Finally the present results indicate that polyphloretin phosphate, polyphlorrhizin phosphate and polyhesperidin phosphate as well as polyoestradiol phosphate inhibit seminal acid phosphatase. These polymeric phosphates inhibit other enzymes as well (Diczfalusy *et al.* 1953, Fernö *et al.* 1953, 1958). It is thus very likely that the inhibition of acid phosphatase by these compounds is just an example of a widespread and non-specific action of high molecular-weight polyelectrolytes on several enzymes, as suggested by Spensley & Rogers (1954). The reversibility of the enzyme inhibition also suggests a macro-anion–macro-cation interrelationship similar to that postulated by these authors for the interaction between polyphloretin phosphate and protamine on hyaluronidase or between heparin and polylysine on pepsin.

SUMMARY

1. The hydrolysis of monophenyl phosphate by unpurified human seminal acid phosphatase has been investigated.

2. The optimum pH was found to lie between 5·4 and 5·7 and was not influenced by the substrate concentration.

3. The Michaelis constant was estimated at several pH values. At the optimum pH a K_m value of 0·18 mM was repeatedly found. High substrate concentrations did not inhibit the enzyme activity.

4. Polyoestradiol phosphate was found to be a powerful, non-competitive inhibitor of seminal acid phosphatase. The inhibitory effect was strongest on the acid side of the pH optimum. The inhibition could be reversed by small amounts of protamine sulphate.

5. The enzyme was also inhibited by polymeric phosphates of phloretin, phlorrhizin and hesperidin. The inhibition by polyphloretin phosphate was non-competitive.

6. The trypanocide suramine proved to be a non-competitive inhibitor of seminal acid phosphatase.

We are indebted to Dr E. Nordlander, Head of the Division of Fertility at the Department of Women's Diseases, for his helpful co-operation in obtaining semen specimens. Thanks are also tendered to AB Leo, Hälsingborg, for a generous gift of polyphosphate analogues.

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Transport of some Mono- and Di-Saccharides into Yeast Cells

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The mechanism of transport of sugars into yeast cells is still subject to discussion. Rothstein (1956) maintains that the transport of fermentable sugars into the cells of *Saccharomyces cerevisiae* is effected by phosphorylating reactions, but other authors, e.g. Sols (1956), assume that this transport in another yeast strain is independent of phosphorylating processes.

In this paper experiments on the transport of some sugars, fermentable and non-fermentable, into yeast cells (*S. cerevisiae* R XII, *Saccharomyces fragilis*, *Saccharomyces cartilis*) are reported. It has been shown that under certain experimental conditions a considerable amount of apparently free sugars is found in the cells. The results of some kinetic studies of the fluxes of D-galactose and

D-arabinose are reported. Furthermore, evidence is presented for a competition between various sugars for their transport into yeast cells and some experiments are recorded showing that the physiological state of the yeast cells is of importance for the rate of sugar penetration into the cells. Finally, some comparative data on the influx of disaccharides into *S. cerevisiae* and *S. fragilis* are presented.

A preliminary account of this was presented at the VIIth International Congress of Microbiology, Stockholm, August 1958.

METHODS

Yeast strains. S. cerevisiae strain R XII and *S. fragilis* were both obtained from the Type Culture Collection, Biological Institute, Czechoslovak Academy of Sciences, Prague. *S. cartilis* was obtained from Professor V. I. Kudriavtsev, Moscow.

Unless otherwise stated, the yeasts were grown aerobically for 24 hr. in a shaker at 30° in 100 ml. of 10° (Balling) malt wort in 250 ml. conical flasks. In some cases (e.g. experiments with trehalose) *S. cerevisiae* was grown for 48 hr. at 30° in a stationary culture in the above medium; thus growth was practically anaerobic, the average yield being 0·4 g. dry wt. of yeast/flask. The yeast cultures were centrifuged and washed three times with 0·15M-NaCl, and resuspended in the same medium and then used for the experiments. This suspension contained usually 30 mg. dry wt. of cells/ml.

Experimental procedures. The following procedure was used for the study of sugar penetration (influx) into the cells. To 5 ml. of the washed yeast suspension, prepared as described above, 25 ml. of 0·1M-phosphate buffer (prepared by adding 0·1M-Na_2HPO_4 to 0·1M-KH_2PO_4 to final pH 5·5) was added, and further additions to a volume of 50 ml. The final suspension usually contained 3–4 mg. of yeast (dry wt.); this will be referred to hereafter as standard suspension. Somewhat higher concentrations of yeast cells were used in some experiments, e.g. those where trehalose influx was examined. The final concentration of the various sugars used was usually 5% (w/v).

In aerobic experiments 7–10 ml. of the above standard suspension was placed into 100 ml. conical flasks and then incubated with shaking in a thermostat at 30°, with air as the gaseous phase. At regular time intervals 2 ml. samples were removed and centrifuged, and the sediment of cells quickly washed three times with ice-cold 0·15M-NaCl, the washing procedure being finished within 6 min. It will be shown below that practically no loss of sugar from the cells takes place during this procedure. The sediment was then suspended in water, final volume 2 ml., and the tubes were placed into a boiling-water bath for 20 min. After cooling, the volume was readjusted to 2 ml., the cellular debris removed by centrifuging and the supernatant extract used for analysis of sugars.

In anaerobic experiments, the suspension was placed into small flasks through which a stream of O_2-free N_2 was passed. The further procedure was as described above.

Where the efflux of sugars from yeast cells into the medium was followed, the yeast suspensions were prepared as stated above and incubated under anaerobic or aerobic conditions at 30° with the appropriate sugar [5% (w/v) final concentration] for 60 min. Preliminary experiments showed that after this time interval the intracellular sugar concentration was at or near equilibrium with the medium. The suspension was then centrifuged, and the sediment quickly washed three times with ice-cold 0·15M-NaCl, and suspended in saline and phosphate buffer (pH 5·5), added to the final concentration of 50 mM. These suspensions were then incubated anaerobically as described previously, and at regular intervals 2 ml. samples were removed and centrifuged, and the sugar contents in both medium and cells were estimated.

In some experiments conventional Warburg manometric techniques were used to determine whether a given sugar was metabolized under stated experimental conditions, i.e. whether O_2 uptake (aerobically) or CO_2 formation (anaerobically) was increased as compared with controls with no sugar added. These experiments were carried out in parallel with those where sugar influx into the cells was examined, and the same suspending media were used.

Analytical methods. The sugar content of the yeast cells was estimated in the extracts after chromatographic separation on paper, a slight modification of the method described by Green & Stone (1952) being used. The method differed from that described by these authors in the following respects: the $AgNO_3$ soln. in acetone for developing chromatograms was prepared by adding only 2·7 ml. of a saturated aq. $AgNO_3$ soln. to 1 l. of dry redistilled acetone; an aq. 1·3% (w/v) NH_3 soln. for clearing the background of the chromatograms was always freshly prepared; for further clearing of the chromatograms and greater contrast of the spots an aq. 20% (w/v) sodium thiosulphate solution was employed. The developing procedure was repeated several times according to the type of sugar analysed.

The spots produced by the various sugars were then quantitatively analysed by photometry in transmitted light. The results of analyses carried out in duplicate did not differ more than 5% from the mean, provided that the sugar content on the spot was within 2–6 μg. The amount of analysed sugar solution placed on the paper was therefore varied in order to keep within the above range. In all cases standards of the appropriate sugars were also analysed by the same procedure.

Trehalose was estimated as described above and also in the eluate of paper chromatograms, developed as described, with the anthrone colorimetric method of Trevelyan & Harrison (1951). Satisfactory agreement between both these methods was obtained.

Where the amount of sugars, released by the cells into the medium, was analysed, the colorimetric method of Somogyi (1952) and Nelson (1944) and also the above chromatographic procedure were used.

Materials. The sugars used were recrystallized commercial preparations from Lachema, Brno.

The results presented below are expressed in μg. of sugar penetrated or released/mg. dry wt. of yeast cells.

RESULTS

Time curve of sugar penetration into yeast cells. On incubation of *S. cerevisiae*, aerobically or anaerobically, with a non-fermented sugar, e.g. D-galactose or D-arabinose, considerable amounts of apparently free sugar can be demonstrated in the

cells, as already indicated previously by Conway & Downey (1950). In Fig. 1 a representative result of a time-curve experiment for the penetration of D-galactose into *S. cerevisiae* cells under anaerobic conditions is shown. This curve appears to be exponential in time and reaches an equilibrium level within 60 min. of incubation. The same type of curve was obtained with D-arabinose and, as will be mentioned below, also with D-galactose and α-D-methylglucoside in *S. fragilis*.

Since D-arabinose is known not to be metabolized by *S. cerevisiae* (see, for example, Kudriavtsev, 1954), it appears that this substance penetrates into the cells as free sugar. Certain experimental evidence suggests that under given experimental conditions galactose also penetrated into the cells as free sugar. Thus it was found that the Q_{O_2} or $Q^{N_2}_{CO_2}$ of the yeast in the first 60 min. was not increased above the endogenous respiration or fermentation rates on addition of D-galactose, and the addition of galactose did not bring about a decrease of the steady-state level of inorganic phosphate, as opposed to glucose (Kotyk, 1958 and personal communication). On the other hand, after more prolonged incubation with galactose, but without nitrogeneous substrate, some adaptation of the yeast cells to galactose utilization manifested itself (see also Spiegelman & Reiner, 1947) by a decrease of the galactose content in the cells, and also by an increased Q_{O_2} (-24, as compared with a Q_{O_2} of -12 without substrate) and a decreased level of inorganic phosphate in the cells. Such cells will hereafter be termed partly adapted, as opposed to fully adapted yeast described by Stephenson & Yudkin (1936).

Mention should be made here that with normal yeast no free glucose could be demonstrated in the cells on incubation with this sugar, neither aerobically nor anaerobically; however, in the presence of mM-iodoacetate glucose could readily be demonstrated intracellularly, as will be shown below (see Fig. 5).

It was considered of interest to calculate from the results the maximum rate of sugar penetration into the cells, i.e. the rate at zero time. This may be conveniently approximated by plotting sugar found (μg./mg. of cells dry wt./min.) against time; the intercept with the ordinate of the straight line obtained gives the zero-time rate of sugar penetration in μg./mg. of cells dry wt./min. (Dr J. B. Chappell, personal communication). This maximum rate was found to average $2{\cdot}06 \pm 0{\cdot}20$ μg., i.e. 124 μg./mg. of dry cells/hr. (eight experiments).

Effect of the concentration gradient of sugar on its intracellular concentration. The effect of the extraneous concentration of D-arabinose on the intracellular level of this sugar in *S. cerevisiae* after incubation for 60 min. is shown in Fig. 2. It will be seen that the intracellular level of sugar increases nearly linearly with increasing concentrations of sugar in the medium. The same relationship was obtained with D-galactose. It has been pointed out above that 1 hr. of incubation was found to be sufficient to establish an equilibrium level intracellularly; accordingly, the values for 90 min. incubation did not differ from the results presented.

The data obtained allow a calculation of the apparent 'galactose or arabinose compartment' (space) within the yeast cell (Conway & Downey, 1950), assuming that this cell contains 65·4 % of intracellular water (White, 1954), that the volume of intracellular water does not change on addition of the sugar and that at equilibrium the sugar concentration in the cellular compartment equals that in the medium. This apparent galactose space represented in our experiments $56{\cdot}4 \pm 2{\cdot}53$ % of the total cell volume (range 38·9–79·6 %, 22 experiments). However, it has been shown by Kotyk & Kleinzeller (1958) that the volume of the yeast cell

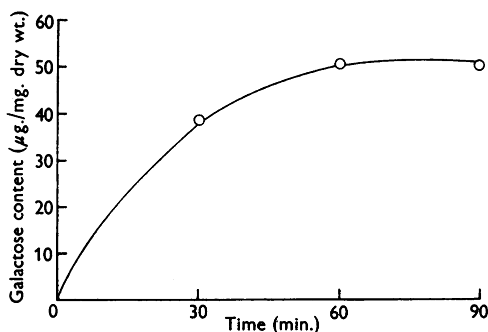

Fig. 1. Time curve of galactose influx into *S. cerevisiae*. Standard yeast suspension, containing 5% (w/v) of galactose (final concn.). Temp. 30°; N_2 as gaseous phase.

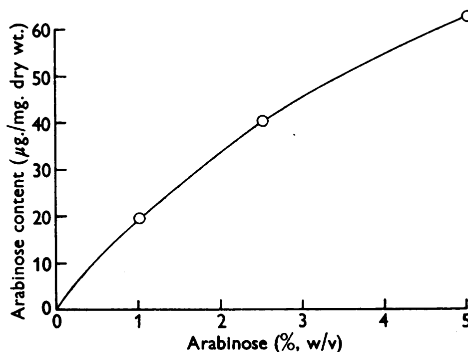

Fig. 2. Effect of arabinose concentration in the medium on its intracellular level. Standard suspension of *S. cerevisiae*. Temp. 30°; 60 min. incubation; air as gaseous phase.

may decrease up to 20% on addition of a sugar solution to the final concentration of 5%. The real galactose (or arabinose) space will therefore be considerably higher, and may even approach 100%. These figures are considerably higher than those reported by Conway & Downey (1950), who calculated the galactose space to be of the order of 10%; the discrepancy might be explained by the indirect method of measurement of the space used by the above authors, and also by some differences in the experimental conditions.

It should be mentioned that in all of the numerous experiments the apparent equilibrium intracellular concentration of the examined monosaccharides was lower than that of the medium; a transport against a concentration gradient thus was not observed under these experimental conditions.

Table 1 shows the calculated apparent sugar spaces for galactose and arabinose in *S. cerevisiae*, and also for α-D-methylglucoside in *S. fragilis*. The spaces of the various sugars in the two yeast species are of the same magnitude.

Effect of temperature on the influx and efflux of galactose in Saccharomyces cerevisiae. The effect of varying temperatures (1–30°) on the movement of galactose into yeast cells and on the efflux of this sugar was examined in order to contribute to the elucidation of the mechanism of sugar transport across the cellular membrane.

The results of a representative experiment on the efflux of D-galactose are shown in Fig. 3. It will be seen that the galactose efflux is very low at 1° and increases considerably with increasing temperature. From the results obtained an average Q_{10} of 2·4 was calculated. The very slow efflux of galactose from the cells at 1° justifies the washing procedure for the estimation of the intracellular sugar level, as outlined in Methods.

The effect of varying temperatures (1–30°) on the galactose influx was similarly examined. Here again a very low influx of galactose at 1° was observed, and the influx rapidly increased on increasing the temperature, with an average Q_{10} of 2·9. The zero-time rates of penetration at different temperatures, plotted on an Arrhenius plot, gave a straight line (Fig. 4).

Identical results, not reported here in detail, were obtained with D-arabinose, both for the efflux and influx of this sugar.

Although values of Q_{10} of the order of 2 and higher for both fluxes do not of themselves constitute conclusive evidence for a biochemical mechanism of sugar transport across the cell membrane, as opposed to transport due to physical forces alone (see Davson & Danielli, 1952), these

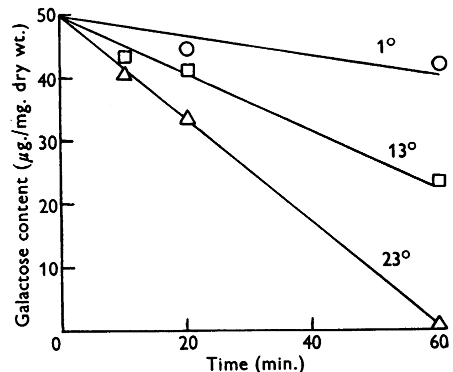

Fig. 3. Effect of temperature on galactose efflux. Standard suspension of *S. cerevisiae*, pre-incubated with 5% (w/v) galactose for 1 hr. N_2 as gaseous phase.

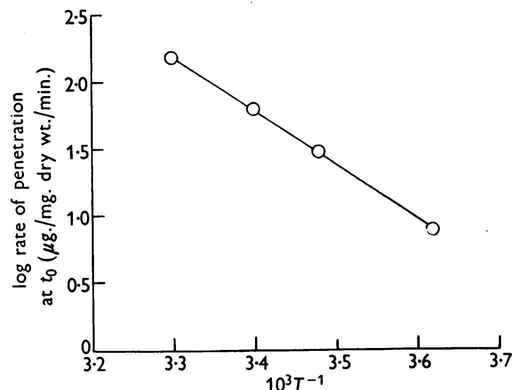

Fig. 4. Effect of temperature on galactose influx. Standard suspension of *S. cerevisiae* containing 5% (w/v) of galactose. N_2 as gaseous phase.

Table 1. *Intracellular concentration of various sugars after equilibration of yeast cells with 5% sugar solutions*

Standard yeast suspensions (3–4 mg. dry wt./ml.) were incubated with 5% (w/v) sugar solutions in a NaCl–phosphate buffer medium (see Methods) at 30° for 60 min. S.E. and range (in parentheses) are given where five or more experiments were carried out.

Yeast	Sugar	No. of expts.	Concn. of sugar (μg./mg. dry wt.)	Calculated apparent space (% of total cell vol.)
S. cerevisiae	Galactose	22	53·3 ± 2·4 (33·2–75·3)	56·4 ± 2·5
S. cerevisiae	Arabinose	5	55·9 ± 3·8 (45·6–65·0)	59·1 ± 3·95
S. fragilis	α-D-Methylglucoside	2	53·8	56·9

results taken in conjunction with those reported below are suggestive for the view that galactose, and equally some other monosaccharides, penetrate the yeast cells by a carrier mechanism.

Competitive phenomena for sugar influx into yeast cells. Further experimental evidence for a possible carrier mechanism of monosaccharide transport across the cell membrane of *S. cerevisiae* was obtained by examining the effect of glucose on the influx of galactose and some other non-utilized sugars.

Preliminary experiments showed that addition of glucose to the medium greatly reduced the influx of galactose into yeast cells, the rate of galactose penetration in the presence of 1% glucose and 5% galactose being practically nil (Table 2). This table also shows that of a number of various sugars and their derivatives only glucosamine, and to some extent also maltose, decreased the influx of galactose into *S. cerevisiae* cells, whereas the effect of α-D-methylglucoside, trehalose or phosphorylated sugars (glucose 6-phosphate and glucose 1-phosphate) was negligible.

Since under the above conditions glucose is rapidly utilized, these experiments were repeated in the presence of iodoacetate of sufficiently high concentration to prevent glucose utilization completely, i.e. 1–5 mM. The same inhibition of galactose influx by glucose was observed (Table 3). The influx of galactose at a concentration of 5% in the medium was decreased to about one-third by the presence of 0·05% of glucose; on further increasing the glucose concentration, the galactose level found in the cells after 1 hr. of incubation was still lower.

Fig. 5 shows that the influx of galactose is decreased by the presence of 5 mM-iodoacetate. Lower concentrations of this inhibitor, i.e. mM, had no such inhibiting effect. When the yeast was incubated with glucose and 5 mM-iodoacetate, glucose penetration into the cells could be readily demonstrated and the glucose influx was even higher than that of galactose under otherwise similar conditions. However, when galactose, glucose and iodoacetate were present, the influx of galactose into the cells was practically nil.

The effect of glucose on the galactose influx was also examined in a different yeast strain, i.e. *S. cartilis*, which was well adapted to the utilization of galactose, under both aerobic and anaerobic conditions. These experiments had to be carried out in the presence of iodoacetate; in the absence of this inhibitor no free galactose could be demonstrated intracellularly, the galactose penetrating into the cells being obviously immediately further metabolized.

Table 4 shows that in this yeast glucose did not appreciably affect the galactose influx. This result may suggest that the transport mechanism for galactose in *S. cartilis* differs from that of *S. cerevisiae*.

Table 2. *Effect of various sugars on galactose influx*

Standard suspensions of *S. cerevisiae* cells were incubated aerobically for 30 min. at 30° with 5% (w/v) galactose (control) or with galactose and additional sugar.

Final concn. of additional sugar	Galactose (μg./mg. dry wt.) Control	With additional sugar
1% Glucose	40·5	0·0
2% Glucose 1-phosphate	50·0	42·0
2% Glucose 6-phosphate	38·0	37·0
2% Maltose	39·5	23·7
5% Trehalose	36·1	35·1
1% α-D-Methylglucoside	28·3	28·3
1% Glucosamine hydrochloride*	34·6	12·5

* This experiment was done at pH 7·5.

Table 3. *Effect of varying glucose concentration on galactose influx*

Standard suspensions of *S. cerevisiae* cells were incubated aerobically (air) at 30° for 60 min. with 5% galactose, mM-iodoacetate and varying glucose concentrations.

Final concn. of added glucose (%)	Galactose found (μg./mg. dry wt.)
0·0	61·5
0·05	23·1
0·1	17·7
0·3	10·3

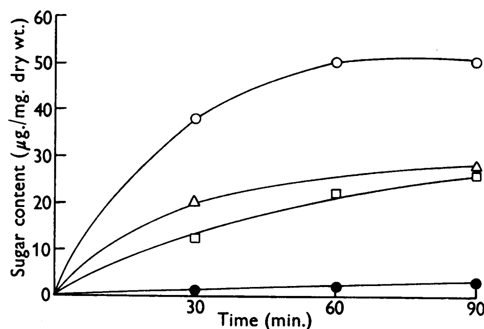

Fig. 5. Influx of galactose and glucose into *S. cerevisiae*. Suspensions of *S. cerevisiae* were incubated at 30° with air as gaseous phase. ○, Galactose (5%) in medium, galactose influx; □, 5% galactose + 5 mM-iodoacetate, galactose influx; △, 5% glucose + 5 mM-iodoacetate, glucose influx; ●, 5% galactose, 1% glucose and 5 mM-iodoacetate, galactose influx.

Effect of glucose on the efflux of galactose from cells of Saccharomyces cerevisiae. On addition of glucose to a yeast suspension which had been incubated previously for some time in the presence of galactose, and in which this sugar had penetrated into the cells, a rapid efflux of galactose from the cells occurred. If, however, iodoacetate (mM final concn.) was added simultaneously with glucose, the intracellular level of galactose did not change. These results are presented in Fig. 6. The same type of response to glucose was observed with arabinose, in the absence or presence of iodoacetate; the loss of arabinose was, however, considerably more rapid than that of galactose, so that 30 min. after the addition of glucose no arabinose could be detected in the cells.

It will be noted that under the above experimental conditions the cells lost galactose or arabinose effectively against the concentration gradient of the respective sugars.

In order to analyse this effect of glucose further under less complex conditions, experiments were carried out as described in Methods for the study of sugar efflux. In parallel experiments the efflux of galactose was studied and the effect thereon of glucose or mM-iodoacetate or both. The results of such an experiment are shown in Fig. 7. Here the efflux of galactose was followed by the measurement of both intracellular level and the amount of this sugar found in the medium; both figures agreed satisfactorily.

It will be seen that the efflux of galactose was decreased only in the presence of glucose plus iodoacetate, glucose or iodoacetate alone being without effect. No such inhibiting effect of glucose was observed when lower concentrations of iodoacetate, i.e. 0·5 mM, were used; under these conditions, also, no glucose could be demonstrated in the cells.

From these experiments it appears that glucose affects the efflux of galactose, or of arabinose, only when free glucose can be demonstrated intracellularly. This last result may explain why glucose brings about an efflux of galactose (or arabinose) from the cells, whereas glucose plus iodoacetate have no such effect, as shown in Fig. 6. Glucose in the medium, but not present intracellularly, inhibits the galactose influx without affecting the efflux; the intracellular concentration of galactose thus decreases. In the presence of iodoacetate glucose is also present intracellularly and under these conditions inhibits both influx and efflux of galactose.

Effect of preliminary aeration on the influx of galactose into yeast cells. It was considered of interest to examine whether preliminary aeration affected the galactose influx into *S. cerevisiae* cells.

Table 4. *Effect of glucose on galactose influx in* Saccharomyces cartilis

Standard suspension of *S. cartilis* cells were incubated at 30° aerobically (air) with 5% galactose + 3 mM-iodoacetate (control), and with added glucose (1%).

Time interval (min.)	Galactose found (µg./mg. dry wt.)	
	Control	Glucose added
20	26·9	23·9
40	43·1	36·6

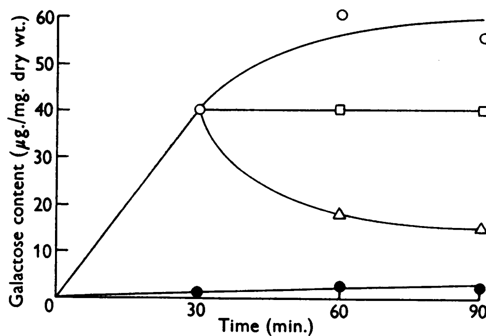

Fig. 6. Effect of glucose and iodoacetate on the transport of galactose. Suspensions of *S. cerevisiae* were incubated anaerobically at 30° with 5% galactose; at 30 min. glucose 1% (final concn.) or glucose and mM-iodoacetate was added. Galactose levels in the cells in µg./mg. dry wt. ○, Control, 5% galactose; △, glucose added; □, glucose and iodoacetate added; ●, galactose and glucose added at zero time.

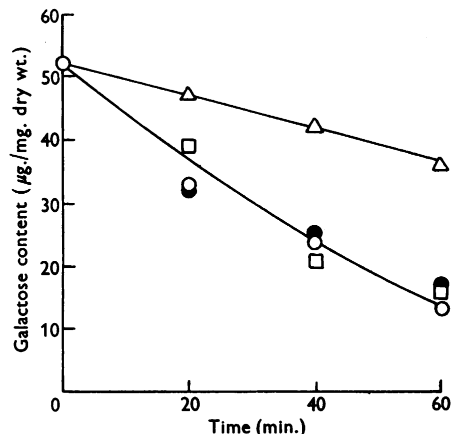

Fig. 7. Effect of glucose on the efflux of galactose. *S. cerevisiae* cells were pre-incubated with galactose, and washed and suspended in buffered saline (see Methods) and incubated at 30° anaerobically with or without additions. ●, Control; □, 1% glucose added; ○, mM-iodoacetate added; △, 1% glucose + mM-iodoacetate added.

Yeast was grown under anaerobic conditions, then centrifuged and washed, as described in Methods. Suspensions of this yeast were aerated for varying time intervals, then galactose (5% final concn.) was added and the intracellular level of this sugar after incubation for 30 min. was estimated. As shown in Fig. 8, the intracellular level decreased with increasing duration of preliminary aeration.

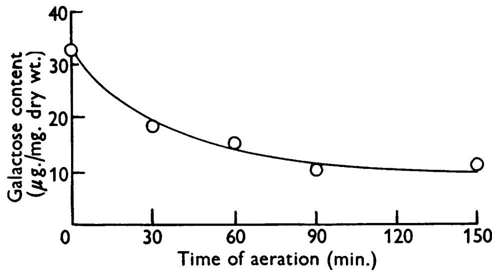

Fig. 8. Effect of aeration on galactose influx. *S. cerevisiae* cells were grown anaerobically, then washed and suspended in buffered saline, aerated for different time intervals without substrate, then galactose was added to 5% final concn. Intracellular galactose (μg./mg. dry weight) was estimated after incubation for 30 min. at 30°, with air as gaseous phase.

Table 5. *Effect of preliminary aeration on the influx of galactose*

S. cerevisiae cells, grown anaerobically, were washed; part of the cells was used for the preparation of a standard suspension (control), the other part was incubated at 30° aerobically for 12 hr. in a shaker with glucose (6%, w/v) as substrate (the glucose was not completely used up), then a standard suspension was prepared with galactose (5% final concn.) as substrate.

Time interval (min.)	Galactose found (μg./mg. dry wt.)	
	Control	Aerated yeast
20	24·8	8·1
40	41·2	24·5
60	53·0	32·0

Similar results were obtained when the preliminary aeration was carried out with glucose as substrate, as shown in Table 5.

These results suggest that aeration brings about changes of the membrane properties associated with a decreased permeability of the cell membrane for galactose. A more detailed investigation of this effect of aeration will be presented in future. The possibility has to be considered that under the above experimental conditions a loss of some nitrogeneous constituent required for the transport of galactose occurred.

Penetration of some oligosaccharides into yeast cells. It has been shown above that monosaccharides can be demonstrated inside yeast cells under conditions when they are not further metabolized. It was therefore considered of interest to compare the penetration of some oligosaccharides into yeast cells and thus to obtain further information on the mechanism of sugar penetration. As with monosaccharides, no intracellular sugar could be demonstrated where this was appreciably metabolized.

Table 6 shows that no penetration of lactose into cells of *S. cerevisiae*, nor of maltose into *S. fragilis*, could be demonstrated, although these yeasts do not metabolize the respective sugars. The mechanism of penetration of these sugars thus appears to differ from that of galactose.

On the other hand, it will be noted that, as opposed to *S. cerevisiae*, the influx of α-D-methylglucoside could be demonstrated in *S. fragilis*. The penetration of α-D-methylglucoside into the latter yeast was decreased by the presence of glucose, which possibly suggests a common transport mechanism for both these sugars. Since maltose penetration into this yeast could not be demonstrated, it would appear that the mechanism of transport of methylglucoside across the cell membrane of *S. fragilis* differs from that of maltose.

A considerable apparent permeability of the cell membrane of *S. cerevisiae* for trehalose follows from the observation that on incubation of this yeast, grown under anaerobic conditions with trehalose

Table 6. *Comparison of influx of various sugars into cells of* Saccharomyces cerevisiae *and* Saccharomyces fragilis

Standard suspensions were incubated at 30° aerobically for 60 min. with various sugars (5% final concn.).

Sugar tested	Addition	Intracellular concn. (μg./mg. dry wt.)	
		S. cerevisiae	*S. fragilis*
Galactose	—	40·2	47·8
α-D-Methylglucoside	—	0	53·0
α-D-Methylglucoside	1% Glucose	—	0
Maltose	—	0	0
Lactose	—	0	0
Trehalose	—	120*	0

* The initial amount of trehalose present in *S. cerevisiae* cells (63·6 μg./mg.) was deducted.

the intracellular level of this sugar considerably increased, whereas no increase was observed in the presence of glucose as substrate (see Fig. 9). The relatively high initial level of trehalose reflects the fact that this oligosaccharide is a normal metabolic constituent of the yeast cells. Further experiments, not reported here in detail, showed that on incubation of yeast in which trehalose had accumulated, no efflux of this sugar could be detected. No suggestion can be made at present as to whether this last observation, and also the fact that the apparent intracellular concentration of trehalose after incubation with this sugar was higher than that of the medium (Table 6), may be due to an intracellular fixation of trehalose or to a specialized transport mechanism across the yeast cell membrane.

DISCUSSION

Rate of penetration of monosaccharides into yeast cells. It has been shown that the maximum rate of galactose (or arabinose) penetration into *S. cerevisiae* was of the order of 120 μg./mg. dry wt./hr. How does this rate compare with the maximum observed rate of galactose utilization in this yeast? In yeast cells partly adapted to the utilization of galactose by aeration with this sugar for 24–48 hr., the following Q_{O_2} values were found: with galactose, 24, without substrate, 12, corresponding to a galactose-utilization rate of about 30 μg./mg. dry wt./hr., assuming here that in the presence of galactose the utilization of endogenous substrate was completely suppressed. It thus follows that under given experimental conditions the galactose influx was more than sufficient to account for the rate of metabolism of galactose. This conclusion is also borne out by the observation (to be published) that in *S. cerevisiae* R XII, fully adapted to the utilization of galactose, this sugar in an apparently free state could be demonstrated intracellularly during the course of galactose utilization; it would appear that here also the influx of galactose exceeded the rate of utilization of this sugar.

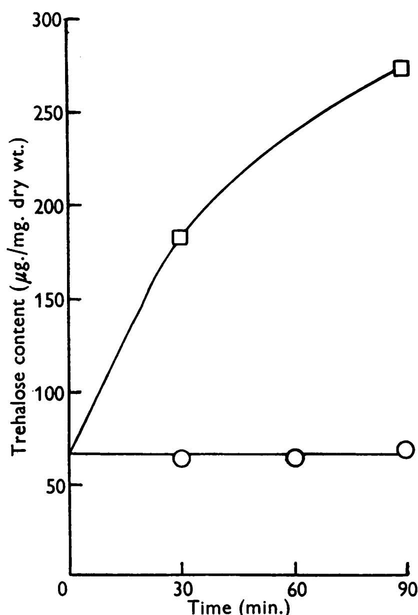

Fig. 9. Trehalose influx into *S. cerevisiae*. Yeast was grown anaerobically, and then the standard suspension was prepared and 5% of trehalose (□) or 1% of glucose (○) added.

The observation that glucose influx appears to be somewhat greater than that of galactose under specified conditions (Fig. 5) suggests that the mechanism transporting glucose across the cell membrane may be responsible for a considerable portion of the rate of metabolism of this sugar in *S. cerevisiae*.

Mechanism of transport of monosaccharides across the yeast cell membrane. The views on the mechanism of monosaccharide transport across the cell membranes vary with different organisms in which this problem has been studied. Whereas some authors maintain that glucose is transported by a phosphorylating mechanism, located in the cell membrane (e.g. Rothstein, 1956, for yeast cells), others suggest in *Escherichia coli* an active transport mechanism for oligosaccharides with the participation of a permease (see Cohen & Monod, 1957). Finally, some authors suggest a facilitated diffusion mechanism for the permeability of erythrocytes to glucose (e.g. Stein & Danielli, 1956), which may in some respects be considered to be equivalent to a carrier-linked mechanism.

The results reported above appear to exclude a phosphorylating mechanism of galactose (or arabinose) transport in yeast cells, since this sugar was found intracellularly in an apparently free, i.e. non-phosphorylated, state, and since no phosphorylation could be directly demonstrated by changes of the steady-state level of inorganic phosphorus in the yeast cells on addition of galactose (Dr A. Kotyk, personal communication). On the basis of a phosphorylating transport it would also be rather difficult to explain the same order of influx of both galactose and arabinose, no evidence being available that the latter sugar is metabolized by yeast cells. Apparently free glucose could also be demonstrated intracellularly when the further metabolism of this sugar was completely inhibited by iodoacetate. This finding may suggest that at least a portion of glucose entering *S. cerevisiae* cells may do so by a non-phosphorylating mechanism. The possibility cannot be excluded at present that free glucose may be secondarily produced in the cells by the action of a phosphatase on primarily

formed glucose phosphate. Such a sequence of events, however, appears to be less likely for the following reasons. First, 1 mole of high-energy phosphate would be required/mole of glucose transported across the cell membrane; this is difficult to visualize since with anaerobic conditions in the presence of iodoacetate little high-energy phosphate would be available in the cells, and also since it was found in experiments not reported here in detail that the intracellular level of glucose was linearly related to the glucose concentration in the medium (1 and 5%, w/v); secondly, it would also be rather difficult then to explain the competition between glucose and galactose or arabinose for the penetration into the cells, glucose phosphates having no such effect.

The suggestion was made above that the transport of D-galactose, D-arabinose and also of some portion of glucose across the yeast cell membrane is carrier-linked, and not due to diffusion only. This suggestion is based on the following evidence: (1) The Q_{10} values for both influx and efflux of galactose or arabinose were of the same order, i.e. 2·4–2·9, thus being considerably higher than would be expected for a simple diffusion process. (2) Competition between various hexoses for the transport mechanism, as shown above, is hardly compatible with transport due to diffusion. However, it should be recalled that under specified conditions galactose and arabinose were transported effectively against a concentration gradient; in view of the rather complex conditions this last result cannot be taken as evidence for an active transport mechanism. Further experiments, especially at lower concentrations of sugar, and the measurement of the kinetics of galactose influx at various concentrations of this sugar in the medium, might help to elucidate further the transport mechanism. It should be mentioned that a carrier-linked transport of monosaccharides (or facilitated diffusion) has been postulated as the transport mechanism in a number of different cells, especially in erythrocytes, both on kinetic grounds (see Bowyer & Widdas, 1956; Wilbrandt, Frei & Rosenberg, 1956; Stein & Danielli, 1956) and on the basis of competitive phenomena of hexose influx (LeFevre & Davies, 1951).

The observation that glucose competes with galactose or arabinose for the influx or efflux across the cell membrane of *S. cerevisiae* cells suggests that the same carrier mechanism is responsible for the transport of the above monosaccharides. From the data presented on the effect of various concentrations of glucose on galactose influx it may be calculated, with the equation of Krebs & Johnson (1948), that the affinity of the postulated carrier is about 100 times as high for glucose as for galactose. No suggestion other than a speculative one can at present be offered as to the character of the carrier system. However, the possible participation of a (phosphorylated? see Lipmann, 1954) hexokinase as carrier in the yeast cell membrane, acting in both directions, might not be excluded in view of the relatively low specificity of this enzyme (Stumpf, 1954) and also since in the above experiments galactose influx was inhibited by those sugars which are known to act as substrates for yeast hexokinase, i.e. glucose and also glucosamine (Brown, 1951). The fact would then have to be explained that yeast hexokinase does not appear to catalyse the phosphorylation of galactose or arabinose.

If it is allowable to argue from the competition between various sugars for the penetration across the cell membrane of *S. cerevisiae* by a common carrier system, it would follow that the membrane of this yeast possesses a carrier capable of transporting glucose, galactose and arabinose. On the other hand, in *S. fragilis*, α-D-methylglucoside appears to be transported by the same mechanism as galactose and glucose, the mechanism of maltose transport being different. In *S. cartilis*, galactose influx was not affected by glucose. Thus differences appear to exist in the specificity of the carrier system or in the mechanism of transport of monosaccharides in various yeasts.

Metabolic aspects of the transport of monosaccharides in yeast cells. Here two aspects will be considered, i.e. the described competitive phenomena and the effect of aerobiosis of yeast on galactose influx.

Monod (1942) described the phenomenon of diauxie in micro-organisms, i.e. that in the presence of two utilizable substrates one substrate after the other is utilized for growth, and the view was advanced that this phenomenon is due to the formation of adaptive enzymes. A similar phenomenon has been shown for a resting culture of the yeast *Rhodotorula gracilis* (Kleinzeller, Málek, Praus & Škoda, 1952) where yeast, previously adapted to the utilization of xylose, in the presence of both glucose and xylose consumed first glucose and only then xylose. It appears that diauxie may find its explanation—at least where monosaccharides are concerned—in the competition of various sugars for penetration into the cells.

The observation that the rate of penetration of galactose was affected by the previous aerobic or anaerobic conditions of the yeast cell, as shown above, might have a bearing on the understanding of the Pasteur effect in yeast. It has been repeatedly pointed out (see, for example, Stickland, 1956) that the Pasteur effect is characterized by a decreased utilization of substrate under aerobic conditions; the decreased permeability of the cell membrane under aerobic conditions might thus contribute to this complex regulating mechanism.

SUMMARY

1. The permeability of the cell membranes of some yeast cells for various mono- and di-saccharides has been studied.

2. The influx of D-galactose and D-arabinose into the cells of *Saccharomyces cerevisiae* followed an exponential curve; an apparent equilibrium concentration of these sugars in the cells was reached within 60 min.

3. Free glucose could be demonstrated intracellularly when the metabolism of this sugar was inhibited, e.g. by mM-iodoacetate.

4. The maximum rate of penetration of D-galactose into *S. cerevisiae* cells was $2{\cdot}06 \pm 0{\cdot}2$ μg./mg. dry wt./min. This rate was sufficient to account for the metabolism of galactose in this yeast, partly adapted to the utilization of galactose by aerobic incubation with this sugar.

5. The apparent galactose or arabinose space in *S. cerevisiae* was found to be 56·4 and 59·1% respectively, and the apparent α-D-methylglucoside space in *S. fragilis* was found to be 56·9% of the cell volume. In the calculation of the apparent sugar space no account was taken of the considerable changes of the intracellular water volume on addition of sugar.

6. The Q_{10} values for both influx and efflux of D-galactose in *Saccharomyces cerevisiae* cells was of the same order, i.e. 2·9 and 2·4 respectively; at a temperature of 1° practically no fluxes of sugar were observed.

7. The influx of D-galactose or D-arabinose into *S. cerevisiae* was inhibited by the presence of glucose and glucosamine.

8. The efflux of D-galactose from *S. cerevisiae* cells was not inhibited by glucose unless glucose could be simultaneously demonstrated intracellularly in the presence of 1–5 mM-iodoacetate.

9. Preliminary aeration of anaerobically grown *S. cerevisiae* cells decreased the influx of D-galactose.

10. The permeability of the cell membrane of *S. fragilis* for α-D-methylglucoside, but not for maltose, and of *S. cerevisiae* for trehalose, was demonstrated.

11. The results obtained are considered from the point of view of a carrier-linked transport of D-galactose and D-arabinose across the cell membrane of *S. cerevisiae* cells, with glucose acting as a competitive inhibitor of considerably higher affinity for the carrier than galactose.

12. The possible significance of the results for the phenomenon of diauxie is discussed.

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