# Transport of D-Xylose and Sugar Space in Baker's Yeast

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ABSTRACT

The cell volume fraction of *Saccharomyces cerevisiae* accessible to D-xylose and other nonmetabolized sugars was found to correspond to 100% of cell water in resting yeast over a range of osmolarities of the medium. This sugar space was decreased to 60% by nitrogen deficiency and to 82 % by phosphorus deficiency. The space of N-deficient cells was significantly raised by pre-incubation with ammonium chloride, amino-acids and nucleotides from the yeast extract and by adenosine triphosphate while that of P-deficient cells was not significantly altered by analogous treatment. The xylose space was found to be markedly lower in growing than in resting cells. Urea (0.3—6%) had a depressing effect on the sugar space size while a variety of metabolic inhibitors were without influence. The $K_m$ and $V$ values of D-xylose uptake by *Saccharomyces cerevisiae R XII* at 30° were found to be 180—192 mM and 680—730 mg. xylose/ml. cell volume/hr., respectively. These values were not affected by N-deficiency and by enrichment with adenosine triphosphate.

The xylose space size was shown to be practically identical with that of urea space and its decrease was related to the increase of "bound" water within the cell under various conditions studied. The changes of space are tentatively attributed to changes of hydration and dehydration of cellular components.

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The mechanism of uptake of sugars by the yeast cell has been satisfactorily explained by presenting extensive evidence on the following points: (1) sugars entering the cell are not phosphorylated (Burger, Hejmová & Kleinzeller, 1958, 1959; Cirillo, 1959; Kotyk, 1961); (2) competition exists between different sugars for the transport into the yeast cell (Burger *et al.* 1959; Cirillo, 1961a, 1962); (3) the transport is markedly stereospecific, a sugar with the D-configuration being transported by some yeast strains and that with the L-configuration not entering the cell (Cirillo, 1961b); (4) a counterflow of sugars against the concentration gradient can be brought about by adding a sugar with high affinity for the transport mechanism to a suspension previously equilibrated with another sugar (Burger *et al.* 1959; Cirillo, 1961a). The above evidence is in agreement with the mobile-carrier hypothesis as advanced by LeFevre (1948, 1954) and developed by Rosenberg and Wilbrandt (1952) and Widdas (1952, 1954) for erythrocytes. Numerous authors have recently demonstrated a similar mechanism in other types of cells, such as tumour cells (Crane, Field & Cori, 1957; Nirenberg & Hogg, 1956, 1958), striated muscle and heart muscle (e.g. Post, Morgan & Park, 1961; Morgan, Post & Park, 1961) as well as bacteria (e.g. Horecker *et al.* 1961).

It is tacitly assumed that nonfermentable sugars reach an equilibrium level in the cell, corresponding to a diffusion equilibrium, in other words, that the intracellular concentration of the sugar is then equal to that in the medium. This assumption is justified by the kinetics of

sugar transport and by its lack of metabolic energy requirements (see Results). If the sugar does not distribute throughout the entire cell volume the volume fraction corresponding to distribution according to diffusion equilibrium is then regarded as the sugar free space (Conway & Downey, 1950). However, the size and character of the space in which non-metabolized sugars are distributed has not been explicitly determined so far. Moreover, there seems to be no uniformity with regard to the reference volume for the free space. In yeast cells it is often referred to packed-cell volume (Conway & Downey, 1950; Cirillo, 1961b) which is not sufficiently accurate or else to the dry weight of yeast (Burger *et al.* 1959) which requires further knowledge of the ratio of fresh to dry volume of the cell. In the present paper use is made of measurements of the cell volume under various conditions and it is suggested (for reasons to be expounded in the Results) that the intracellular water volume be set equal to the potentially free space for sugars; if a sugar distributes throughout the intracellular water volume and reaches diffusion equilibrium with the external medium it is said to occupy 100% space.

For characterizing the cell volume accessible to sugars and for defining its size D-xylose was used as it is not metabolized by baker's yeast and in most strains of *Saccharomyces cerevisiae* attains a 100% distribution in the cell-water volume. Various factors bearing on the size of the accessible space were investigated.

## MATERIALS AND METHODS

*Microorganism and its cultivation.* Three production strains of *Saccharomyces cerevisiae* and the collection strain *R XII* were used, all the fundamental experiments being carried out with the *R XII* strain. It was maintained on wort agar slopes and propagated aerobically on a shaker at 30° in a liquid synthetic medium of the following composition: glucose 10.0 g.; urea 3.14 g.; $KH_2PO_4$ 5.2 g.; $MgSO_4 . 7H_2O$ 0.25 g.; sodium citrate 1.0 g.; $ZnSO_4 . 7H_2O$ 1.05 mg.; $CuSO_4 . 5H_2O$ 0.1 mg.; biotin 0.02 mg.; calcium pantothenate 0.50 mg.; pyridoxine 1.2 mg.; thiamine 4.4 mg.; water to 1 l. The final pH was 5.6.

After 18 hrs. the suspension was centrifuged, washed and the pellet left overnight at +3° C. Deficient cells were prepared by 6-hour growth in the above medium from which either urea (N-deficient) or phosphate (P-deficient) was omitted. After centrifugation and washing the pellet was again left overnight. The contents of the respective elements after such precultivation are shown in Table 1.

Table 1. Contents of elements in yeast cells impoverished by precultivation in deficient media (in mg./g. dry weight, averages from 8 determinations)

| Cell type | N | P |
|---|---|---|
| Normal | 78.1 | 13.4 |
| N-deficient | 54.0 | 12.0 |
| P-deficient | 69.8 | 9.9 |

*Synchronized cells* were prepared according to Nosoh and Takamiya (1962); *protoplasts* were prepared according to de Kloet, van Wermeskerken and Koningsberger (1961).

*Incubation and removal of samples.* Suspensions of cells were incubated in a water-bath and bubbled with air or oxygen-free nitrogen, depending on the character of the experiment (the density of cells was 5—10 mg. dry weight/ml.). After adding D-xylose 1 ml. samples were removed at various time intervals, filtered through a Millipore filter (HA, 0.45 μ pore size) fitted in an ice-cold stainless--steel funnel, washed twice with 1 ml. of ice-cold water and the pellet washed down from the filter into a test-tube. The washed cells in suspension were boiled for 20 min. to extract the intracellular D-xylose, the suspension centrifuged and

an aliquot of the supernatant used for the determination of pentoses by the orcinol method (Umbreit, Burris & Stauffer, 1957).

*Isotopic experiments.* $^{14}$C-Xylose was used in some experiments where interference of other pentoses might occur (when applying ATP, ADP etc). Radioactivity was measured on aluminium planchets using a Frieseke-Hoepfner counter with thin end-window Geiger-Müller tubes.

In the experiments where $^{14}$C-urea space was measured incubation was stopped by filtering a suspension sample through a silicone oil layer into 10 % trichloroacetic acid, as described by Werkheiser and Bartley (1956). Radioactivity was measured in an aliquot of the trichloroacetic acid layer in a Tracerlab liquid scintillation counter. The space was expressed as above for xylose.

*Dry weight and volume of yeast.* Samples of the suspension were used for determining the dry weight (6 hrs. at 105° C) and the cell volume (specially calibrated haematocrit tubes, 1300 ***g***, 15 min.). The aqueous volume was calculated from the values obtained, taking the density of the yeast dry matter to be 1.1 (as determined pyknometrically) and subtracting the extracellular volume estimated as inulin space. The amount of bound, osmotically inactive water was assessed by plasmolyzing the cells for 30 min. with a saturated sodium chloride solution and observing the amount of water lost from the cells, the water remaining in cells being considered, with due reserve, as immobile.

*Kinetics of D-xylose uptake.* The maximum rate of xylose uptake ($V$) and the apparent $K_m$ of the process were determined from the initial rates of uptake at various external concentrations and compared with rates of exit from xylose-saturated cells into distilled water.

The uptake or loss of D-xylose against known concentrations of the sugar could not be analyzed with the aid of the simplification suggested by Wilbrandt (1961) because the $K_m$ of the process is so high that it could not be neglected as compared with either the internal or the external concentrations practically applicable.

All the sugars and reagents used were of analytical purity.

## RESULTS

Factors bearing on the size of the cell space accessible to xylose tested here were of three general types: (1) physico-chemical, viz. osmolarity of the medium, pH and temperature; (2) chemical, viz. the presence of different components in the medium; (3) physiological, viz. nutritional states and phase of the growth cycle.

### Physico-chemical factors

*Osmolarity of the medium.* The suspension of resting cells was prepared in NaCl concentrations between 0.1M and 2.0M and incubated at 30° C with 2% xylose. Fig. 1 shows that the changes in xylose space exactly parallel those in the cell-water volume, the distribution of xylose being always very nearly 100% of the cell-water volume. This is taken as

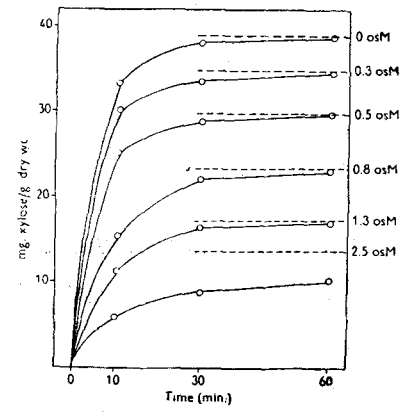


Fig. 1. Distribution of D-xylose in baker's yeast at different osmolarities of NaCl. Incubation with 1.8% xylose, aerobically at 30° C. The dotted lines correspond to 100% distribution in cell-water volume at the given osmolarities.

a strong indication that D-xylose can distribute throughout the entire cell water volume unless prevented by some of the factors studied here and henceforth the percentage xylose space will always be referred to the cell-water volume as being equal to 100%.

The lower xylose distribution at the highest NaCl concentration used is very likely due to cell rupture and denaturation of proteins then taking place.

Yeast protoplasts were used to check the results and here, too, the distribution of xylose was in 100% of the cell-water volume.

*The pH value.* No effects on xylose space were observed when using potassium phthalate and tris buffers in their respective ranges between pH 3.0 and 9.0.

*Temperature.* Yeast strains differed in their response to temperature changes. Two of them (among them the *R XII* collection strain) were found to have the xylose space unaltered by temperature while particularly in one of the production strains maximum distribution of xylose could not be attained below 30° C. The behaviour is shown in Fig. 2, where also the decrease in xylose space on prolonged incubation may be observed (this is not due to death of cells).

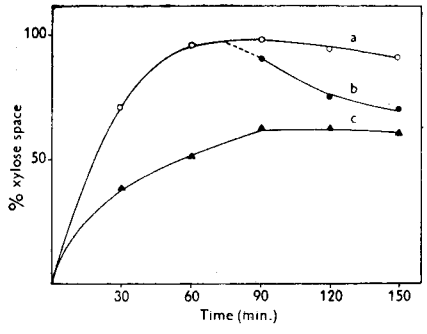


Fig. 2. The effect of temperature on the distribution of D-xylose in a production strain of baker's yeast. Incubation with 1% xylose, aerobically, in distilled water. *a* — 30° C; *b* — 30° C, after 75 min. switched to 15° C; *c* — 15° C.

## Chemical factors

Various metabolic inhibitors were examined with respect to their effect on xylose space in yeast. 2,4-Dinitrophenol ($5\times10^{-4}$ and $10^{-3}$M), sodium iodoacetate ($5\times10^{-4}$M), potassium cyanide ($10^{-4}$M), phloridzin ($5\times10^{-4}$M) and oligomycin (0.2/μg./ml.) were found to have no effect whatever.

On the other hand, urea, which is the source of nitrogen in some cultivation media, displayed an appreciable effect on the size of the xylose distribution space (Table 2).

Table 2. The effect of urea on xylose distribution space in yeast (30° C, aerobic conditions, 1% xylose)

| Concentration of urea, mM | Xylose space, % |
|---|---|
| 0 | 98 |
| 5 | 98 |
| 50 | 91 |
| 500 | 69 |
| 1000 | 64 |

Urea does not compete with xylose for the carrier and its effect is thus solely on the final distribution level. A conjectural explanation might be that urea brings about local changes in protein hydration by virtue of its denaturing capacity.

Other sugars competing for the carrier with D-xylose such as D-galactose and L-arabinose, used in concentrations commensurate to that of D-xylose decreased the rate of uptake of xylose but not its final level, which shows that their effect is only on the carrier but not on the space of distribution.

## Physiological factors

Yeast cells deficient in N and P displayed a generally decreased xylose space as shown in Table 3.

Factors were then investigated that could restitute the original 100% space in such deficient yeast. It may be seen

Table 3. The effect of cultivation conditions on xylose space in baker's yeast (pre-incubation took place at 30° C for 30 min., aerobically)

| Defective element | Pre-incubated with | Xylose space % ± S. E. | Number of determinations |
|---|---|---|---|
| None | — | 96.7 ± 2.1 | 14 |
| N | — | 60.3 ± 3.7 | 14 |
| | 0.1M-$NH_4Cl$ | 74.4 ± 3.8 | 5 |
| | 1.5mM ATP | 80.3 ± 3.6 | 5 |
| | 1.5mM ADP | 62.3 ± 2.1 | 5 |
| | Nucleotides from yeast extract | 84.8 ± 4.3 | 5 |
| | Amino-acids from yeast extract | 72.5 ± 6.1 | 3 |
| P | — | 82.0 ± 2.2 | 8 |
| | 0.1M-$KH_2PO_4$ + 1% glucose | 82.5 ± 3.8 | 5 |
| | 1.5mM ATP | 85.6 ± 3.9 | 5 |
| | Nucleotides from yeast extract | 85.4 ± 3.9 | 5 |
| Protoplasts from normal cells | | 99.1 ± 3.0 | 6 |
| Protoplasts from N-deficient cells | | 66 | 2 |
| Anaerobically grown yeast | | 98.5 | 2 |

from Table 3 that a 30-minute pre-incubation with 0.1M-$NH_4Cl$ amino-acids from the yeast extract, nucleotides from the yeast extract and adenosine triphosphate alone could significantly raise ($p < 0.01$) the xylose space of N-deficient yeast. Adenosine diphosphate was ineffective in this respect. It should be pointed out here that the cell volume was not appreciably affected by any of these procedures.

A dual effect suggests itself here. Ammonium chloride and amino-acids apparently merely replenish the nitrogen pool of the cell while the increase brought about by the nucleotides might be more directly associated with the chemical state (apparently hydration) of cell components (no detectable rise in N content was observed on pre-incubation with the nucleotides, in contrast with that in the presence of ammonium chloride and amino-acids). When ATP or $NH_4Cl$ were added to the cells in the presence of 0.2mM chloramphenicol their effect was unaltered, this suggesting that we are not dealing here with a synthesis of proteins *de novo*.

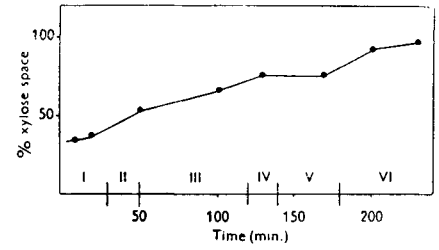


Fig. 3. Changes in the xylose space of yeast during a single growth cycle and in resting cells. Incubated at 30° C in the medium described in Methods. Abscissa: time in min.; ordinate: % xylose space. *I* - Induction phase (during this phase the very low xylose space observed was accompanied by a decreased and somewhat anomalous rate of uptake). *II* - budding, *III* - bud maturation, *IV* - bud separation, *V* - aeration in $H_2O$ at 30° C, *VI* - centrifuged pellet at 4° C.

In P-deficient yeast neither ATP nor potassium phosphate with 1% glucose effected any rise in the xylose space, the inference being that the changes in P-deficient yeast are of a different character.

The possible effect on xylose space of the growth stage of cells was tested in a synchronized culture of *Saccharomyces cerevisiae R XII*. The changes observed are recorded in Fig. 3.

The findings indicate that in growing yeast generally the entire water volume of the cell is not available for solution but rather that there is a considerable part of cell water which is not accessible to hydrophilic solutes.

## Kinetic aspects of D-xylose uptake

The substantial differences between the D-xylose space of normal and N-deficient yeast raised the question whether the maximum rate of uptake and the apparent Michaelis constant of the process differed in those two types of yeast. It was found that the uptake of xylose by fresh cells displayed the same $K_m$ in normal, N-deficient and ATP-enriched cells. The maximum rates of xylose uptake were also the same in the three types of yeast (Table 4). The respective values of $K_m$ and $V$ for the release of D-xylose from the cells were identical with those for the uptake.

## Urea space

In order to elucidate whether the variability of xylose space is due to non-accessibility of some of the cell water to xylose (and other sugars) as opposed to other solutes it was considered useful to apply a substance which would be water-soluble and nontoxic for the cell and which would penetrate through the cell membrane by free diffusion. Urea labelled with $^{14}C$ was used for this purpose (its total concentration was less than 0.5 mM) and it was found that it distributes in the various types of cells in a volume closely resembling that of xylose distribution (Table 5).

## "Free" vs. "bound" water of yeast cells

Since it was shown that urea occupies a cell space comparable to that of xylose the explanation suggested itself that

Table. 4. Apparent $K_m$ and $V$ of D-xylose uptake by *Saccharomyces cerevisiae R XII*

| Type of yeast | $K_m$ (mM) | $V$ (mg. xylose/ml. cell/hr.) |
|---|---|---|
| Normal | 180—184 | 680—710 |
| N-deficient | 180—190 | 690—730 |
| ATP-enriched | 182—192 | 690—730 |

Table 5. Distribution of urea in various types of yeast cells (in % cell water)

| Experiment no. | Type of yeast | Urea space | Xylose space |
|---|---|---|---|
| 1 | Normal, resting | 95 | — |
| | N-deficient, resting | 69 | — |
| 2 | Normal, resting | > 100 | 100 |
| | Normal, growing (bud separation phase) | 79 | 80 |

under various conditions different portions of cell water are available for solutes. Therefore, the amount of this "free" water was determined in the cells, the results being shown in Table 6.

Table 6. "Free" and "bound" water in yeast cells

| Type of cell | Cell water, in % | |
|---|---|---|
| | Free | Bound |
| Normal resting cells | 86 | 14 |
| N-deficient cells | 59 | 41 |
| ATP-enriched cells | 65 | 35 |
| Synchronized cells from the bud-maturation phase *Saccharomyces cerevisiae R XII* cells growing on beer wort* | 60 | 40 |
| | 67 | 33 |

* Values from the paper of Kalyuzhnyi (1963)

Even if the method of determining the amount of "bound" water is by no means free of considerable approximations the results indicate a general agreement between the xylose or urea space and the amount of free cell water.

## DISCUSSION

The results shown indicate that a great variety of factors bear on the size of the sugar space in yeast. Several explanations might be advanced to account for the decreased sugar space.

(1) The efficiency of the sugar carrier is greater in the direction out of the cell; this could be simulated by a substance entering the cell together with xylose and consumed inside (e.g. glucose) but this possibility can be ruled out by the arrangement of the incubation experiment. Moreover, kinetic measurements show that the $K_m$ and $V$ of the transport process are equal in both directions.

(2) There are discrete spaces within the cell which become unaccessible to the solute under certain conditions. This again seems to be unlikely for a variety of reasons: (*a*) the changes are not step-like but rather gradual, depending e.g. on the length of treatment, even if this by itself is not a rigorous refutation of the possibility mentioned; (*b*) yeast cell protoplasts also exhibit a 100% xylose space although the cell wall and the "space" between it and the protoplasmic membrane are missing in them; (*c*) the absence of mitochondria in anaerobically grown yeast (Linnane, Vitols & Nowland, 1962) does not affect the xylose space in any respect.

(3) Changes in hydration of cell components are brought about by the various types of treatment. This would result in different amounts of water being fixed in an osmotically immobile form as was actually indicated experimentally. The only classes of cell components that are present in amounts sufficient to account for the great changes observed are proteins and polysaccharides and it is therefore tentatively inferred that the changes described above reflect changes in the hydration of cell proteins and polysaccharides. Whether this is limited to certain types of protein and/or polysaccharide localized at definite sites in the protoplasm might possibly be decided by applying electron microscopy to $^{3}H$-histoautoradiograms. Histoautoradiograms obtained here with $^{14}C$-labelled xylose and studied under the optical microscope were not sufficiently distinct to discern any details. The possibility of the hydration of cellular components affecting the sugar space will have to be examined further.

The values of sugar space and the rate of uptake reported above deserve of being compared with analogous data obtained in other types of cells or with different sugars. The 100% sugar space is equal to that found in baker's yeast by Cirillo (1961b) and, after corrections for cell volume contraction, to that for galactose in baker's yeast by Burger *et al.* (1959), in ascites tumour cells by Crane, Field and Cori (1957), in erythrocytes by Lacko *et al.* (1961) and in tissue culture L-cells

(in the absence of active transport) by Rickenberg and Maio (1961).

In rat diaphragm and muscle generally, however, D-xylose was found to be distributed to the maximum of 30% intracellular water (Kipnis & Cori, 1959).

No data on the $K_m$ and $V$ of xylose penetration have been found in the literature on baker's yeast. The $V$ for glucose fermentation is given by Sols and de la Fuente (1961) as about 250 mg. glucose/g. yeast/hr.; from the data of Stickland (1956) the $V$ for anaerobic glucose uptake can be assessed as 600 mg. glucose/ml. cells/hr., which is in agreement with the value found by Scharff and Kremer (1962) and in this laboratory. It will be seen that these values are much lower than those found in human erythrocytes by Wilbrandt (1961), viz. 5.4 g./ml. cells/hr. Conversely the $K_m$ for D-xylose in erythrocytes was found to be 21 mM, much lower than in the yeast. An interesting comparison can be made with respect to the $V$ for xylose and glucose. The value is lower for glucose both in yeast (3.3 m-moles glucose vs. 4.5 m-moles D-xylose/ml./hr.) and in erythrocytes (31 m-moles glucose vs. 51 m-moles D-xylose/ml./hr.).

The variability of the yeast cell volume accessible to transported, but nonmetabolized, sugars is a phenomenon which should be taken into account insofar as the kinetic investigation of sugar uptake by cells has so far disregarded the effect of the sugar space changes on the equilibrium level reached.

Conversely, provided electron-microscope histoautoradiography should prove feasible it could furnish a method for distinguishing between the true water for solution and other parts of the cell.

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## ТРАНСПОРТ D-КСИЛОЗЫ И РАСПРОСТРАНЕНИЕ САХАРОВ В ПЕКАРСКИХ ДРОЖЖАХ

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Участок объема клетки дрожжей Saccharomyces cerevisiae, доступный для D-ксилозы и других не подвергающихся обмену сахаров, отвечает 100% клеточной воды у дрожжей в сосотянии покоя в пределах широкого спектра осмолярности среды. При недостатке азота этот участок уменьшался до 60%, а при недостатке фосфора — до 80%. У N-дефицитных клеток это пространство можно было существенно увеличить с помощью предварительной инкубации с хлористым аммонием, аминокислотами и нуклеотидами из дрожжевого экстракта, а также с аденозинтрифосфатом, тогда как на P-дефицитные клетки подобные вмешательства не оказывали действия. У растущих клеток ксилозодоступное пространство заметно меньше, чем у клеток в покое. Обработка мочевиной (0,3—6,0%) уменьшала размеры ксилозодоступного пространства, тогда как различные ингибиторы метаболизма не оказывали влияния. Величины $K_m$ и V для включения D-ксилозы клетками Saccharomyces cerevisiae R XII составляют 180—192 мМ или же 680—730 мг ксилозы/мл клеточного объема/час. На эти величины не оказывали действия ни недостаток азота, ни обогащение аденозинтрифосфатом. Было установлено, что размеры ксилозодоступного пространства практически сходны с размерами пространства, доступного для проникновения мочевины, и что их уменьшение находится в связи с увеличением содержания воды, связанной в клетках при различных условиях опыта. — Изменения этого пространства предположительно относятся за счет изменений в гидратации и дегидратации белков.