Properties of the Sugar Carrier in Baker's Yeast

II. Specificity of Transport*

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ABSTRACT. A total of 16 hexoses and pentoses were investigated with respect to transport into *Saccharomyces cerevisiae* cells. All monosaccharides were transported across the cytoplasmic membrane but only those with an equatorial hydroxyl group in positions 1 and 4 of the *C1* chair conformation and those with an equatorial hydroxyl group in position 2 and an equatorial $-CH_2OH$ group in position 5 of the *1C* chair conformation reached an equilibrium distribution in the entire cell water volume. Other monosaccharides reached a distribution in only 20—66% of the intracellular water. The two groups of sugars are apparently transported by different carriers (either in parallel or in series), each of them showing countertransport and an apparent activation energy of 6,700—7,800 cal/mol.

The carrier transporting the perfectly distributing sugars (Group 1) is affected by uranyl nitrate but not by 2,4-dinitrophenol, the other carrier (Group 2) is apparently not susceptible to uranyl ions but is influenced by 2,4-dinitrophenol. The space of distribution of the Group 1 sugars is reduced in hypertonic media in accordance with changes of intracellular water, that of the Group 2 sugars is altered only very slightly. The carriers differ in their kinetic parametres (mobility of the loaded carriers, maximum rate of transport). There is only a very indistinct competition for transport between representatives of the two groups. Preincubation with D-galactose induces the formation or unmasking of a transport system whereafter even the Group 2 sugars reach equilibrium in the entire cell water.

Although a number of papers have been published that deal with monosaccharide transport in baker's yeast (for a review see Cirillo, 1961) little systematic study has been done in the field of specificity of the sugar-transporting system. Suggestions of structural specificity of sugar transport by baker's yeast can be found in Cirillo (1967) and Sols (1967). We considered it useful to establish whether there is any structural feature of the sugar molecule governing the affinity and the capacity of monosaccharide transport across the cell membrane of *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Yeast. In most experiments described here we used the *R XII* strain of *Saccharomyces cerevisiae* from the collection of this Institute. The qualitative properties of this strain with respect to sugar transport were found to be identical with those of the distillery yeast produced at Kolín and in some cases (only for verification's sake) this yeast was used. The *R XII* strain was maintained on wort agar slopes and propagated in the following medium: KH_2PO_4 1 g; $MgSO_4 \cdot 7H_2O$ 0.7 g; NaCl 1 g; $(NH_4)_2HPO_4$ 4 g; $FeSO_4 \cdot 7H_2O$ 0.03 g; glucose 30 g; water to 1,000 ml. Yeast extract was added after sterilization. Growth took place on a reciprocal shaker at 30° C for 24 h. Before incubation the yeast cells were washed with distilled water, aerated for 3 h and again washed.

Incubation. Suspensions of 5—8 mg dry weight/ml in distilled water were incubated with the sugars (and inhibitors) either in a Dubnoff incubator or in a

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water bath with tube holders and gas inlets permitting continuous passage of gas through the suspension (oxygen, air or argon, as the case may be). The bath could also be attached to a cooling unit for achieving temperatures down to 5° C. Samples of suspension were filtered through a membrane filter (0.3—0.5 μ pore diameter, HUFS Synthesia, Uhříněves, Czechoslovakia), washed twice with ice-cold water (within 15 sec) and the pellet on filter transferred either into 2 ml boiling water (where it remained for 20 min, was deproteinized with zinc sulphate and barium hydroxide and aliquot of the supernatant was used for analysis) or into 1 ml boiling 0.1% solution of Triton X (where it remained for 10 min and was used as such for radioactivity determination).

To check that the washing procedure does not cause any of the intracellular sugar to be lost to the cell, suspension samples were placed in small centrifuge tubes containing 0.4 ml silicone H-oil (Merck and Co.) of a density corresponding to 1.05, quickly centrifuged in an Ecco-Quick centrifuge and cells thus separated from the medium (an analogy of the procedure used by Werkheiser and Bartley, 1957, for mitochondria). The analysis of the sedimented cells (using D-xylose and L-xylose as representative sugars) showed an agreement within 8% with the washing procedure.

Estimation of sugars. Pentoses were determined with orcinol according to Meijbaum (Umbreit *et al.*, 1957), aldohexoses by the reducing-sugar method of Somogyi (1945) and Nelson (1944), with the exception of glucose which was estimated by the enzyme method (glucose oxidase, peroxidase, *o*-dianisidine), adding concentrated sulphuric acid after development of the *o*-dianisidine colour to the final concentration of 12N. 2-Deoxyglucose was estimated with periodic acid, arsenite and thiobarbituric acid according to Waravdekar and Saslaw (1957), ketohexoses then by the resorcinol method in the modification of Kulka (1956). Optical density was read on a Hilger Spekker or on a Lange VII colorimeter at 650 nm for pentoses and reducing sugars, at 525 nm for glucose, 2-deoxyglucose and the ketohexoses.

With ^{14}C-labelled sugars, the suspension after boiling was transferred to aluminium planchets treated with tertiary sodium phosphate to ensure homogeneous distribution of the sample over the entire area, and radioactivity counted either in a Frieseke-Hoepfner apparatus using a thin end-window tube or a methane-flow attachment, or in a low-background Tracerlab counter, depending on the activity available.

Chemicals. D-Glucose, D-fructose, D-galactose, L-rhamnose and D-ribose were from Lachema, Czechoslovakia; D-xylose from Fluka, A. G., Buchs, Switzerland; D-mannose, L-xylose, L-sorbose and D-fucose from Hoffmann-La Roche, Basel, Switzerland; L-glucose from Karl Roth, Karlsruhe, Germany,; L-arabinose from British Drug Houses, Poole, England; 2-deoxy-D-glucose from Sigma Chem. Co., St. Louis, USA; D-arabinose from Carlo Erba, Milan, Italy and D-lyxose and D-gulose from the Chemical Institute, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

D-Ribose-1-^{14}C, D-arabinose-1-^{14}C, D-galactose-1-^{14}C and D-xylose-^{14}C(U) were obtained from the Radiochemical Centre, Amersham, England; L-arabinose-1-^{14}C was from Calbiochem, Los Angeles, USA; D-glucose-^{14}C(U) from the Institute for Research, Production and Application of Radioisotopes in Prague.

The purity of all the sugars was checked by paper chromatography in butanol-acetic acid-water (4 : 1 : 5) on Whatman no. 1 paper, in the descending direction (8 h at 20°C), detecting with $AgNC_3$, NaOH, NH_4OH and $Na_2S_2O_3$ as modified from Green and Stone by Burger and co-workers (1959). The samples contained no detectable reducing impurities.

2-Thiobarbituric acid was prepared by

Mr. I. Beneš of this laboratory, the other reagents were commercial products of analytical purity.

RESULTS

Determination of apparent K_M and V of uptake.

The first aim was to determine the apparent Michaelis constants (K_M) of transport of sugars into the cell and the maximum rates of uptake (V). This was done by plotting the initial rate of uptake (v_0) against $v_0/(S)$, this method being preferred to the common Lineweaver and Burk plot (cf. Dowd & Riggs, 1965). The results are shown in Tab. 1. For practically all the sugars, data on which have been published by Sols (1967), the values of K_M were in rough agreement with his, with the exception of D-galactose, which was found to possess a much lower K_M here (0.021—0.046M) than reported by Sols (about 1M). This, just as some other minor deviations, can of course be due to the strain used. The K_M for D-glucose was in agreement with the value reported by Scharff and Kremer (1962) who found 5.3 mM, the values for D-mannose and D-fructose were lower in our yeast strain. The values of V for glucose, mannose and fructose found here were higher by two orders of magnitude than those of Scharff and Kremer (cf. also Kotyk & Kleinzeller, 1963).

Table 1. Apparent Michaelis constants and maximum rates of uptake of various monosaccharides by baker's yeast. Incubated at 30° C, anaerobically. Range of values from 3—8 experiments is given

Sugar	K_M* (mM)	V (mg sugar/g dry weight/min)
D-Glucose	4—6	20—28
2-Deoxy-D-glucose	4—5	20—26
D-Mannose	27	20—30
D-Galactose	21—46	10—13
L-Glucose	10^2	1
L-Rhamnose	10^3	3
D-Xylose	95—170	22—32
D-Arabinose	75—155	18—30
D-Lyxose	80—110	15—22
D-Ribose	300—800	3
L-Arabinose	10^2	3
L-Xylose	200—600	2.5
D-Fructose	17	20—30
L-Sorbose	21	7—16

* It should be noted that this is not necessarily the dissociation constant of the carrier-substrate complex (cf. Kotyk, 1967b).

On the whole, the sugars seem to fall into two groups as far as the maximum velocity of transport is concerned, the first with V values ranging from 15—30 mg sugar/g dry weight/min, the second in the neighbourhood of 1—3 mg sugar/g dry weight/min. L-Sorbose and D-galactose are intermediate. As will be seen in subsequent sections, this difference is only one of the distinguishing features of the two groups of sugars suggested above.

Steady-state space of distribution.

In the course of this study there emerged an unexpected aspect of the transport process, namely that only a part of the monosaccharides tested reach an intracellular concentration that would correspond to equilibration of the sugar in question in the entire cell water (what might be called a 100% space of distribution). Many sugars never reached this perfect distribution, their apparent steady state intracellular concentration corresponding roughly to one-half (but frequently less) that of the medium. Tab. 2 gives the calculated space of distribution for the sugars tested here, Fig. 1 shows a typical uptake experiment for representatives of both groups. It is readily seen that a plateau has been reached even by the imperfectly distributing sugar and that no further net uptake occurs after some 90 min of incubation. The figure also includes information on the effect glucose which will be taken up later.

It is perhaps noteworthy that all the investigated sugars penetrated the cell interior, this indicating a rather broad overall specificity of the transport system.

Table 2. Space of distribution of various sugars in baker's yeast. The values (R) express the % fraction of cell water in which the sugar is distributed at a concentration equal to that in the external medium. The range of values from 4—12 estimations at different monosaccharide concentrations is given

Sugar	R	Sugar	R
D-Glucose*	87—103	D-Xylose	89—110
2-Deoxy-D-glucose*	87— 96	D-Arabinose	95—109
D-Mannose*	95—104	D-Lyxose	98—104
D-Gulose	58— 62	D-Ribose	19— 41
D-Galactose	57— 66	L-Arabinose	31— 42
D-Fucose	26— 35	L-Xylose	38— 50
L-Glucose	88— 98	D-Fructose*	87— 95
L-Rhamnose	19— 37	L-Sorbose	96—119

* In the presence of 5×10^{-4}M iodoacetamide to block metabolism (cf. Burger *et al.* 1959).

The space of distribution of any sugar was far in excess of the so-called outer cell region of Conway and Downey (1950) which was reported to correspond to some 10% of the cell volume.

The shape of the uptake curves of the imperfectly distributing sugars and the number of experiments performed (over 20 experiments with incubation lasting for more than 4 h) convince us that the reduced space of distribution is a reality rather than an artifact due to slow uptake. Just as with the perfectly distributing sugars, some 90% of the final intracellular level was reached within at most 60 min.

Structural requirements of transport.

It was now investigated whether a general structural feature of the sugar molecule could account for the above-mentioned difference in the space of distribution.

If the conformation formulae (cf. LeFevre & Marshall, 1958) of the sugars tested are drawn in such a way that both the *C1* and *1C* chair conformations have the same formal space arrangement with respect to the oxygen (the numbering of their C atoms is then reversed) it can be observed (Fig. 2) that the perfectly distributing sugars all possess an equatorial (β) hydroxyl group in position 1 of the *C1* conformation or an equatorial $-CH_2OH$ group in position 5 of the *1C* conformation, and an equatorial hydroxyl in position 4 of *C1* or in position 2 of *1C*. Thus D-glucose, 2-deoxy-D-glucose, D-mannose, D-xylose, D-arabinose, D-lyxose and also L-glucose conform to the structural requirements of the Group 1 transport across the membrane. D-Ribose fits also into this group but apparently the position of the hydroxyls at C_2 and C_3 is prohibitive for binding to the Group 1 carrier. The other sugars all possess an axial hydroxyl at the crucial position (C_4 of the *C1* conformation and C_2 of the *1C* conformation) in one or both of the conformational forms. If the axial hydroxyl in question is a structural requirement for the second (Group 2) carrier then D-glucose, 2-deoxy-D-glucose, D-xylose and L-glucose, but not the other sugars belonging to Group 1, may be envisaged to be also transported by the Group 2 carrier. However, it may be seen on the example of competition between L-arabinose (Group 2) and D-glucose (possible competitor) and D-mannose (should not compete if axial hydroxyl at C_4 of *C1* or C_2 of *1C* is required) that the effect of both hexoses is similar. Hence it appears that the Group 2 carrier does not require a particular position of the crucial hydroxyl group. Actually, its specificity is likely to be very broad as there is no structural feature of the sugar molecules transported

by it that could distinguish it positively from the Group 1 carrier. Thus we must content ourselves with a negative distinction in the sense that the Group 2 carrier probably transports all the monosaccharides tested.

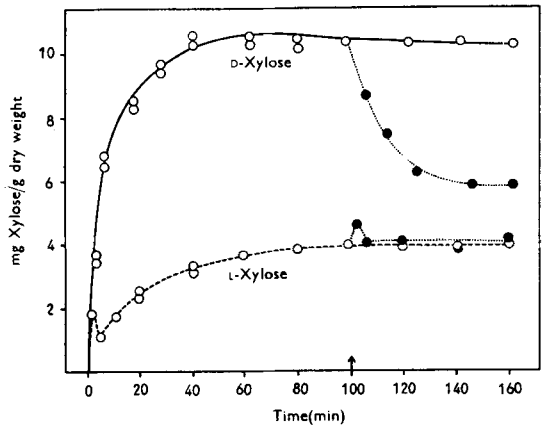


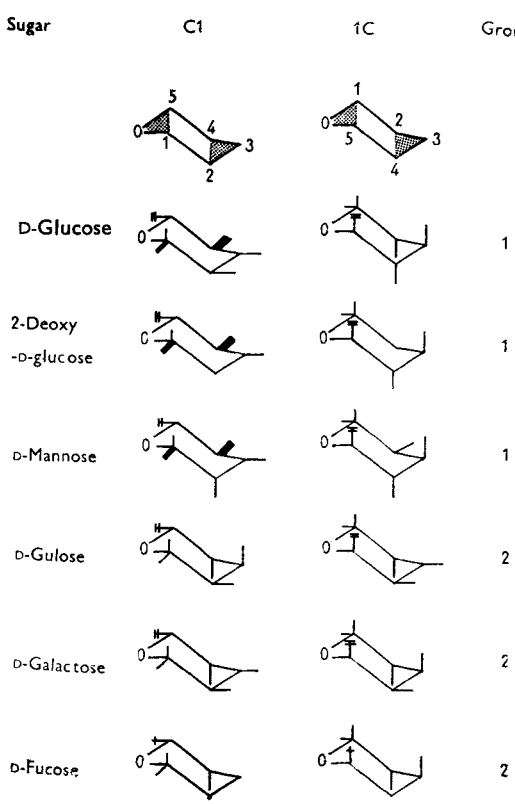
Fig. 1. Uptake of 0.5% D-xylose and 0.5% L-xylose by baker's yeast. After 100 min glucose was added to the final concentration of 0.5%, its effect being shown by the dotted curves.

Since the ketohexoses probably occur predominantly in the furanose form, the above rules are not directly applicable to them. The fact remains that both D-fructose and L-sorbose are apparently transported by the Group 1 carrier.

Differentiation between the two groups of monosaccharides.

It was now attempted to characterize the two types of transport in greater detail.

Participation of a mobile carrier. The existence of uphill countertransport has been considered as indicative of the



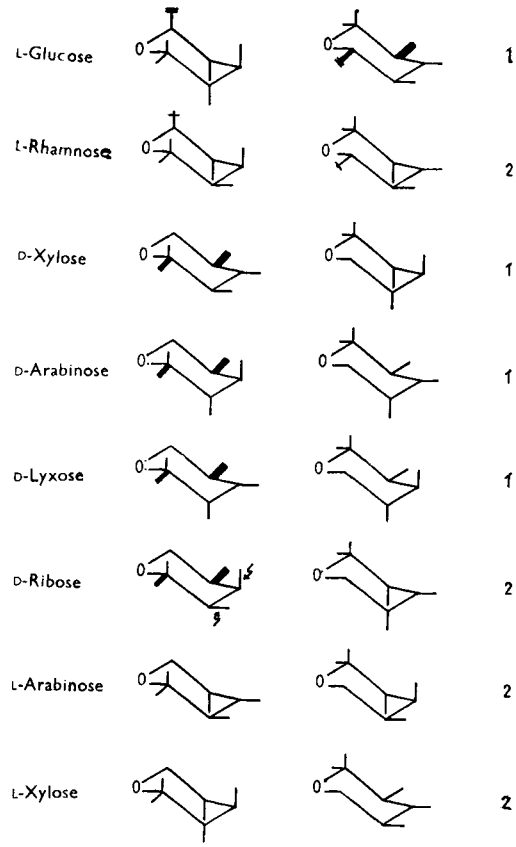


Fig. 2. Conformation formulae of the aldoses investigated here. In position 1, both the α and β hydroxyls are shown in the formulae.

participation of a mobile carrier in the transport process (see, however, Heckmann, 1965). A test described by Miller (1965) was used with D-xylose and L-arabinose (Fig. 3) and a countertransport peak was found in both cases, this being taken as evidence that the transport of both sugars is mediated by a mobile carrier.

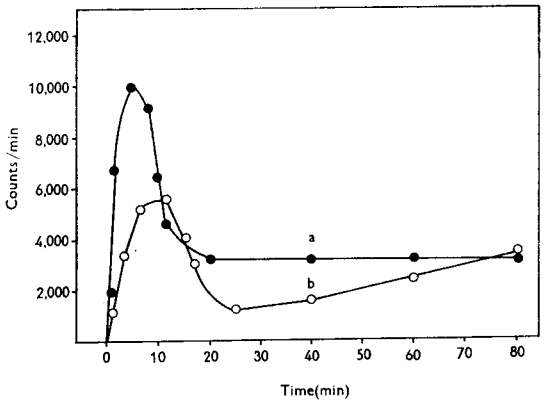


Fig. 3. Countertransport of D-xylose and L-arabinose in baker's yeast. Cells were incubated for 3 h with 1% D-xylose (a) or 1% L-arabinose (b), separated from the medium, washed with ice-cold water and resuspended in a solution of ^{14}C-D-xylose (a) or ^{4}C-L-arabinose (b) of negligible concentration. Intracellular activity was then estimated at the time Intervals shown. The positive slope of the L-arabinose curve (b) after some 30 min might be due to an incorporation of the sugar into cell components.

Effect of osmolarity. Suspension of yeast cells in hypertonic solutions of NaCl results invariably in considerable cell volume shrinkage, due to extrusion of a part of the cell water. Therefore, if a sugar is distributed in an osmotically active form in the intracellular water, any reduction of the water space should be accompanied by a reduction of the amount of intracellular sugar. Fig. 4 shows the result of such treatment of cells with respect to the transport of D-xylose and L-xylose. The amount of intracellular D-xylose follows closely that of intracellular water whereas that of L-xylose decreases much less in the hypertonic medium. The reduction of intracellular water after placing the cells in 3 osM NaCl amounts to 64%, that of the amount of intracellular D-xylose to 63% but that of the amount of intracellular L-xylose only to 39%. Two explanations of this phenomenon are possible: (1) L-xylose distributes itself in a space that does not shrink substantially when the cell is placed in a hypertonic medium; (2) L-xylose is partly adsorbed within the cell. The second possibility is rather unlikely, however, since then the presence of another sugar competing with L-xylose should result in a decreased steady-state distribution which has not been observed.

Effect of temperature. As seen in Fig. 5, showing an Arrhenius plot of the rate of transport of several pentoses, both types of transport are characterized by a low temperature quotient, the apparent activation energy ranging from 6,700 cal/mole for L-xylose to 7,800 cal/more for D-xylose and D-ribose. This might indicate that the actual movement of the carrier-substrate complex across the membrane is limiting for both groups of sugars, at least within the temperature range from 15° to 30° C. Particularly at lower temperatures (not shown in the graph) the Q_{10} quotient

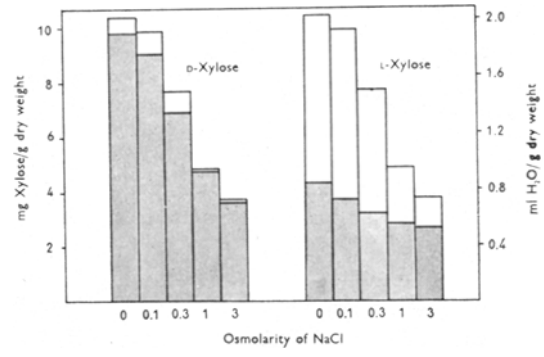


Fig. 4. Effect of hypertonic NaCl on the amount of cell water and intracellular D-xylose and L-xylose. Cells were incubated with 0.5% pentose in the presence of NaCl at the osmolarity shown, for 120 min, in air at 30° C. The hatched parts of the columns correspond to the amount of intracellular sugar, the total height of the column shows the intracellular water determined in specially calibrated hematocrit tubes.

for sugar uptake reaches values well above 2, such as found by Burger and co-workers (1959) or Cirillo and co-workers (1963), the activation energies involved ranging from 11,000 to 14,500 cal/mole.

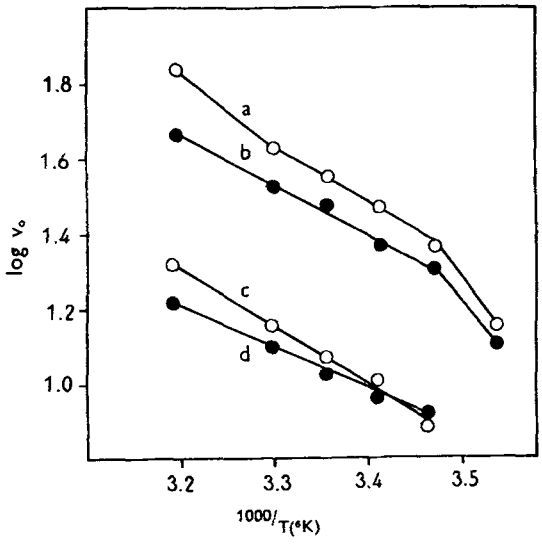


Fig. 5. Effect of temperature on the rate of transport of D-xylose (a), D-arabinose (b), D-ribose (c) and L-xylose (d) by baker's yeast. The initial rates of uptake (v_0) from a 0.8M sugar solution in arbitrary units were used for the demonstration.

Effect of inhibitors. Two representative inhibitors are selected here to demonstrate the different character of the two transport processes investigated. 2,4-Dinitrophenol was used as a powerful inhibitor of energy metabolism and uranyl nitrate as an inhibitor of glucose transport (cf. Rothstein, 1954). Fig. 6 shows that whereas DNP does not affect the transport of D-xylose it affects the distribution of L-xylose and, on the contrary, UO_2^{2+} suppress the rate of uptake of D-xylose but do not substantially influence that of L-xylose. This may well have to do with a phosphorylation reaction being indirectly involved in the sugar transport process of D-xylose but not of L-xylose (cf. Kotyk, 1967a). The effect of DNP appears to be on the initial phase of L-xylose uptake where irregularities in the uptake curve occur, their significance being rather obscure (Kotyk, 1967c).

Competition between various sugars. A clearer picture of the distinction between the two carriers assumed to operate in yeast might be obtained from mutual competition between representative sugars of the two groups. Tab. 3 was compiled from uptake experiments with various sugar pairs, the selection being rather restricted by the availability of labelled compounds. The nonlabelled sugar was always present in a 200-fold excess over the radioactive one. It may be seen that the mutual inhibition is not clearly limited to the individual groups in question but is slightly overlapping (D-glucose vs. D-galactose and D-ribose etc.) which can be accounted for by the fact that the Group 2 carrier is probably shared by all sugars and, due to great differences in affinity, a competitive effect can be observed in the one but not in the other direction.

Since the initial rates of uptake are not the ideal parameter for more refined kinetic measurement the competition between various sugars was tested also by

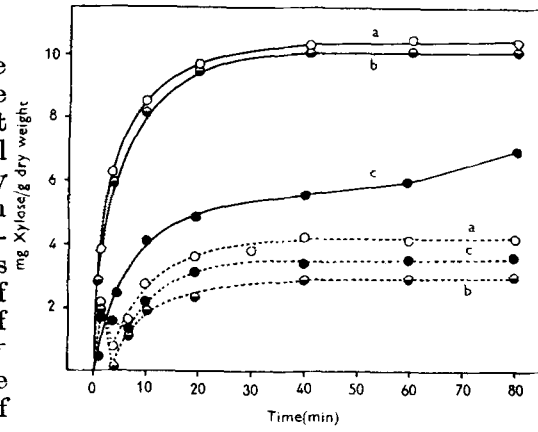


Fig. 6. Effect of 2,4-dinitrophenol and uranyl nitrate on the uptake of D-xylose and L-xylose by baker's yeast. 0.5% sugar solutions were used and incubated in air at 30° C. Solid curves — D-xylose, broken curves — L-xylose. a — no inhibitor; b — 5×10^{-4}M 2,4-dinitrophenol; c — 3×10^{-4}M $UO_2(NO_3)_2$.

Table 3. Inhibition of uptake of ^{14}C-labelled monosaccharides by the presence of various nonlabelled sugars. Incubated anaerobically at 30° C; both sugars were added simultaneously. The number of plus signs corresponds to the intensity of inhibition by the nonlabelled sugar

^{12}C-Sugar \ ^{14}C-Sugar	D-Glucose	D-Xylose	D-Arabinose	D-Galactose	D-Ribose	L-Arabinose
D-Glucose	++	++	++	+	+	+
2-Deoxy-D-glucose	++	++	++	+	+	?
D-Mannose	++	++	++	?	+	+
D-Galactose	—	—	+	++	+	++
L-Rhamnose	—	—	—	?	—	?
D-Xylose	++	++	++	—	—	?
D-Arabinose	++	++	++	—	—	?
D-Lyxose	+	+	+	—	—	?
D-Ribose	—	—	—	+	+	+
L-Arabinose	—	—	—	+	—	+
L-Xylose	—	—	—	+	—	—
D-Fructose	++	++	++	+	+	?
L-Sorbose	++	++	++	+	—	?

adding one sugar to an equilibrium concentration of another, whereafter a counterflow minimum would be expected when neither of the sugars is metabolized or a drop of the intracellular concentration of the equilibrated sugar when the added one is metabolized. These experiments, even better than those reported above, bore out the lack of competition between sugars of Group 1 and Group 2. An example of this approach is shown in Fig. 1 where D-glucose added to an equilibrium concentration of D-xylose caused the intracellular concentration of this sugar to decrease sharply but was without effect on the level of L-xylose.

An even more convincing demonstration of the difference between the two groups of sugars was obtained in experiments where a thin yeast suspension was incubated with glucose and a two-hundred-fold greater concentration of various nonmetabolized sugars. The uptake of glucose due to metabolic utilization wasdetermined and it was found (Tab. 4) that the Group 1 sugars (L-sorbose, D-xylose and D-arabinose) blocked the utilization of glucose almost completely whereas the Group 2 sugars showed practically no effect whatsoever. That the effect takes place at the membrane and not in subsequent metabolism was shown by determining the intracellular level of labelled free D-glucose in the presence of the various nonmetabolized sugars where an identical picture emerged: Group 1 sugars reduced the intracellular level of free glucose whereas the Group 2 sugars left it intact.

Table 4. Effect of various monosaccharides on glucose utilization by baker's yeast. Incubated anaerobically at 30° C with 10% nonmetabolized sugar and 0.05% D-glucose

Nonmetabolized sugar	Utilization of glucose (mg/g dry weight/min)	Inhibition (%)
None	22.0	—
D-Galactose	20.0	9
D-Xylose	3.7	83
D-Arabinose	4.8	78
D-Ribose	21.5	1.5
L-Xylose	22.0	0
L-Sorbose	4.0	82

Effect of adaptation to galactose on the transport of L-xylose.

It has been indicated by Sols (1967) and by Cirillo (1967) that an adaptation of yeast cells to galactose metabolism changes the uptake rate for various other sugars.

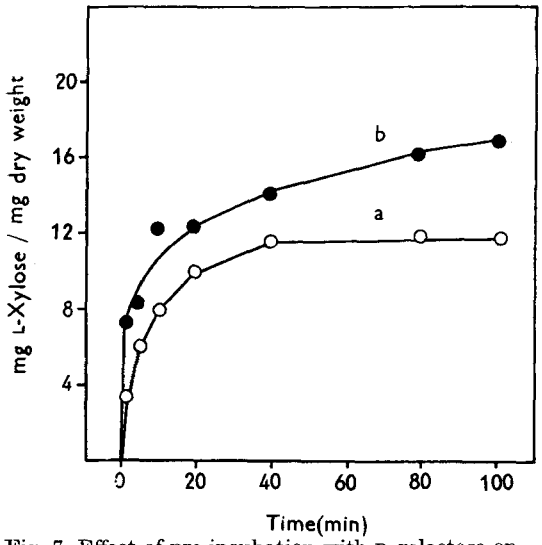


Fig. 7. Effect of pre-incubation with D-galactose on L-xylose uptake. a — Normal cells in aqueous medium; b — cells pre-incubated aerobically for 2 h with 1% D-galactose, washed thoroughly in distilled water and resuspended in distilled water. L-Xylose was then added to a final concentration of 1% to both suspensions and the uptake followed aerobically at 30° C.

Therefore, yeast was incubated aerobically for 2 h in the presence of 1% D-galactose, washed thoroughly and placed in the presence of L-xylose. A comparison of the uptake by this and by nonadapted yeast is shown in Fig. 7, wherefrom it follows that, indeed, an essential part of the carrier of Group 2 sugars is inducible in the broad sense of the word. Both the initial rate of uptake and the space of distribution are increased significantly, the latter almost to the 100% value of the Group 1 sugars.

DISCUSSION

The finding of two different types of transport for monosaccharides in baker's yeast can be related to previously observed differences in the uptake of various sugars. Cirillo (1967) found a rapid uptake followed by a slower process in some of the sugars designated here as Group 1 but did not find the rapid part in Group 2. Both he and Sols (1967) report that growth in the presence of galactose changes the uptake pattern of the "slowly" transported sugars (cf. the remarkable work of Robichon-Szulmajster, 1961). These findings being all of preliminary nature, it still remains a matter for conjecture where the difference between the two types of transport may lie. Summarizing the data on the properties of the two transport types as have been gathered here (Tab. 5)

Table 5. Distinction between the two postulated monosaccharide carriers in yeast

Feature investigated*	Carrier 1	Carrier 2
Distribution of sugar in cell water	100%	~50%
Effect of hypertonic medium on sugar distribution	Great	Slight
Effect of uranyl ions on transport	Great	Slight
Competition with glucose for uptake	Yes	No
Occurrence of countertransport	Yes	Yes
Mobility ratio for loaded vs. free carrier**	2—3	1
Apparent activation energy between 15 and 30° C	6—8,000 cal/mole	6—8,000 cal/mole

* In addition to the features listed here substantial differences were observed between the effect of D-xylose and L-xylose on the endogenous production of CO_2 and on the intracellular level of high-energy phosphate, D-xylose being very effective, L-xylose showing practically no effect (to be published).

** From Kotyk (1967b).

one is compelled to assume the existence of more than one barrier which the sugars must cross to distribute themselves in the entire cell water. The following hypothesis is advanced to account for the observations.

The process of uptake involves, at least for Group 1 sugars, two spatially separated stages. One of them, possessing a broad specificity, would be common to both sugar groups, the other would apply only to Group 1, unless induction has caused the second-stage carrier either to appear or to become unmasked or to change its affinity pattern. This hypothesis might be visualized on the morphological level as follows: All monosaccharides enter (with a carrier) into a certain cell compartment, perhaps the vacuole or another structure in direct communication with the cytoplasmic membrane. Only certain sugars (Group 1) can enter the rest of the cell by virtue of a carrier localized at the further barrier. This possibility is suggested by the fact that the space of distribution for the Group 2 sugars varies from experiment to experiment as the size and the amount of e.g. the vacuoles may change from batch to batch. This hypothesis might be tested by (1) selecting cells with large vacuoles and comparing them with cells containing no vacuoles and (2) investigating the efflux curves of various sugars as to the number of exponentials (and hence intracellular compartments) involved. Some preliminary experiments indicate indeed that the efflux curves of D-xylose and D-lyxose contain one more component than those of D-ribose and L-xylose.

The fact that the uptake of some Group 2 sugars is inhibited by Group 1 sugars but not vice versa can be explained by competition for the stage I carrier (which is shared by all sugars) where the effect of Group 1 sugars may be quite pronounced. There is no competition on the part of the Group 2 sugars for the stage II of transport so that the possible competition for stage I is obscured, the affinities of the Group 2 sugars being very low. The only exception, D-galactose, is actually found to decrease the rate of uptake of such Group 1 sugars as D-arabinose.

Results along similar lines were recently published by van Steveninck and Rothstein (1965) who, on the basis of changes in Ni^{2+} binding by the cell surface, assume glucose, fructose and mannose to be transported by an altered (perhaps phosphorylated) form of carrier whereas other monosaccharides cannot use this carrier form. Nevertheless, competition between both classes of monosaccharides can take place by virtue of their sharing the "core" of the carrier. Induction of a "galactose permease" could also be explained by this hypothesis. It is hard to decide at present whether these findings fit in directly with the hypothesis of two carrier types advanced here or whether they supplement it with still a third type of transport which would be restricted to the metabolizable sugars. At any rate, there does not seem to be any distinction made in the paper by van Steveninck and Rothstein as to sugars equilibrating in the entire water space and those penetrating into only a part of it.

It is hoped that an investigation of the inducibility of the carrier for Group 2 sugars might clarify the localization and the mutual relation of the two transport systems involved, as well as the character of the carrier as a molecular entity.

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References

Burger, M., Hejmová, L., Kleinzeller, A.: *Transport of some mono- and di-saccharides into yeast cells.* Biochem. J. 71 : 233, 1959.

Cirillo, V. P.: *Sugar transport in microorganisms.* Annual Rev. Microbiol. 15 : 197, 1961.

Cirillo, V. P.: *The characteristics of sugar transport in*

yeast protoplasts. Symp. über Hefe-Protoplasten, Jena 1965, Akademie-Verlag, Berlin 1967.

Cirillo, V. P., Wilkins, P. O., Anton, J.: *Sugar transport in a psychrophilic yeast*. J. Bacteriol. 86 : 1259, 1963.

Conway, E. J., Downey, M.: *An outer metabolic region of the yeast cell*. Biochem. J. 47 : 347, 1950.

Dowd, J. E., Riggs, D. S.: *A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations*. J. biol. Chem. 240 : 863, 1965.

Heckmann, K.: *Die Permeabilität biologischer Membranen*. In *Mechanism of Hormone Action*, Academic Press, New York 1965.

Kotyk, A.: *Effect of metal ions on monosaccharide transport in baker's yeast*. Symposium on Yeast, Bratislava 1966, Academia Praha 1967a.

Kotyk, A.: *Mobility of the free and of the loaded monosaccharide carrier in Saccharomyces cerevisiae*. Biochim. biophys. Acta 135 : 112, 1967b.

Kotyk, A.: *Sugar space in yeast cells and protoplasts*. Symp. über Hefe-Protoplasten, Jena 1965, Akademie- Verlag, Berlin 1967c.

Kotyk, A., Kleinzeller, A.: *Transport of* D-*xylose and sugar space in baker's yeast*. Fol. microbiol. 8 : 156, 1963.

Kulka, R. G.: *Colorimetric estimation of ketopentoses and ketohexoses*. Biochem. J. 63 : 542, 1956.

LeFevre, P. G., Marshall, J. K.: *Conformational specificity in a biological sugar transport system*. Am. J. Physiol. 194 : 333, 1958.

Miller, D. M.: *The kinetics of selective biological transport. II. Equations for induced uphill transport of sugars in human erythrocytes*. Biophys. J. 5 : 417, 1965.

Nelson, N.: *A photometric adaptation of the Somogyi method for the determination of glucose*. J. biol. Chem. 153 : 375, 1944.

de Robichon-Szulmajster, H.: *Contribution à l'étude génétique et physiologique du métabolisme du galactose chez la levure*. Ann. Technol. agric. 10 : 81 + 185, 1961.

Rothstein, A.: *Enzyme systems of the cell surface involved in the uptake of sugars by yeast*. Symp. Soc. Exp. Biol. VIII, Cambridge University Press, 1954.

Scharff, Th. G., Kremer, E. H. III: *A tentative mechanism for the anaerobic transport of glucose, fructose and mannose in yeast*. Arch. Biochem. Biophys. 97 : 192, 1962.

Sols, A.: *Regulation of carbohydrate transport and metabolism in yeast*. Symp. on Some Aspects of Yeast Metabolism, Dublin 1965, Blackwell Scient. Publ., Oxford 1967.

Somogyi, M.: *Determination of blood sugar*. J. biol. Chem. 160 : 69, 1945.

van Steveninck, J., Rothstein, A.: *Sugar transport and metal binding in yeast*. J. gen. Physiol. 49 : : 235, 1965.

Umbreit, W. W., Burris, R. H., Stauffer, J. P.: *Manometric Techniques*, p. 274. Burgess Publ. Co., Minneapolis 1957.

Waravdekar, V. S., Saslaw, L. D.: *A method of estimation of 2-deoxyribose*. Biochim. biophys. Acta 24 : 439, 1957.

Werkheiser, W. C., Bartley, W.: *The study of steady-state concentrations of internal solutes of mitochondria by rapid centrifugal transfer to a fixation medium*. Biochem. J. 66 : 79, 1957.