine
pyr-3(KS23)[a]	3.45	No growth	3.43

[a] This strain lacks both aspartate transcarbamylase and pyrimidine-specific carbamyl phosphate synthetase activities (6).

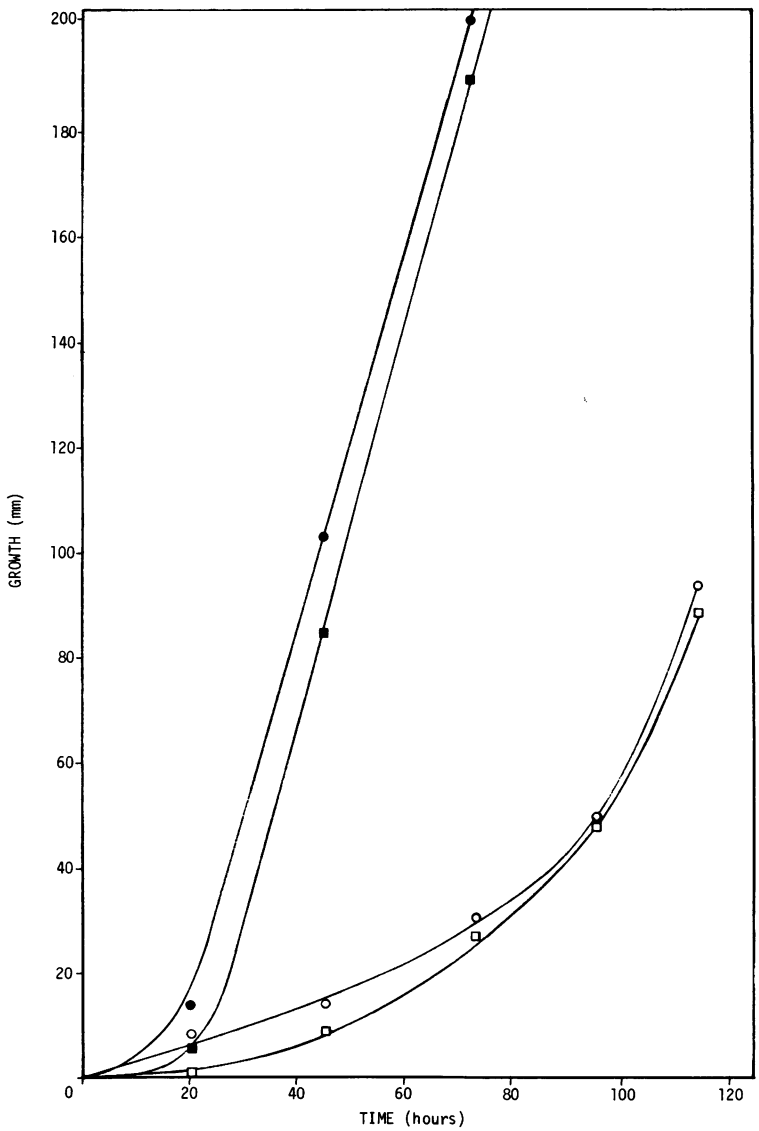

FIG. 2. *Growth curves of ad-8 conidia and mycelium grown on adenosine or adenosine plus inosine. Mycelia* (●) *and conidia* (■) *were taken from 0.1 mM adenosine medium and grown on the same medium. Mycelia* (○) *and conidia* (□) *were taken from 0.1 mM adenosine medium and grown on 0.1 mM adenosine plus 0.5 mM inosine.*

other bases suggests that these compounds do not compete for a common transport system.

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