The Entry of D-Ribose into some Yeasts of the Genus *Pichia*

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SUMMARY

The utilization of D-ribose by yeasts of the genus *Pichia* was examined with respect to aerobic growth, respiration and entry of ribose into the cells. *Pichia etchellsii* (CBS2011) could respire D-ribose, but not use it for aerobic growth. *Pichia fermentans* (CBS187) neither respired nor grew on D-ribose, though it entered the cells of this yeast either by simple diffusion or, possibly, by the D-glucose carrier, this having a very low affinity for D-ribose. *Pichia pinus* (CBS5097) respired and grew on D-ribose; kinetic evidence is given for this yeast having two ribose carriers, one inducible and the other constitutive.

INTRODUCTION

It is remarkable that most yeasts which are able to use D-ribose as a sole source of carbon for aerobic growth can also grow aerobically on ribitol. However, the converse is not true. For example, of 495 strains of species of various genera, examined by N. J. W. Kreger-van Rij and D. Yarrow (cited by Barnett, 1975), none appeared to utilize D-ribose which did not also utilize ribitol: 145 strains were D-ribose +, ribitol +; 233 were D-ribose -, ribitol -; 117 were D-ribose -, ribitol +. This association of the ability of yeasts to utilize D-ribose and ribitol is consistent with the following hypotheses for yeasts in general: (i) the first step in the catabolism of D-ribose is its reduction to ribitol; (ii) D-ribose and ribitol are equally free to enter the cells across the plasmalemma; (iii) hence, they are equally accessible to the appropriate enzymes of catabolism.

To investigate the validity of these generalizations, a few yeasts were selected from each of three categories (Table 1): D-ribose -, ribitol +; positive for both substrates; negative for both substrates. This paper records the first part of an investigation into the utilization of D-ribose by these selected yeasts, which all belong to species of the genus *Pichia*. The main question considered here is whether D-ribose enters yeasts that do not utilize it.

METHODS

Yeasts. These are listed in Table 1.

Media. The following media were used: (i) Difco Bacto YM agar (pH 6.2); (ii) the chemically-defined carbon-free medium (N-base), pH 4.8, of Wickerham & Burton (1948), without amino acids (Barnett & Ingram, 1955), to which different organic compounds were added, each to a final concentration of 50 mM.

Chemicals. Where possible, the chemicals were of analytical grade. Ribitol was recrystallized twice from ethanol, then twice from water by the addition of acetone. Commercial D-ribose was purified through its 'anilide' and recrystallized from ethanol (Whistler &

Table 1. *Aerobic growth of eight yeasts of the genus Pichia on D-ribose or ribitol*

The yeasts were grown in T-tubes, using a recording nephelometer (Barnett, 1968*b*), with 50 mM D-ribose, ribitol or D-glucose as sole source of carbon. Inoculation was from 1-day-old slopes of YM agar. Results with D-glucose are given for comparison.

Pichia species	Taxonomist's assessment*		Specific growth rate (generations/h)†		
	D-Ribose	Ribitol	D-Ribose	Ribitol	D-Glucose
<i>P. farinosa</i> CBS185	+++	+++	0.05	0.1	0.3
<i>P. robertsii</i> ‡ CBS2934	+++	+++	0.03	0.2	0.3
<i>P. pinus</i> CBS5097	+++	+++	0.2	0.2	0.4
<i>P. trehalophila</i> CBS5361	+++	+++	0.2	0.3	0.3
<i>P. etchellsii</i> CBS2011	—	+++	—	0.3	0.4
<i>P. vini</i> CBS4050	—	+++	—	0.07	0.2
<i>P. polymorpha</i> CBS186	+	+	0.1	0.2	0.3
<i>P. fermentans</i> CBS187	—	—	—	—	0.3

+++ , Strong growth; + , weak growth; — , no growth; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.

* Results of Dr N. J. W. Kreger-van Rij (personal communication; for methods see Kreger-van Rij, 1964).

† Mean results of duplicated experiments.

‡ Now called *Wingea robertsii* (van der Walt, 1970).

Bemiller, 1962). The melting points of both ribitol and D-ribose were checked. D-[U-¹⁴C]-glucose (2 to 4 mCi/mmol) and D-[1-¹⁴C]ribose (5 to 10 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Buckinghamshire; their specific activities and purities were not checked.

Rates of growth. For aerobic growth at 25 °C, rocked T-tubes of 40 ml total capacity, containing 15 ml N-base and a source of carbon, were used with a recording nephelometer, calibrated in terms of dry weight (Barnett, 1968*b*). Growth was measured hourly.

Dry weights. Yeast dry weights were measured as described by Barnett (1968*b*). Between 280 and 350 µg dry wt/ml, the dry weight was proportional to the number of cells of *P. pinus*, as counted with a Model A Coulter counter (Coulter Electronic Corp., U.S.A.) (300 µg/ml $\cong$ 17×10^6 cells/ml).

Rates of respiration. Yeast from a 1-day-old YM slope was used to inoculate 100 ml of N-base with an appropriate carbon source, in one or several Pyrex Fernbach culture flasks of 1000 ml total capacity. The flasks were rocked at 1 cycle/s through an angle of about 6° either side of horizontal for 20 h at 25 °C. The contents of each Fernbach flask were centrifuged and the yeast washed and resuspended in 50 ml of 0.1 M-KH₂PO₄. To reduce the endogenous rate of oxygen uptake, the suspension was replaced in a clean Fernbach flask and starved for 3 h at 25 °C; it was then centrifuged and resuspended in 0.1 M-KH₂PO₄ at a final concentration of about 2 mg dry wt/ml. All yeast suspensions were checked visually by phase-contrast microscopy for microbial contamination. Oxygen uptake was measured with Warburg manometers, as described by Barnett (1968*b*).

Rates of entry of D-ribose. The yeast suspensions were prepared as for the respiratory measurements, but starved for only 2 h. The methods of measuring entry of D-ribose were based on those described by Kotyk & Janáček (1970). Yeast suspension (5 to 10 ml, depending on the number of samples to be taken) was incubated in a 25 ml conical flask either at 25 or 30 °C in a water bath, or in an ice and water mixture over an immiscible magnetic stirrer (Rank Brothers, Bottisham, Cambridge) stirred vigorously with a small Teflon-covered magnet in the flask.

Inhibitors, 5 μ M-carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), or 25 μ M- $\text{UO}_2(\text{NO}_3)_2$, when used, were added to the yeast suspension 10 min before zero time. The molarities given are the final concentrations used.

At zero time, D-[1- ^{14}C]ribose (usually at about 1.25 nCi/ μ mol) was added. In certain experiments, ribitol, D-glucose or 2-deoxy-D-glucose were also added.

At timed intervals, usually every minute for 10 min, samples of about 500 μ l (s.d. $\approx \pm 2 \mu$ l) were taken with an Eppendorf piston pipette. Each sample was discharged immediately on to either a Millipore (DAWP 02500) cellulose ester membrane filter, 25 mm diameter, pore size 0.65 μ m, or a Sartorius (SM 11305025) cellulose nitrate filter, pore size 0.6 μ m. The filter was held in a chilled stainless steel holder (Kotyk & Janáček, 1970) on a Buchner flask under reduced pressure. The yeast on the membrane was washed twice with 1 ml ice-cold water. Filtering and washing together took less than 20 s. Each membrane was then transferred to a polyethylene vial and dried immediately in an oven at 80 °C. To each vial and its contents, when cool, were added 15 ml of toluene with 0.7% (w/v) 2,5-diphenyloxazole.

Two adverse criticisms might be made of these techniques for measuring the uptake of D-ribose: entry into washed, starved, resting cells might differ greatly from that into exponentially-growing cells; since changes in radioactivity were measured solely on the yeast itself, kinetic analysis might be vitiated by changes in the proportion of ^{14}C remaining in the yeast as compared with that lost from it. To meet these criticisms, additional uptake experiments were done on cultures growing exponentially, and also the disappearance of radioactivity from the suspending medium was used as a further criterion of uptake.

An exponentially-growing yeast suspension (15 h) was transferred to a flask equipped with a stirrer, in a water bath at 25 °C. Samples were taken from this flask at timed intervals for measuring growth spectrophotometrically. For each measurement of uptake, 10 ml of the suspension were filtered with a Millipore URWP membrane of 25 mm diameter and 1.5 μ m pore size. The yeast was washed on the membrane with 3.5 ml of sugar-free N-base medium at 25 °C. The washed yeast and the membrane filter carried with them about 40 μ l exogenous water. At zero time, a membrane filter and yeast were put into an experimental flask (50 ml Quickfit, B24 stopper) containing 10 ml of N-base at 25 °C to which D-[^{14}C]ribose had been added. The yeast was dispersed into the medium by vigorous mechanical agitation for 15 s, and a Teflon-covered magnetic stirrer put in the experimental flask which was stirred vigorously at 25 °C in a water bath. Samples of about 1 ml were taken, usually at 2 min intervals, for about 14 minutes. The samples were centrifuged with an Eppendorf Microcentrifuge 3200 at about 8000 *g* for about 30 s, and 200 μ l (s.d. $\approx \pm 2 \mu$ l) of the supernatant were added to 15 ml of scintillation fluid (7 g of 2,5-diphenyloxazole in 1 l toluene and 500 ml Triton X-100).

For measurements of loss from the suspending medium, it was only practicable to work with concentrations of from 0.2 to 3 mM D-ribose for a given amount of yeast (about 280 μ g dry wt/ml). With concentrations much below 0.2 mM, the rate of loss fell steadily after incubation for 3 min or less; above 3 mM, the errors of measurement were excessive because too small a proportion of D-ribose was lost from the medium.

In addition to the kinetic constants, the ratio of ^{14}C incorporated into the yeast (net uptake) to loss from the medium was measured for *P. pinus* at 500 and 50 μ M D-ribose. Samples were taken alternately over about 20 min of (i) the supernatant fluid after centrifugation and (ii) the yeast after filtration and washing.

Counting was done with an Intertechnique SL 30 liquid scintillation spectrometer for at

Fig. 1. Time-course for growth of *P. etchellsii* (CBS2011) on D-glucose or ribitol. (a) Direct plot; (b) semi-logarithmic plot. T-tubes contained initially 15 ml of N-base, 0.75 mmol of substrate (as indicated) and about 10 μ g dry wt of yeast from a 1-day-old YM slope. The T-tubes were rocked and incubated at 25 °C and growth was measured hourly with a recording nephelometer. ●, D-Glucose; ■, ribitol; ○, D-ribose or no substrate.

least 10^4 counts or for 100 min. The d.p.m. were calculated from the c.p.m. by a channel ratio method (Glass, 1970). The efficiency of counting was about 93 %.

RESULTS

Growth rates

Rates of aerobic growth, on D-ribose or ribitol, of each strain during the exponential phase are given in Table 1, where they are compared with the results of a taxonomist's qualitative assessment of growth. When growth occurred, the specific growth rate was not associated with that assessment; this was not surprising, since a taxonomist's assessment of growth on different substrates is often based on observations taken over a number of days. However, there was agreement on whether or not each strain utilized D-ribose and ribitol. Growth rates on D-glucose are given for comparison. Figure 1 shows linear and logarithmic plots of the time course of growth of *Pichia etchellsii* on D-glucose or ribitol.

Oxidation of D-ribose and ribitol by suspensions of washed yeast cells

The rates of oxidation of D-ribose, ribitol and D-glucose by four strains of yeast are shown in Table 2. Starvation lowered the endogenous rate to $< 0.3 \mu\text{mol O}_2/\text{mg dry wt yeast/h}$. Those yeasts that grew on D-ribose or ribitol could respire them at rates comparable to that at which they oxidized D-glucose.

Pichia fermentans did not grow on D-ribose or ribitol (Table 1). Nor did it respire them,

Table 2. Rates of respiration on D-ribose, ribitol or D-glucose as substrates by intact cells of four yeasts of the genus *Pichia*

Manometer flasks contained about 4 mg dry wt of washed, starved yeast in 2 ml of 0.1 M-KH₂PO₄ in the main compartments; 20 μ mol of substrate (as indicated) in 0.2 ml of water in the side arms; 0.2 ml of 2 M-KOH with filter paper in each centre well. Incubation was in air at 25 °C and substrates were added at zero time; 20 h cultures were used. The rates are given as μ mol O₂ consumed/mg dry wt yeast/h, uncorrected for endogenous respiration.

Carbon sources in growth medium	Respiratory substrate			Endogenous
	D-Ribose	Ribitol	D-Glucose	
<i>P. fermentans</i> (CBS187)				
Succinate	0.18	0.18	3.8	0.18
Succinate + D-ribose	0.27	0.27	3.2	0.27
Succinate + ribitol	0.27	0.27	3.7	0.22
D-Xylose	0.09	0.09	1.8	0.09
<i>P. etchellsii</i> (CBS2011)				
Succinate	0.22	1.5	3.3	0.13
Succinate + D-ribose	0.40	1.4	2.9	0.22
Citrate	0.31	2.1	3.5	0.13
Citrate + D-ribose	0.63	3.1	4.6	0.13
Ribitol	0.54	2.6	3.3	0.27
<i>P. pinus</i> (CBS5097)				
Succinate	0.33	1.4	6.3	0.25
D-Ribose	3.0	3.5	4.2	0.17
Ribitol	0.29	3.3	2.7	0.24
<i>P. trehalophila</i> (CBS5361)				
Succinate	0.32	0.80	4.5	0.17
D-Ribose	3.5	0.17	4.1	0.15
Ribitol	0.25	3.1	3.0	0.13

even after growth on (i) succinate in the presence of 50 mM D-ribose or ribitol or (ii) D-xylose. Growth on D-xylose was tried because it induces at least two enzymes of ribose and ribitol metabolism in a strain of *Candida utilis* (CBS5947) which utilizes neither compound if supplied exogenously (Barnett, 1968*a, b*).

Pichia etchellsii grew on ribitol but not D-ribose (Table 1). This yeast respired ribitol quite quickly, irrespective of the growth substrate (Table 2). Although oxygen uptake in the presence of D-ribose was low, it is striking that it was appreciably greater than the endogenous rate and, moreover, highly correlated with that for ribitol (+0.85) over nine experiments.

Both *Pichia pinus* and *P. trehalophila* (CBS5361) grew on D-ribose and on ribitol (Table 1). However, these yeasts differed in the inducibility of their utilization of these compounds. With *P. pinus*, only ribose-grown yeast respired D-ribose appreciably, whereas ribitol was respired after growth on succinate, ribose or ribitol. By contrast, *P. trehalophila* respired ribose only when ribose-grown, and respired ribitol only when ribitol-grown (Table 2).

Entry of D-ribose into yeast cells

Table 3 gives the results of measuring the apparent half-saturation constants and maximum velocities of D-ribose entry for *P. pinus* grown on D-ribose or succinate. Estimates of the K_m were between 0.09 and 0.20 mM D-ribose for D-ribose-grown yeast; two estimates for succinate-grown yeast were 3.3 and 4.9 mM D-ribose.

In Fig. 2, the effect of varying concentrations of exogenous D-ribose on the rate of its uptake in *P. pinus* is compared with that in *P. fermentans*, both succinate-grown. The

Table 3. *Measurements of apparent kinetic constants of D-ribose entry into cells of Pichia pinus* (CBS5097)

Yeast grown on	Preparation	Method of measurement	Half-saturation constant, K_m (mM-D-ribose)	Maximum velocity, v_{max} (nmol D-ribose/min/mg dry wt yeast)
D-Ribose	Starved, resting	Net uptake by yeast	$\begin{cases} 0.2 \\ 0.11 \end{cases}$	$\begin{cases} 40 \\ 43 \end{cases}$
D-Ribose	Exponentially growing	Net uptake by yeast	0.11	27
D-Ribose	Exponentially growing	Disappearance from medium	$\begin{cases} 0.11 \\ 0.09 \end{cases}$	$\begin{cases} 56 \\ 40 \end{cases}$
Succinate	Starved, resting	Net uptake by yeast	$\begin{cases} 4.9 \\ 3.3 \end{cases}$	$\begin{cases} 14 \\ 39 \end{cases}$

Fig. 2. D-Ribose uptake at different concentrations of D-ribose by two succinate-grown yeasts. Incubations were in 0.1 M-KH₂PO₄ at 30 °C. (a) Direct (Michaelis-Menten) plots; (b) reciprocal (Lineweaver-Burk) plots. ●, *P. pinus*; ○, *P. fermentans*.

relationship for *P. pinus* shows typical saturation kinetics, whilst that for *P. fermentans* appears to be linear with respect to substrate concentration, at least up to about 32 mM D-ribose. Figure 3 compares reciprocal plots of D-ribose transport by D-ribose-grown *P. pinus*, measured (i) by increase in radioactivity in cells which were collected on membrane-filters or (ii) by disappearance of radioactivity from the suspending medium. For two concentrations of exogenous D-ribose, Fig. 4 compares the results of the two methods, namely loss from the medium and net uptake by the yeast. The ratio of net uptake to loss from the medium was 0.61 for 2.5 mM and 0.60 for 0.25 mM D-ribose.

The effect of change in temperature on the rate of entry of D-ribose into both yeasts is shown as an Arrhenius plot in Fig. 5. With *P. fermentans* the plot is linear between 15 and

Fig. 3. Reciprocal (Lineweaver-Burk) plots of D-ribose uptake by D-ribose-grown *P. pinus* (CBS5097) at different concentrations of D-ribose. Measurements were made of (●) net uptake into starved yeast, and (○) loss from the suspending medium into exponentially-growing yeast.

Fig. 4. Net uptake by yeast (●) and loss from the suspending medium (○) of D-ribose for D-ribose-grown *P. pinus* (CBS5097), with initial exogenous concentrations of D-ribose of (a) 2.5 mM, and (b) 0.25 mM.

35 °C, whereas that for *P. pinus* may be interpreted as showing a 'break' (cf. Kumamoto, Raison & Lyons, 1971), with a maximum at between 25 and 35 °C. Heller, Winter & Höfer (1974) obtained comparable results for D-xylose transport into *Rhodospiridium toruloides* (*Rhodotorula gracilis*). Table 4 gives the corresponding apparent energies of activation (for non-saturating conditions) and values for Q_{10} .

Measurements were made of the relative rates of uptake of D-ribose into succinate- and D-ribose-grown *P. pinus*, and into succinate-grown *P. fermentans*. These rates, at 0 and 30 °C and in the presence of various potential inhibitors, are given in Table 5. The effects of these on *P. fermentans* are shown in Fig. 6.

Fig. 5. Arrhenius plots showing effects of change of temperature on rate of entry of D-ribose into two succinate-grown yeasts: ●, *P. pinus* CBS5097; ○, *P. fermentans* CBS187. Incubations were in 6 ml of 0.1 M-KH₂PO₄. At zero time for each incubation, 20 μmol of D-[¹⁴C]ribose were added, and 0.5 ml samples were taken every minute for 10 min.

Fig. 6. Effects of inhibitors on the rates of uptake of D-ribose by succinate-grown *P. fermentans* (CBS187). Initial concentrations were D-[¹⁴C]ribose, 1.7 mM; D-glucose and 2-deoxy-D-glucose, 17 mM; CCCP, 1.0 mM. ●, No inhibitor; ■, CCCP; □, D-glucose; ○, 2-deoxy-D-glucose.

Table 4. Values for Q_{10} and E_a , for entry of 25 mM D-ribose into succinate-grown yeasts

Incubations were in 0.1 M-KH₂PO₄, using 25 ml conical flasks in a shaking water bath, at various temperatures (every 5 ± 0.2 °C, from 10 to 35 °C).

	Q_{10}	E_a (J/°C/mol)
<i>P. fermentans</i> (CBS187)	1.5	2190
<i>P. pinus</i> (CBS5097)	2.5	6590

Table 5. Relative rates of uptake of D-[¹⁴C]ribose or D-[¹⁴C]glucose in the presence of various inhibitors

Initial concentrations: D-[¹⁴C]ribose and D-[¹⁴C]glucose, 1.7 mM; sugars and ribitol as inhibitors, 17 mM; CCCP, 1.0 mM; uranyl nitrate, 25 µM. Absolute rate of entry of D-glucose into *P. fermentans* at 30 °C: 18 nmol D-glucose/mg dry wt yeast/min.

Incubation temperature	Inhibitor	<i>P. pinus</i> (CBS5097)		<i>P. fermentans</i> (CBS187) Succinate-grown	
		D-Ribose-grown D-Ribose uptake	Succinate-grown D-Ribose uptake	D-Ribose uptake	D-Glucose uptake
0 °C	None	0	5	40	NT
30 °C	None	100	100	100	100
	2-Deoxy-D-glucose	89	90	5	32
	2-Deoxy-D-ribose	89	84	NT	98
	Ribitol	69	84	100	96
	D-Glucose	101	94	20	—
	D-Ribose	—	—	—	98
	CCCP	1	NT	40	18
	Uranyl nitrate	100	NT	NT	NT

NT, Not tested.

DISCUSSION

Neither *P. fermentans* nor *P. etchellsii* utilized D-ribose for aerobic growth, though the sugar appeared to enter their cells. The results of studying the entry of D-ribose into *P. fermentans* are open to alternative interpretations.

(i) Since over a wide range of concentrations, the rate of uptake of exogenous ribose was directly proportional to the external concentrations of the sugar (Fig. 2*a*), entry could occur by simple diffusion. This explanation was given by Herzenberg (1959) for his analogous results with cryptic mutants of *Escherichia coli* with respect to their uptake of *o*-nitrophenyl β-D-galactopyranoside. The effects of change of temperature on entry (Fig. 5), with a low Q_{10} (1.5) and an apparent activation energy (E_a) of 2190 J/°C/mol (Table 4), are also consistent with entry by simple diffusion (cf. Stein, 1967). This is how Horák & Kotyk (1969) interpreted the results of their experiments on the uptake of D-ribose by a strain of *Rhodospiridium toruloides* (*Rhodotorula gracilis*). However, there were some indications from their results (e.g. the effect of 2,4-dinitrophenol on uptake) that this process might not be fully explicable in terms of simple diffusion.

(ii) An alternative interpretation of the apparently linear plot for *P. fermentans* in Fig. 2(*a*), as well as for the results with *R. toruloides* (Horák & Kotyk, 1969), is that this line could correspond to the lower part of a rectangular hyperbola, representing the activity of a carrier-mediated system with a very low affinity for D-ribose, although this view is not

borne out by the reciprocal plot in Fig. 2(b). Accordingly, in view of the obvious possibility that D-ribose might enter by means of a constitutive carrier with a high affinity for another sugar, some experiments were done on the transport of D-glucose, for comparison. Indeed, the effects of various inhibitors on the rate of entry into *P. fermentans* of D-ribose, on the one hand, and of D-glucose on the other (Table 5), were consistent with the suggestion that ribose entered by means of the glucose carrier. This conclusion accords with the findings of Heredia, Sols & Delafuente (1968) for *Saccharomyces cerevisiae* with respect to D-ribose and a number of non-metabolizable sugars (for review, see Barnett, 1975).

Table 5 compares the effects of certain possible inhibitors on the uptake of D-ribose by *P. pinus* and *P. fermentans*, as well as of D-glucose by succinate-grown *P. fermentans*. With *P. pinus*, D-glucose hardly affected D-ribose uptake, so the two sugars probably enter by different carriers. Furthermore, uptake was unaffected by uranyl ions, which are fast-acting inhibitors of sugar uptake by yeasts, including facilitated diffusion (Rothstein, 1954; Cirillo, 1961*a, b*). CCCP uncouples oxidative phosphorylation, inhibiting active transport of sugars in some bacteria (for review, see Cirillo, 1972); therefore its inhibition of uptake (almost total for *P. pinus* and partial for *P. fermentans*) suggests that metabolic energy is involved in the uptake of D-ribose by both yeasts. The action of ribitol in inhibiting uptake by *P. pinus* might at least partly be due to it affecting the metabolism of D-ribose. However, should this action prove instead to be competitive inhibition of transport, it will be pertinent to ask in what molecular form D-ribose crosses the plasmalemma of *P. pinus*. Since ribitol is acyclic, possibly it is *aldehydo*-D-ribose that enters (Barnett, 1975). The effect of the inhibitors on *P. fermentans* was immediate (Fig. 6), so they could have acted directly on the carrier of D-ribose. The action of inhibitors on the uptake of D-ribose by *P. fermentans*, and the similarity of this action to that on uptake of D-glucose, are evidence that carrier-mediated uptake of D-ribose occurs in this yeast by means of a D-glucose carrier.

Since D-ribose was catabolized by *P. pinus*, experiments were done to find out whether the measurements of kinetic constants of uptake were confuted by variations in the proportion of ^{14}C lost as CO_2 . Accordingly, D-ribose-grown yeast was studied, because it utilized D-ribose fastest and so would be most likely to give the highest variations. However, the different estimates of K_m were similar (90 to 200 μM , Table 3), whether based on measurements of net uptake of D-ribose or on its disappearance from the medium. Furthermore, the ratios of the rates of loss from the medium to those of net uptake by the yeast (Fig. 4) were similar for exogenous concentrations of 50 and 500 μM D-ribose. Hence the results of the present experiments on the kinetics of D-ribose uptake were probably not invalidated by changes in the proportion of sugar metabolized when its exogenous concentration was varied. On the other hand this might possibly be true for differences associated with changes in temperature, measured with succinate-grown *P. pinus*.

Unlike *P. fermentans*, *P. etchellsii* utilizes ribitol for growth, though not D-ribose (Kreger-van Rij, 1970; and see Table 1). In addition, Table 2 shows that *P. etchellsii* respire D-ribose slowly, and that this rate of respiration by cells grown on citrate in the presence of D-ribose is double that for those grown on citrate alone. This combination of characteristics – ability to grow on and respire ribitol, and inability to grow on but ability to respire D-ribose – cannot be explained simply in terms of conventional concepts of ribose and ribitol catabolism (for review, see Barnett, 1968*a*, 1975):

or

Haškovec & Kotyk (1973) reported an identical observation on *Torulopsis candida*, but Barnett (1968*b*) found that the same strain would grow on ribose. *Pichia etchellsii* is being investigated further.

The estimates of E_a for D-ribose entry into succinate-grown yeast were higher for *P. pinus* than for *P. fermentans* (Table 4). These observations are consistent with the view that for *P. pinus* the rate-determining step of entry involved binding or release. The different values for K_m and v_{max} with succinate- and D-ribose-grown *P. pinus* (Table 3) are evidence of there being two carriers (or sites) for D-ribose entry, one of them inducible. The non-induced carrier in *P. pinus* differs markedly from that of *P. fermentans*, in exhibiting saturation-kinetics (Fig. 2), in its temperature relations (Fig. 5 and Table 4) and in its reactions to inhibitors (Table 5).

Ribitol never clearly induced the utilization of D-ribose (Table 2), though *P. etchellsii* may have been an exception. In *P. pinus*, ribitol induced the respiration of ribitol, and ribose induced both ribose and ribitol utilization, whereas in *P. trehalophila*, ribose utilization was induced only in D-ribose-grown yeast and ribitol respiration only in ribitol-grown yeast.

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