ACCUMULATION OF TREHALOSE AND SUCROSE IN RELATION TO THE METABOLISM OF α-GLUCOSIDES IN YEASTS OF DEFINED GENOTYPE

GAD AVIGAD*

Laboratory of Microbiological Chemistry, Department of Biochemistry, Hebrew University, Hadassah Medical School, Jerusalem (Israel)

(Received July 21st, 1959)

SUMMARY

1. Glucose-grown haploid yeast hybrids lacking the ability to ferment sucrose and affording an extract devoid of sucrose activity failed to accumulate [^{14}C]maltose or methyl-α-glucoside but accumulated [^{14}C]sucrose up to 3 % of cell dry weight. The accumulated [^{14}C]sucrose could be removed by incubation with [^{12}C]glucose or fermentable α-glucosides. Its accumulation was inhibited by 10^{-3} *M* NaN_3 or 10^{-3} *M* 2,4-dinitrophenol.

2. Although the cells when grown on maltose or methyl-α-glucoside contained an α-glucosidase which can hydrolyse sucrose, they failed to ferment or hydrolyse sucrose. They accumulated sucrose as did glucose grown cells. The possibility that a fragile intracellular barrier separating sucrose from the α-glucosidase exists in these cells is considered.

3. The bulk of radioactivity fixed by cells incubated with ^{14}C fermentable sugars (glucose, maltose, methyl-α-glucoside) was shown to reside in trehalose. In washed cells the amount of the accumulated trehalose is fairly stable but a rapid turnover and a small accretion of trehalose occurs in the presence of the fermentable sugar.

INTRODUCTION

In a study of carbohydrases in *Saccharomyces* haploid stocks of defined genotype, HESTRIN AND LINDEGREN[2,3] found that cells grown on maltose or methyl-α-glucoside, failed to hydrolyze sucrose though the presence of sucrase activity in the cells could be demonstrated after drying or ageing. Selective permeability of cell membranes has been proposed as one of the possible explanations for this phenomenon of "cryptic" enzymic activity. Further studies of these haploids have indicated that the enzyme responsible for the hydrolysis of sucrose is the same α-glucosidase which hydrolyzes methyl-α-glucoside and/or maltose and that its formation and specificity is controlled by the yeast's genotype and the growth sugar[4]. The fact that intact cells metabolize methyl-α-glucoside and maltose but not sucrose although they contain a single enzyme

* This study is part of a Ph. D. thesis submitted to the Senate of the Hebrew University, May 1958. An abstract presenting part of the results has appeared[1].

which can split these α-glucosides is consistent with the presence of a selective barrier which prevents sucrose from access to the enzyme site within the cells.

Recent work has presented evidence to the presence of specific "permease" systems which control the penetration of various substrates into cells[5]. ROBERTSON AND HALVORSON[6] have suggested the presence of an α-glucoside permease in yeasts. It seemed therefore of interest to study aspects of permeation and accumulation of α-glucosides in haploid yeasts of defined genotype in relation to the genetic constitution and to the contents and specificity of the cells inducible α-glucosidases. This communication presents results of experiments exploring these relationships.

MATERIALS AND METHODS

Yeasts: The haploid strains MA MG su mz (12836) and MA mg su mz (8293) of the Carbondale stock[3] were grown for 32 h on a rotatory shaker at 30° in 1 l batches of nutrient medium[2] containing 4 % of the desired sugar. Cells were washed three times with cold distilled water and were finally suspended in water to give 30–40 mg dry wt./ml. The cells were kept at 4° and used within 24 h except when stated otherwise.

Enzymes: Invertase concentrate was obtained from the British Drug House and diluted 1:10 in 0.1 *M* acetate buffer pH 5.0.

Levansucrase was an extract prepared from *Aerobacter levanicum*[7] cells disrupted mechanically by shaking in the Nossal disintegrator[8] for 30 sec.

Sugars: ^{14}C uniformly labelled sugars, viz. glucose, sucrose and maltose, were purchased from the Radiochemical Centre, Amersham, Bucks. Methyl-α-[^{14}C]glucoside (9.1 mC/mmole; m.p. and mixed m.p. 162°) was synthesized on a microscale from ^{14}C uniformly labelled glucose according to HELFERICH AND SCHAFER[9].

Chromatographic methods: Whatman No. 1 filter paper and the following solvent systems were employed: (a) upper layer of *n*-butanol–acetic acid–water (4:1:5, v/v); (b) *n*-butanol–ethanol–water (5:2:2 v/v). Sprays used for detection of sugar spots were: (a) *p*-anisidine–HCl for reducing sugars and β-fructosides[10]; (b) urea–phosphoric acid for ketoses[11]; (c) aqueous alkaline triphenyltetrazolium chloride for reducing sugars[12]; (d) alkaline silver nitrate for reducing and non-reducing carbohydrates[13,14]; (e) 0.5 % ninhydrin in acetone for the detection of amino acids.

Radioautograms for detecting ^{14}C compounds on paper chromatograms were prepared by a 7-day exposure to an X-ray sensitive film. This technique enabled a margin of detection of 0.5 mμC/cm^2 of filter paper.

Quantitative methods: Total hexose content was determined by the anthrone[15] or by the phenol–sulphuric acid[16] methods. Sucrose was determined by the resorcinol method for fructose[17].

Measurements of ^{14}C accumulated in washed cells

A cell suspension (containing 9–15 mg dry wt./ml)) was incubated in 0.033 *M* K-Na phosphate buffer pH 6.8 and 0.166 *M* ^{14}C sugar (0.10–0.15 μC/ml) at 30° in a water bath with gentle agitation. Samples (1–2 ml) were withdrawn at various time intervals and the cells were collected with suction on a 1″ millipore filter disc of 0.8 μ porosity (AA filter, Millipore Filter Co., Bedford, Mass.). The cake of cells was washed with two volumes of cold 0.033 *M* phosphate buffer, pH 6.8. After the addition of two drops of 15 % glycerol the disc was immediately dried under an infrared lamp,

mounted on E-7A rings (Tracerlab Inc., Boston, Mass.) and measured in a conventional Geiger counter. Time needed to prepare a dry sample by this procedure did not exceed 3 min. The value of actual radioactivity was obtained after corrections, taking into account the activity of a zero time control and the value of self absorption (read from a calibration curve obtained by determining the decrease of emission in relation to the amount of dry cells on the disc).

Measurements of the water content of the yeast cells

The water space of the yeast cake was measured by a modification of known methods[18] so as to enable the use of a small amount of cells. The determination was performed as follows: A sample of a washed cell suspension (equivalent to about 20–30 mg dry wt.) in 0.033 *M* phosphate buffer pH 6.8 was collected by suction on an AA (1″ diameter) millipore filter disc of known weight. The cell cake was washed with 5 ml of a 4.0 % solution of inulin and dried at 80° over P_2O_5 *in vacuo* for 10 h. The discs were weighed accurately and the dry weight of the sample and the total water content of the pressed cake (76 ± 1.5 % of its fresh weight) was obtained by difference. The dried cells were dispersed in 2 ml water and the inulin content was determined colorimetrically[17]. A sample of dried cells washed with water instead of the inulin solution served as a blank. The inulin space thus obtained was found to be 15 ± 1 % of the fresh cake weight and the weight of intracellular water calculated by difference was found to be 71.0 ± 0.8 % of the yeast fresh weight. The values presented were obtained with many samples of fresh MA MG or MA mg cells which were grown on glucose, maltose or methyl-α-glucoside.

EXPERIMENTAL

Fixation of ^{14}C by yeast cells incubated with ^{14}C sugars

The results presented in Fig. 1 show that after contact with ^{14}C fermentable sugar the cells fixed an amount of ^{14}C equivalent to 4–5 % of their dry wt. (calculated as glucose). The ^{14}C fixed could not be removed from the cells by repeated washing with cold water or a phosphate buffer of pH 6.8. Under these conditions no ^{14}C was fixed by cells unable to metabolize methyl-α-glucoside or maltose when allowed contact with these ^{14}C sugars. Only prolonged incubation (> 1 h) which might favour induction towards the utilization of these α-[^{14}C]glucosides resulted in a slow increase in ^{14}C uptake. It can be seen (Fig. 1) that the rate of net increase of ^{14}C in the cells on contact with a fermentable sugar falls gradually until it levels off to a very low value. Though MA MG cells grown on maltose fermented methyl-α-glucoside at about 1/5th the rate of maltose[3] the uptake of ^{14}C in presence of [^{14}C]maltose was about 30 % of the total amount fixed in presence of methyl-α-[^{14}C]glucoside under the same conditions of test. MA mg cells grown on maltose, *i.e.* cells which do not ferment methyl-α-glucoside nor can be induced to its utilization as a substrate, showed no uptake of radioactivity when in contact with methyl-α-[^{14}C]glucoside.

On contact with [^{14}C]sucrose an uptake of ^{14}C, which could not be removed by repeated washings, occurred. This intracellular ^{14}C uptake reaches a maximal constant value after about 1 h of incubation and was equal (when calculated as glucose) to 1.5–3.0 % of the cells' dry wt. It is interesting to note that this ^{14}C fixation in presence of sucrose occurred both in the case of glucose and α-glucoside grown cells. It will be

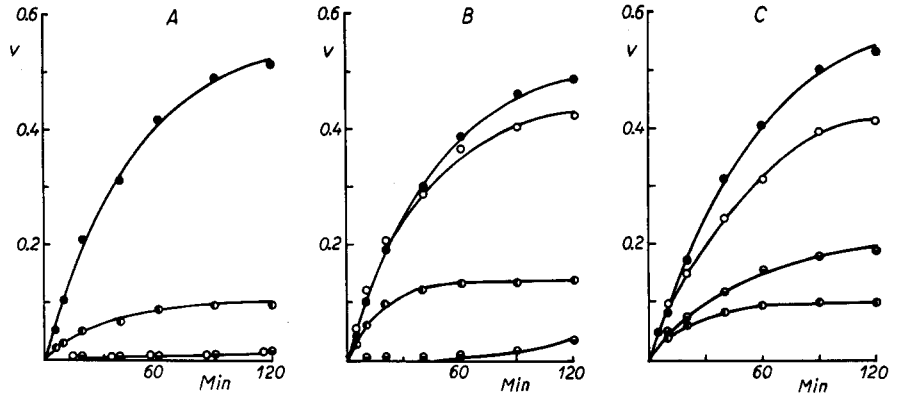

Fig. 1. Uptake of ^{14}C sugars into washed yeast cells. MA MG cells suspension was incubated under standard conditions and samples withdrawn for analysis of ^{14}C uptake as described under METHODS, v = μmoles substrate fixed/mg dry cells; ●, ○, ◓, ◐, represent [^{14}C]glucose, methyl-α-glucoside, maltose and sucrose respectively; A, glucose grown cells; B, methyl-α-glucoside grown cells; C, maltose grown cells.

recalled that in all of these cases the intact cells cannot hydrolyze sucrose though an α-glucosidase which hydrolyzes sucrose can be extracted from α-glucoside grown cells[3,4]. The total amount of ^{14}C from sucrose fixed by glucose grown cells was usually somewhat lower than the amount fixed by those grown on an α-glucoside but no constant ratio could be established.

Washed cells kept in suspension at 4° were tested at several time intervals during 14 days for their ability to fix ^{14}C when in contact with ^{14}C sugars for 1 h under the standard conditions. Only a slight decrease of ^{14}C fixation (> 20 %) was found towards glucose, sucrose and methyl-α-glucoside, while with maltose the rate of ^{14}C fixation decreased at a gradual rate down to 30 % of the initial value. This phenomenon was found to accompany a decrease in maltase activity found during the ageing of the yeast suspensions (*cf.* ROBERTSON AND HALVORSON[6]). Added inorganic phosphate in the range of 0.12–0.007 *M* did not alter appreciably the amount of ^{14}C fixed by fresh washed cells.

Identification of the accumulated ^{14}C compounds

More than 80 % of the radioactivity acquired by the cells after 30 min of contact with ^{14}C fermentable sugar and all of that acquired after contact with [^{14}C]sucrose could be extracted with 5 % cold trichloroacetic acid (TCA). Most of the residual ^{14}C could be extracted with alkali at room temperature (Table I).

Cold TCA extracts were repeatedly extracted with ether, neutralized and concentrated *in vacuo*. Chromatographic analysis showed that except for traces of a very slow moving reducing component (probably a hexose phosphate) which corresponds to less than 1 % of the total sugar content when analyzed quantitatively, only a single sugar component was detected in the extracts prepared from the various cells samples. This non-reducing compound was indistinguishable from trehalose in its chromatographic behaviour. Extracts of washed cells which were in contact with sucrose showed in addition to the trehalose spot a non-reducing ketose spot which behaved chromatographically like sucrose. An amount of about 15 mg of this sugar was eluted after

TABLE I

EXTRACTION OF FIXED ^{14}C

MA MG cells grown on maltose were incubated 30 min in standard conditions with ^{14}C sugars. Cells were washed and dispersed in a cold 5 % TCA solution equal to the initial volume and shaken 30 min at 4°. Cells were collected by centrifugation, washed with 5 % cold TCA, extracted 30 min with 10 % NH_4OH at 30°, and precipitated again. Samples of the supernates and cells were taken to measure ^{14}C.

		^{14}C substrate		
		Glucose	Methyl-α-glucoside	Sucrose
Amount of ^{14}C fixed*		205	110	51
Percentage of ^{14}C in fraction	Cold TCA	85	82	95
	NH_4OH	6	10	2
	Cell debries	5	11	0

* μmole substrate/g dry cells.

quantitative separation on sheets of filter paper. A sample treated with yeast invertase resulted in total hydrolysis to glucose and fructose as revealed by paper chromatography. A second sample was tested with levansucrase[7] and found to serve as a substrate for the formation of levan, glucose and fructose.

In addition, the chromatographic analysis of concentrates of the cold TCA extracts revealed the presence of several amino acids. Major components were identified as glutamic acid, alanine and lysine. Aspartic acid, arginine, serine and histidine were present in smaller quantities whereas about 7 other amino acids appeared in trace amounts.

Isolation of crystalline trehalose

Washed yeast cells (equivalent to about 2 g of dry wt.) were extracted for 30 min with cold 5 % TCA. After four extractions with ether the concentrated solution was applied to a 1.8 × 15 cm Darco G-60; Celite 535 column[19], washed with 500 ml water and then eluted with 500 ml 10 % ethanol. The alcoholic solution was reduced in volume in vacuo at 40°, passed through a small column of Amberlite MB-1 mixed-bed resin, and concentrated under reduced pressure into a syrup which crystallized from ethanol at 4°. Yields obtained from several yeast samples varied from 4.3 to 5.8 % of crystals with respect to cell dry weight. Recrystallization gave a material with $[\alpha]_D^{20} = +178°$ (c = 2, in H_2O); m.p. and mixed m.p. with authentic αα-trehalose dihydrate: 95–96°.

Location of the radioactivity in the TCA extracts

Radioautograms made from chromatograms which resolved the TCA extract of cells previously kept in contact with ^{14}C fermentable sugars for 60 min showed the radioactivity mainly at the level of the trehalose spot, in addition to a small activity in the glutamic acid and alanine spots. Quantitative studies, such as those presented in Table II show that 75–85 % of the ^{14}C found in the cold TCA extracts was recovered in trehalose and 9–15 % in amino acids. In the case of ^{14}C fixed sucrose, the only radioactive spot found in the cold TCA extracts was that of the sucrose spot, which on quantitative analysis proved to contain all of the ^{14}C fixed in the cells (Table II).

TABLE II

QUANTITATIVE ANALYSIS OF THE COMPONENTS OF THE COLD TCA EXTRACTS

An 8-ml suspension of MA MG maltose or methyl-α-glucoside grown cells (120 mg dry wt.) were incubated with 0.066 M ^{14}C sugar (about 5 mμC/μmole) at 30° for 60 min. Cells collected by centrifugation, washed repeatedly with cold 0.03 M pH 6.5 phosphate buffer and extracted with 5% cold TCA as described under Table I. After extractions with ether the TCA solutions were neutralized, concentrated and resolved quantitatively by paper chromatography. The position of ^{14}C compounds was located by radioautography and then eluted into counting planchets. This eluate after drying and counting was dissolved in a known volume of water in order to make quantitative analysis.

^{14}C substrate	*(a) Per cent of fixed ^{14}C extracted with cold TCA*	*Isolated ^{14}C (per cent of (a))*			
		Alanine	*Glutamic acid*	*Trehalose**	*Sucrose**
Glucose	74.0	4.5	3.9	87.4 (1.05)	—
Methyl-α-glucoside	73.4	6.0	10.5	77.0 (0.92)	—
Maltose	85.2	4.8	5.6	82.0 (0.66)	—
Sucrose	98.0	0	0	0 (0)	92.0 (0.94)

* Numbers in parenthesis indicate the specific radioactivity attained in the isolated material in relation to the external ^{14}C sugar employed.

Influence of various inhibitors on the ^{14}C uptake

The rate of ^{14}C uptake by washed cells in contact with either ^{14}C fermentable sugar or sucrose, could be inhibited up to 90% by $1.6 \cdot 10^{-3}$ M 2,4-dinitrophenol or sodium azide and to about 30% by $1.6 \cdot 10^{-3}$ M NaF. 0.014 M tris (hydroxymethyl) aminomethane which is a powerful competitive inhibitor of the α-glucosidase of these cells[4], did not inhibit either the fermentation or the ^{14}C uptake by intact cells. Several detergents such as cetyltrimethylammonium bromide (Cetavlon) and sodium monolauryl sulphate (Duponol) at $2 \cdot 10^{-3}$ M completely inhibited ^{14}C fixation. Sodium dodecyl sulphate and a polyoxyethylene sulphonate ether (Triton 720, Rohm and Hass Co.) at these concentrations inhibited only to a smaller extent (20–30%). Neutral surface active agents such as polyethenoxy ether (Triton x-100, Rohm and Haas Co.) and polyoxyethylene sorbitan mono-oleate (Tween 80, Atlas Power Co.) at 0.15% did not show inhibition nor a significant increase in the amount of ^{14}C fixed. It should be noted that Cetavlon at $2 \cdot 10^{-3}$ M stopped the fermentation of external sugars and microscopic examination showed incipient cell lysis[23]. In addition, treatment of ^{14}C labelled washed cells with 10^{-2} M Cetavlon resulted in the total extraction of the [^{14}C]-trehalose or sucrose[15].

Effect of ^{12}C sugars on the accumulation of ^{14}C trehalose and sucrose

From experiments such as those presented in Fig. 2 it could be seen that any dilution of the external ^{14}C fermentable sugar by the same or other ^{12}C fermentable sugars, caused a rapid decrease in the ^{14}C content of the cells. This decrease reached 80–90% of that expected from the dilution of the external ^{14}C substrate. It is evident that dilution with [^{12}C]maltose or methyl-α-glucoside usually proceeded more slowly than with glucose. [^{12}C]sucrose could dilute only the accumulated [^{14}C]sucrose but not the accumulated [^{14}C]trehalose, whereas any ^{12}C fermentable sugar caused rapid elimination of the [^{14}C]sucrose accumulated in the cells (Fig. 2). Some preliminary experiments indicated that fermentable sugar (*e.g.* glucose) caused the release of intact accumulated sucrose from the cells into the medium.

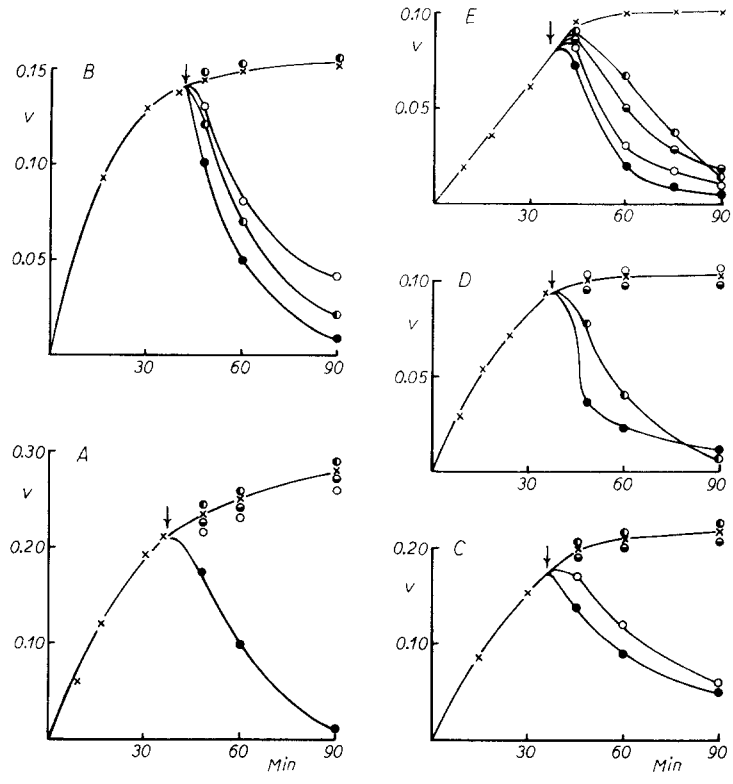

Fig. 2. Dilution of fixed ^{14}C by ^{12}C sugars. MA MG cells were incubated in standard conditions with 0.066 *M* ^{14}C sugars and samples withdrawn for ^{14}C analysis as described under METHODS. At time indicated by an arrow a concentrated solution of ^{12}C sugar was added to bring its concentration to 0.118 *M* and that of the ^{14}C sugar to 0.059 *M*. The values given for homologous pair of ^{12}C–^{14}C sugar are those of the experimental values corrected according to the percentage of maximal dilution expected; v = μmoles substrate fixed/mg dry wt.; x, ^{14}C uptake; ●, ○, ◒, ◑, residual ^{14}C in presence of [^{12}C]glucose, methyl-α-glucoside, maltose and sucrose respectively; A, glucose grown cells on [^{14}C]glucose; B, maltose grown cells on [^{14}C]maltose; C, methyl-α-glucoside grown cells on methyl-α-[^{14}C]glucoside; D, glucose grown cells on [^{14}C]sucrose; E, maltose grown cells on [^{14}C]sucrose.

Turnover and net synthesis of trehalose

The amount of trehalose found in fresh harvested washed cells was fairly constant (Table III) and decreased to a limited extent (< 20 %) during two weeks storage of a thick aqueous suspension at 4° either aerobically or anaerobically. It was also found that cells which accumulated [^{14}C]trehalose and were then washed free from the external sugar, lost about 20–30 % of the fixed ^{14}C within one hour but retained the rest of ^{14}C during 48 h at 30°. Likewise, the [^{14}C]sucrose accumulated in the cells showed no decrease during that period.

Incubation of the cells for periods of up to 1 h with a fermentable sugar caused an increase of 30–40 % in the net amount of trehalose (Table III). When ^{14}C sugars were used, the trehalose was rapidly labelled up to a specific radioactivity equal to that of the external ^{14}C substrate (Table II). It was found that the amount of [^{14}C]-hexose which was fixed aerobically by the resting cells during 1 h at 30° was about 15 % of the total sugar utilized (when related to the CO_2 produced under the same

TABLE III

CHANGES IN THE AMOUNT OF TREHALOSE AFTER CONTACT WITH ^{14}C SUGARS

MA MG cells were incubated 60 min under standard condition with ^{14}C sugars, washed and extracted with cold TCA as described under Tables I and II and the amount of trehalose was determined colorimetrically. Numbers indicate μmoles hexose/g dry cells.

Growth sugar	*(a) Initial trehalose in washed cells*	*Δ trehalose in relation to (a) after incubation with substrate*				*Δ sucrose*
		Glucose	*Methyl-α-glucoside*	*Maltose*	*Sucrose*	*Sucrose*
Glucose	232	+ 92	— 6	—12		+32
Methyl-α-glucoside	260	+ 82	+ 88	— 6	—11	+48
Maltose	264	+120	+116	+74		+42

conditions). The fact that at least 60 % of this ^{14}C assimilation represents turnover means that net increase of the trehalose content amounts to only about 5 % of the external sugar utilized by the resting cells.

Effect of substrate concentration on the rate of trehalose and sucrose accumulation

The uptake of ^{14}C by cells in contact with ^{14}C fermentable sugar was found to conform with the MICHAELIS-MENTEN relationship. The K_m was 0.08 M for glucose, maltose or methyl-α-glucoside as substrates (Fig. 3). This value may be taken as that of a rate limiting reaction concerned with trehalose synthesis or breakdown. It should be mentioned here that the K_m for maltose and methyl-α-glucoside as substrates of the α-glucosidase in these cells varied between 0.07–0.22 M[4], whereas K_m of fermentation in yeasts, as measured by CO_2 formation, is about ten times smaller[21,22]. ROBERTSON AND HALVORSON[6] found a K_m of 0.12 M for fixation of cold TCA extractable ^{14}C by yeast cells in contact with methyl-α-[^{14}C]glucoside.

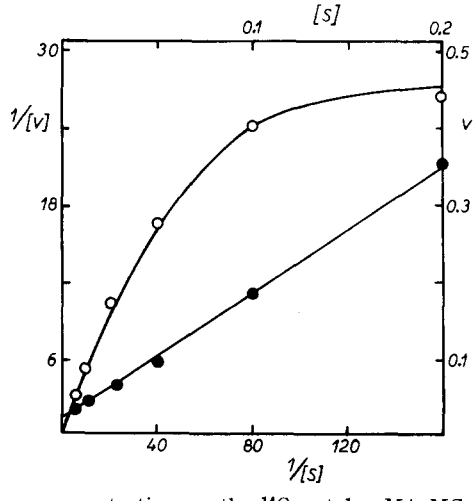

Fig. 3. Influence of sugar concentration on the ^{14}C uptake. MA MG maltose grown cells were incubated 30 min under standard conditions with varying concentration of [^{14}C]glucose and samples taken for analysis of ^{14}C uptake as described under METHODS. S = substrate concentration; v = μmole substrate fixed/mg dry cells; ○, rate of ^{14}C uptake; ●, reciprocal values of ^{14}C uptake. Results in experiments with methyl-α-glucoside or maltose as substrates, yielded similar curves.

In contrast to the process of trehalose synthesis, accumulation of [^{14}C]sucrose was found to be a linear function of the external sucrose concentration within a wide range (0.02–0.40 *M*).

The concentration of trehalose and sucrose in the cells

Since the analysis of the water content has shown that in the washed cells there is an equivalent of 2.45 g of intracellular water per gram of dry matter, the concentration of trehalose or sucrose accumulated can be calculated assuming that they are homogeneously dissolved in the total water space of the cells. From the trehalose found in many samples of washed cells (aged 4–24 h) the values calculated were 0.050 ± 0.002 *M* for the initial and 0.075 ± 0.007 *M* for the trehalose concentration after 60 min contact with 0.166 *M* glucose or fermentable α-glucoside. While the initial concentration of sucrose in the cells was zero, it reached a value of 0.018 ± 0.02 *M* after 60 min of incubation with 0.166 *M* sucrose. It should be pointed out, however, that since these sugars are probably stored in discrete compartments within the cell and not in the whole intracellular water space, the values calculated above represent minimum values for the concentration of trehalose and sucrose accumulated by the cells.

DISCUSSION

In the light of recent evidence on the presence of specific permeases in microorganisms (*cf.* Review by COHEN AND MONOD[5]) and the suggestion that yeasts, possess a similar system controlling the entry of α-glucosides into the cells[6], the possibility could be entertained that some of the genes known to govern the utilization of glucosides (*i.e.* the MA MG or GA genes[24]) might be responsible for the permeation step and not for the formation of the α-glucosidase. LINDEGREN has opposed this hypothesis on the basis of prevailing knowledge on the utilization of α-glucosides by various genetically defined yeast hybrids[20]. ROBERTSON AND HALVORSON have shown[6] that yeast cells adapted to maltose can actively accumulate ^{14}C when in contact with methyl-α-[^{14}C]-glucoside, in amounts of about 1 μg α-glucoside/h/mg dry cells. These authors characterized the accumulated ^{14}C substance by its extractability in cold TCA and did not indicate to what extent the methyl-α-glucoside had been fermented under the same experimental conditions. One has to hesitate in concluding that maltose behaves similarly to methyl-α-glucoside with respect to penetration into the cells only on the grounds that it competes with ^{14}C accumulation in the presence of methyl-α-[^{14}C]-glucoside and in the absence of analysis of the accumulated compounds when using a fermentable α-glucoside as substrate.

The results presented in this communication demonstrate that in the haploid hybrids the ^{14}C fixed in the cells after contact with [^{14}C]glucose or a fermentable α-glucoside was predominantly trehalose. No [^{14}C]glucose, maltose or methyl-α-glucoside could be detected in extracts prepared from cells which had been in contact with these ^{14}C sugars. Although these results do not necessitate the existence of a specific α-glucoside-permease, they do not contradict the possibility that such a mechanism is operative. It is evident that in these yeast hybrids some new experimental approach has to be sought in order to detect the presence of a specific permease system for fermentable α-glucosides. Consequently, it cannot yet be decided whether the MA and MG genes are linked with the formation of a permease system.

The presence of trehalose as a carbohydrate reserve in yeast cells is well known[25,26]. The amount of trehalose and other reserve carbohydrates in washed yeast is fairly constant and their endogenous metabolism is known to be exceedingly low[26]. In addition, trehalose was shown to be one of the major components labelled by ^{14}C after baker's yeasts were brought in contact with [^{14}C]glucose[27,28]. The present results are in agreement with these observations. Trehalose was found to be the component which contained about 70 % of the ^{14}C accumulated during incubation of the cells up to 1 h with a ^{14}C-fermantable sugar. There was only a 30 % net synthesis of trehalose whereas the trehalose already existing in the cells was quickly labelled. The fast turnover of trehalose in the fermenting cells could also be demonstrated by the rapid dilution of [^{14}C]trehalose in cells when brought in contact with ^{12}C fermentable sugars. It seems probable that similarity in behaviour between the fermentable α-glucosides and glucose with respect to trehalose metabolism is due to hydrolysis by the α-glucosidase.

It is noteworthy that reaction equilibrium in the *in vitro* enzymic synthesis of trehalose phosphate from uridine-diphosphateglucose and glucose-6-phosphate has been shown to be far toward the synthesis of trehalose-phosphate and that reversibility could not be demonstrated[29]. The fact that the magnitude of the trehalose pool in the intact washed yeasts is fairly constant fits this *in vitro* result. Yet, the rapid turnover of the trehalose pool when the cells are fermenting an external hexose indicates that a highly active reaction of trehalose dissimilation is also operative. It might be probable that physiological conditions within fermenting cells favor the reversibility of trehalose synthesis by glucose transfer to uridine-diphosphate. The possibility that another enzyme which catalyses trehalose hydrolysis is operative within the fermenting cells is however not excluded. In this respect, the observations that sodium azide and 2,4-dinitrophenol cause a marked decrease in the trehalose pool of resting yeast cells[26,28,30] are of special interest.

It has been shown that washed cells, grown either on glucose or on an α-glucoside, could accumulate [^{14}C]sucrose. The fact that this accumulation could be inhibited by sodium azide and 2,4-dinitrophenol and that the sucrose once accumulated was released from the cells when brought in contact with fermentable sugars, indicate that this phenomenon cannot be considered as a simple absorption. On the other hand, cells unadapted to maltose or methyl-α-glucoside when incubated with the α-[^{14}C]-glucosides were found not to be able to accumulate these, although the possibility of their penetration into the cells by simple diffusion cannot be excluded.

The ability of cells grown on glucose, maltose or methyl-α-glucoside to accumulate sucrose is unexpected and particularly surprising in the light of the fact that the cells grown on the α-glucosides actually are known to contain a sucrose cleaving α-glucosidase[3,4]. To explain this phenomenon one may assume the presence of an intracellular barrier between sucrose and the α-glucosidase. This barrier is probably specific for sucrose as it is evident from the fact that other α-glucosides susceptible to cleavage by the same α-glucosidase (*e.g.* maltose, methyl-α-glucoside) are hydrolysed by whole cells. This barrier can be damaged by mechanical treatment such as ageing or drying of the cells thereby initiating sucrose hydrolysis. Such a change in properties of the barrier might explain some of the known instances of delayed sucrose fermentation by several strains of yeast[31].

In comparison, attention is to be drawn to some recent work concerned with the

penetration of certain carbohydrates into yeast cells[32] and *E. coli*[33]. Patterns of accumulation of sugars within the cells, as was found in these studies, bear much resemblance to the phenomenon of the sucrose accumulation described in the present communication.

ACKNOWLEDGEMENT

The author is deeply indebted to Professor S. HESTRIN for much helpful advice and criticism.

REFERENCES

1 G. AVIGAD, *Abstr. Commun. 4th Intern. Congr. Biochem., Wien*, 1958, p. 133.
2 S. HESTRIN AND C. C. LINDEGREN, *Arch. Biochem.*, 29 (1950) 315.
3 S. HESTRIN AND C. C. LINDEGREN, *Arch. Biochem. Biophys.*, 38 (1952) 317.
4 G. AVIGAD, *Bull. Research Council Israel Sect. A*, 7 (1958) 112.
5 G. N. COHEN AND J. MONOD, *Bacteriol Rev.*, 21 (1957) 169.
6 J. J. ROBERTSON AND H. O. HALVORSON, *J. Bacteriol.*, 73 (1957) 186.
7 S. HESTRIN, D. S. FEINGOLD AND G. AVIGAD, *Biochem. J.*, 64 (1956) 340.
8 P. M. NOSSAL, *Australian J. Exptl. Biol. Med. Sci.*, 31 (1953) 533.
9 B. HELFERICH AND W. SCHAFER, *Org. Synthesis*, Collective Vol. I, p. 364.
10 L. HOUGH, J. K. N. JONES AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702.
11 C. S. WISE, R. S. DIMLER, H. A. DAVIS AND C. E. RISTS, *Anal. Chem.*, 27 (1955) 33.
12 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
13 W. E. TREVELYAN, D. P. PROCTOR AND J. S. HARRISON, *Nature*, 166 (1950) 444.
14 E. F. L. J. ANET AND T. M. REYNOLDS, *Nature*, 174 (1954) 930.
15 W. E. TREVELYAN AND J. S. HARRISON, *Biochem. J.*, 63 (1956) 23.
16 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
17 J. H. ROE, J. H. EPSTEIN AND N. F. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.
18 E. J. CONWAY AND M. DOWNEY, *Biochem. J.*, 47 (1950) 347.
19 R. L. WHISTLER AND D. F. DURSO, *J. Am. Chem. Soc.*, 72 (1950) 667.
20 C. C. LINDEGREN, *J. Bacteriol.*, 74 (1957) 689.
21 R. H. HOPKINS AND R. H. ROBERTS, *Biochem. J.*, 29 (1935) 931.
22 L. HURWITZ AND A. ROTHSTEIN, *J. Cellular Comp. Physiol.*, 38 (1951) 437.
23 W. MCD. ARMSTRONG, *Arch. Biochem. Biophys.*, 71 (1957) 137; 74 (1958) 427.
24 C. C. LINDEGREN AND G. LINDEGREN, *J. Gen. Microbiol.*, 15 (1956) 19.
25 K. MYRBÄCK, *Ergebn. Enzymforsch.*, 10 (1949) 168.
26 W. E. TREVELYAN, in H. A. COOK, *The Chemistry and Biology of Yeasts*, Academic Press Inc,. N.Y., 1958, p. 369.
27 J. P. AUBERT AND G. MILHAUD, *Ann. inst. Pasteur*, 90 (1956) 320.
28 H. L. BERKE AND A. ROTHSTEIN, *Arch. Biochem. Biophys.*, 72 (1957) 380.
29 E. CABIB AND L. F. LELOIR, *J. Biol. Chem.*, 231 (1958) 259.
30 W. E. TREVELYAN AND J. I. HARRISON, *Biochem. J.*, 62 (1956) 177.
31 D. PAPPAGIANIS AND H. J. PFAFF, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 22 (1956) 353.
32 M. BURGER, L. HEJMOVA AND A. KLEINZELLER, *Biochem. J.*, 71 (1959) 233.
33 B. ROTMAN, *Biochim. Biophys. Acta*, 32 (1959) 599.