

Glucose and Fructose Metabolism in a Phosphoglucosomeraseless Mutant of *Saccharomyces cerevisiae*

P. K. MAITRA

Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay 5, India

Received for publication 5 May 1971

A mutant of *Saccharomyces cerevisiae* deficient in phosphoglucosomerase (EC 5.3.1.9) is described. It does not grow on glucose or sucrose but does grow on galactose or maltose. Addition of glucose to cultures growing on fructose, mannose, or acetate arrests further growth without altering viability; removal of glucose permits resumption of growth. Glucose causes accumulation of nearly 30 μ moles of glucose-6-phosphate per g (wet weight) of cells and suppresses synthesis of ribonucleic acid. Inhibition of growth by glucose does not appear to be due to a loss of adenosine triphosphate or inorganic orthophosphate. The mutant, however, utilizes glucose-6-phosphate produced intracellularly. Release of carbon dioxide from specifically labeled glucose suggests a C-1 preferential cleavage. The kinetics of glucose-6-phosphate accumulation during glucose utilization in the mutant is not consistent with the notion that the utilization of glucose is controlled by glucose-6-phosphate.

Mutations affecting phosphoglucosomerase (EC 5.3.1.9) have been described in enteric bacteria (12, 13, 15). Loss of this enzyme seems to have marginal effects on the physiological behavior of such mutants, the most notable one being a reduction in growth rate on glucose. While studying the induction of glycolytic enzymes in yeasts, we came across a mutant of *Saccharomyces cerevisiae* lacking phosphoglucosomerase. This was characterized by its inability to form colonies not only on glucose-mineral medium but on any medium with a permissible source of carbon to which glucose was incorporated. Inhibition of growth after accumulation of nonmetabolizable phosphate compounds is a well-documented phenomenon (1, 2, 6, 11, 16, 25). Despite the generality of this effect, the metabolic basis for such growth inhibition has been traced to diverse causes (1, 11, 16). Nor have we been able to offer a general explanation for such growth inhibition. However, a number of interesting facts has emerged in the course of these studies that throw some light on the control of glucose and fructose metabolism in yeasts. This paper reports some of those experiments.

MATERIALS AND METHODS

Microbiological. A wild-type haploid strain of *S. cerevisiae* was used in these experiments. It was grown in a yeast extract-peptone medium supplemented with

extra carbon sources such as sugars or acetate. A salt-vitamin medium was also used. These have been described elsewhere (18).

The following procedure was employed in isolating the phosphoglucosomeraseless mutant. An overnight culture of *S. cerevisiae* in salt-vitamin-glucose was resuspended in fresh medium lacking glucose; mutagenesis was initiated by adding to the suspension 0.1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml and incubating for 20 min. The mutagen was washed off by centrifugation and the culture was allowed to grow overnight in a number of separate tubes containing salt-vitamin-fructose medium. Washed cells from each tube were plated in a number of petri dishes containing the salt-vitamin-fructose medium to yield nearly 100 colonies per plate. After 5 days the colonies were replicated (17) on salt-vitamin-fructose and salt-vitamin-glucose media. Among nearly 8,500 colonies examined, approximately 0.4% did not grow on glucose plates. After purification by restreaking on the fructose medium, the glucose-negative clones were tested for phosphoglucosomerase activity after growth in liquid yeast extract-peptone-fructose. One clone, labeled 9520, was found to contain less than 1% of phosphoglucosomerase activity of the wild type and was selected for these studies.

The concentration of yeast in suspensions was determined by filtering a sample over membrane filters and washing with 2 ml of distilled water. Filters were weighed after the adherent water was removed.

Assay of enzymes and substrates. Most of these procedures have been described (18, 19). Enzymes were assayed generally in toluene lysates; cell extracts pre-

pared with a French pressure cell were also used in some experiments. Fluorometric methods were employed for assay of enzymes (9). The reaction mixture for phosphoglucoisomerase assay contained 1 mM fructose-6-phosphate free of glucose-6-phosphate, 0.05 mM nicotinamide adenine dinucleotide phosphate (NADP⁺), and 0.3 unit of glucose-6-phosphate dehydrogenase. The enzyme activity of the mutant was always checked for reduced NADP (NADPH) oxidase activity. By using a concentration of 10 to 20 μ M NADPH, no significant oxidase activity could be detected. Linearity of the assays was also established by mixing the mutant and wild-type extracts; the resultant rates were essentially additive in the range of concentrations used in these experiments. A unit of enzyme activity referred to 1 μ mole of substrate converted per min at 22 C.

Nucleotides and substrates were assayed fluorometrically by using a set up described earlier (18). Sugars were measured enzymatically by using the fluorometer; glucose was also assayed by glucose oxidase-peroxidase-coupled assay (J. D. Teller, 1956. 130th Meeting Amer. Chem. Soc. Abstr., p. 69 C). Fructose-1,6-diphosphate and triose phosphates were measured together as trioses. Inorganic phosphate was estimated by the procedure of Fiske and SubbaRow (10). Respiration was measured by the Clark oxygen electrode (7) in 50 mM potassium phosphate buffer, pH 7.4, by using 100 mM ethanol as a substrate.

Experiments with labeled compounds. The release of radioactive carbon dioxide from labeled glucose was followed by doing the experiment in Warburg flasks. Alkali in the central cup absorbed the carbon dioxide, and radioactivity of a suitable sample was measured with a liquid scintillation spectrometer. The incorporation of labeled precursors into macromolecules was monitored by withdrawing samples of cell suspensions in a final concentration of 10% trichloroacetic acid at 0 C and filtering and washing on membrane filters (3). These were counted in a gas-flow counter.

Chemicals. Sugars used in experiments on growth of the mutant were freed from contaminating glucose by treatment with glucose oxidase as described elsewhere (20). Fructose and galactose were from Sigma Chemical Co., mannose was from British Drug Houses, and maltose was from Difco Laboratories, Inc. Other substrates and enzymes were from Boehringer. Radioactive chemicals were obtained from the Bhabha Atomic Research Centre, Bombay.

RESULTS

Characterization of the mutant. When the mutant strain 9520 was plated on either the salt-vitamin or yeast extract-peptone media supplemented with fructose, two types of colonies were observed. One of these, called 9520*b*, yielded relatively large-sized colonies, measuring about 1 mm in diameter after 5 days of growth. The other colony type, called 9520*s* constituting nearly 90% of the total colonies, was small, averaging 0.3 mm in diameter. Whereas the latter

was a stable mutant, the former, 9520*b*, segregated in the same ratio as 9520, giving consistently 90% of type 9520*s* and 10% of its own type. This behavior was maintained in repeated transfers with purified single clones. This property, together with the growth behavior of the two colony types, suggested that 9520*s* was a petite mutation (24). The type 9520*b* grew on acetate at a very slow rate. The type 9520*s*, however, neither grew nor reverted on acetate as a carbon source; the reversion frequency was less than 10^{-9} . Measurements of respiration showed that 9520*s* had negligible respiration compared to the wild type, whereas 9520*b* retained nearly a third of the respiratory capacity of wild-type parent. Both of these mutants, however, were deficient in phosphoglucoisomerase. Compared to the wild type which had, in one experiment, 1,200 milliunits of phosphoglucoisomerase activity per mg of protein, 9520*b* had 10 and 9520*s* 7 milliunits per mg of protein. Both the colony types 9520*b* and 9520*s* therefore had lost, respectively, 99.2 and 99.4% of the phosphoglucoisomerase activity of the wild type. Except for this enzyme, the mutant 9520*b* possessed all the other glycolytic enzymes in amounts comparable to the wild-type parent (20). Unless otherwise stated, we used the mutant 9520*b* in the experiments described here.

When the mutant 9520*b* was plated on either the salt-vitamin or yeast extract-peptone media containing glucose, on an average about 2 out of 10^7 cells gave rise to a colony. Examination of a number of these purified glucose revertants showed partial recovery of phosphoglucoisomerase activity in all of these to the extent of 10 to 30% of wild-type activity. When 9520*b* was reverted on glucose plates containing *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, as much as half of the wild-type phosphoglucoisomerase activity could be recovered. The growth rates of all the revertants on glucose were, however, slower than that of the wild type. The reversion frequency of 9520*s* on glucose was quite similar to that of 9520*b*.

To decide whether the mutant 9520*b* was a structural or a regulator gene mutant, we had examined the thermal stability of the residual phosphoglucoisomerase activity of the mutant 9520*b*. One of the spontaneous glucose revertants was also examined in this regard. Results indicated in Fig. 1 showed that the wild-type enzyme was fairly stable to heat, losing half of the initial activity in 19 min of exposure to 60 C. The activity from the mutant, however, was very sensitive to this temperature as shown by its half-time ($t_{0.5}$) of 25 sec. The enzyme activity in the revertant was even more sensitive, $t_{0.5}$ being 10 sec.

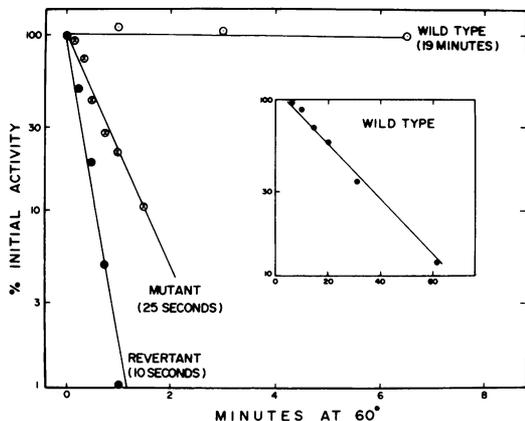


FIG. 1. Thermal inactivation of phosphoglucosomerase from *S. cerevisiae*. Cultures of the wild type, the mutant 9520b, and its glucose-revertant 9520bR13 were grown aerobically in yeast extract-peptone-fructose for 24, 48, and 80 hr, respectively; washed cells were extracted in 50 mM potassium phosphate buffer, pH 7.4, 2 mM β -mercaptoethanol, and 2 mM ethylenediaminetetraacetic acid with a French press. The supernatants obtained by centrifuging the clarified homogenates at $20,000 \times g$ for 10 min were heated at 60°C for the periods indicated, chilled immediately, and assayed for phosphoglucosomerase in 1-ml final volume. The specific activities of the unheated extracts from the wild type, the revertant, and the mutant were, respectively, 1.60, 0.24, and 0.01 units per mg of protein. The results were expressed as percentage of activities of the respective extracts before heating. The numbers within parentheses indicate the time taken to reach half of the initial activities. The inset shows the inactivation kinetics of the wild type in a compressed time scale. The ordinates for both the figures refer to the percentage activity remaining and are expressed in exponential scale.

Both the forward and the back mutations, therefore, seemed to have altered the polypeptide chain, enhancing thermosensitivity. The simplest interpretation of this observation is that the mutation 9520b has affected the structural gene of phosphoglucosomerase.

Growth behavior of 9520b. Unlike in *Escherichia coli* (12), the loss of phosphoglucosomerase in yeast resulted in complete absence of growth on media containing glucose either as a sole source of carbon or as a supplement to otherwise permissible media. Addition of 2 mM glucose to a culture growing on yeast extract-peptone containing 50 mM fructose inhibited growth as long as glucose was present. Smaller amounts of glucose had the same effect except that the interval of inhibited growth was proportionately shorter. Glucose continued to be utilized during growth inhibition; complete disappearance of glucose from the medium led to resumption of

growth. Such an experiment, illustrating the effect of adding 10 mM glucose to an exponentially growing culture of the mutant, is shown in Fig. 2. The results show the kinetics of growth inhibition measured by turbidity as well as by viable counts. The parallelism of the curves B and C indicated that glucose did not decrease the viability of the culture. It, however, caused a gradual arrest of growth. The control culture, curve A, with fructose alone continued to grow exponentially. When glucose was removed after growth had completely stopped, the culture resumed exponential growth after a lag period of 2 hr. The onset of growth inhibition was found to be sharper when higher concentrations of glucose were used.

In subsequent experiments we attempted to determine the amount of glucose-6-phosphate accumulated in the cell during growth on a number of carbon sources. For this purpose, cells from exponentially growing cultures were concentrated by centrifuging and resuspending the cells in the same medium. The concentrated cell

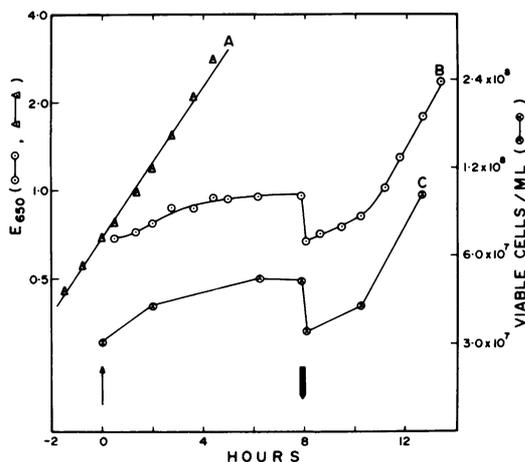


FIG. 2. Inhibition by glucose of growth of a mutant (9520b) of *S. cerevisiae* lacking phosphoglucosomerase. An overnight culture of 9520b in yeast extract-peptone-fructose (50 mM) was diluted in fresh medium. Glucose (10 mM) was added to one-half of the culture after one generation. Nearly 8 hr after the addition of glucose, the sugar was removed by centrifugation and washing with sterile glucose-free medium. The washed cells were resuspended in yeast extract-peptone-fructose and allowed to grow. Periodic samples were examined for growth by measuring the optical density at 650 nm (E_{650}) and also viable counts on the fructose medium. Curves A and B represent the growth curves of the culture containing fructose alone and fructose plus glucose, respectively. In curve C the viable count of the culture B is plotted. Ordinates are in exponential scale. Glucose addition is indicated by the thin arrow and removal by the thicker arrow.

suspension was incubated in a stream of oxygen containing 5% carbon dioxide for 1 hr to approximate the aerobic steady state of the dilute suspension of the growing culture. Separate experiments indicated that oxygenation in presence of 5% carbon dioxide did not alter the profile of glycolytic metabolites as obtained during aeration. Perchloric acid was added to a concentration of 0.7 N. Glucose-6-phosphate was determined in the neutralized supernatants (Table 1). The decreased rate of growth of the mutant in the acetate medium compared to the wild type might perhaps be traced to its decreased respiratory capacity. On fructose and mannose, however, the growth rates were comparable. An unexpected feature of these results was that the mutant was capable of growing on galactose and maltose both of which are metabolized through glucose-6-phosphate. However, the level of intracellular glucose-6-phosphate during growth on these two sugars was much less than when glucose was present in the growth medium. Glucose, when added to the culture growing in presence of any of the other sugars listed in Table 1, inhibited growth. Growth of the mutant on untreated commercial mannose, for example, was associated with an unusually long lag period which increased with increasing concentration of mannose; the delay could not be repaired by prior adaptation of the cells on this sugar. This was traced to the presence of contaminating glucose in mannose. Treatment of mannose with glucose oxidase eliminated the lag period. Unlike maltose, sucrose did not support growth of the mutant. Growth response of the mutant in salt-vi-

tamin medium was quite similar to that in yeast extract-peptone medium.

Metabolism of glucose and fructose. Results in Fig. 3 indicate the pattern of fluorescence changes associated with the metabolism of glucose and fructose by intact cells of the mutant. The characteristic changes in fluorescence emission under these conditions were contributed primarily by reduced nicotinamide adenine dinucleotide (NADH) and NADPH as shown by Chance (5) as also by the correspondence be-

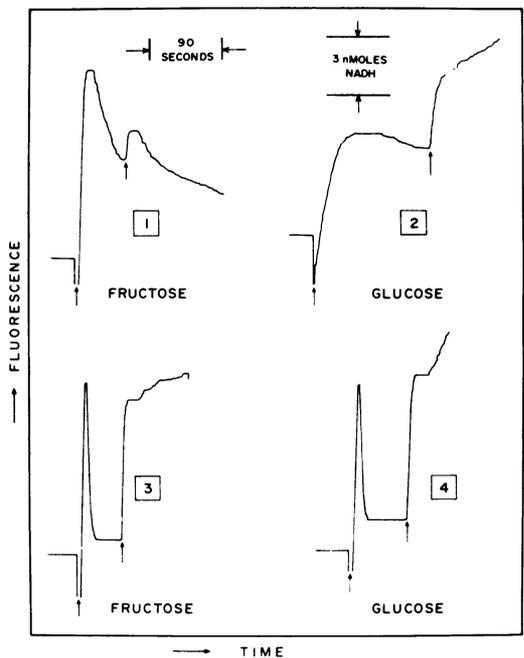


FIG. 3. Sugar-induced changes in reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate levels of intact cells of the phosphoglucosomeraseless mutant and the wild type as measured by fluorescence. Cell suspensions in 50 mM triethanolamine buffer, pH 7.4, were irradiated with an actinic light beam (366 nm) and the emitted fluorescence signal was measured after passage through a filter passing 450 nm and higher wavelengths (9). An upward deflection of the traces indicates a rise of fluorescence corresponding to reduction of nicotinamide adenine dinucleotide (NAD^+) and NAD^+ phosphate; time flows from left to right. The insets indicate the scales for both. The mutant 9520b, shown in curves 1 and 2, was present at a concentration of 46 mg (wet weight) of cells per ml and the wild type (curves 3 and 4) at 21 mg per ml. The first arrow under each curve represents addition of 10 mM sugars and the second arrow indicates the instant at which the suspension became anaerobic, as indicated in separate polarographic records. The cells for these experiments were grown to end of stationary phase and possessed high respiratory activity.

TABLE 1. Growth rates of a phosphoglucosomeraseless mutant of *Saccharomyces cerevisiae* on a number of sugars and glucose-6-phosphate levels thereon^a

Sugars	Doubling time (hr)		Glucose-6-phosphate [μ mole/g (wet weight) of yeast]	
	Wild type	9520b	Wild type	9520b
None	4.9	16	0.2	0.01
Glucose . . .	1.8	>24	0.8	5.8
Fructose . . .	1.9	2.6	0.9	0.03
Mannose . . .	1.9	3.2	0.9	0.1
Galactose . .	2.2	12	0.6	0.2
Maltose . . .	3.7	8	1.0	1.2

^a Cultures of the mutant 9520b and the wild-type parent were grown in yeast extract-peptone-acetate medium containing the indicated additional carbon sources. The initial concentration of glucose was 10 mM; maltose, 25 mM, and all others, 50 mM. Cultures were shaken in air in water both at 30 C. For the determination of glucose-6-phosphate, concentrated suspensions were incubated for 1 hr in a stream of oxygen containing 5% carbon dioxide as described in the text.

tween the rise of the fluorescent signal and drop of nicotinamide adenine dinucleotide (NAD^+) and NADP^+ (not shown). The reduction of these nucleotides after sugar addition, as shown in Fig. 3, was quite rapid in the wild type; fructose addition to the mutant also elicited comparable speed of reduction. The metabolism of glucose in the mutant, however, was slow, as suggested by the reduced velocity of increase in fluorescence (trace 2, Fig. 3). The steady-state oxidation also was lower compared to the traces 1, 3, and 4, reflecting presumably a decreased concentration of glycolytic metabolites which serve to accept electrons from these reduced nucleotides. The increased duration of the aerobic phase during glucose metabolism by the mutant compared to that during fructose metabolism also confirmed the decreased respiration with glucose as substrate. In a separate experiment with 50 mM potassium phosphate buffer, pH 7.4, the mutant 9520*b* respired in the presence of fructose and glucose at rates of 2.3 and 1.2 (endogenous, 0.3) $\mu\text{moles of oxygen per min per g (wet weight) of cells}$. A discernible stimulation of respiratory rate by glucose took as much as 20 sec, whereas fructose oxidation started within 5 sec of fructose addition. With the wild-type parent, no distinction could be made between the kinetics of fructose and glucose oxidation; the respiratory rate was nearly 8 $\mu\text{moles of oxygen per min per g (wet weight) of cells with either substrate}$. Enzymatic assay during aerobic glucose metabolism in the mutant showed a faster reduction of NADP^+ than that of NAD^+ . The percentage of reduction of NADP^+ was also higher than in the wild type. The level of NADP^+ was reduced from 20 nmoles per g (wet weight) of yeast in absence of sugar to nearly 7 nmoles per g when the mutant was treated with glucose. In the wild type only marginal decrease of NADP^+ level could be seen. The maximal extent of reduction of NAD^+ , however, was similar in the two cases, although in the mutant this took place with a delay.

Addition of glucose or fructose to a culture of the wild-type yeast or of fructose to a culture of the mutant 9520*b* growing on acetate resulted in an immediate increase in growth rate. As indicated earlier, addition of glucose to the mutant culture arrested growth. Since this might lead to an abortive overproduction of glucose-6-phosphate, we examined if glucose addition caused a drainage of the adenosine triphosphate (ATP) pool in the mutant. We show in Fig. 4 the adenine nucleotide and inorganic orthophosphate (P_i) analyses of nongrowing cultures of the mutant during glucose and fructose metabolism. Parallel experiments with the wild-type parent are also shown for purposes of comparison. The

results show that in the wild type the kinetics of changes in levels of adenine nucleotides and P_i after glucose or fructose addition were indistinguishable and bore resemblance with those in the mutant culture treated with fructose. In all of these cases, the addition of sugars led to rapid rise of ATP and a drop of adenosine diphosphate (ADP), adenosine monophosphate (AMP), and P_i . Addition of glucose to the mutant, however, led to the same end result, although the kinetics were different. There was a lag of nearly 4 min before the drop of P_i and AMP or the rise of ATP was evident. This was surprising, because in the mutant it was the utilization of fructose rather than that of glucose which showed a lag. This has been verified in a number of other experiments where early kinetics of sugar utilization had been examined. The behavior of ADP was the most striking in this respect. Clearly, the time to turn over the adenine nucleotide pool was much longer with glucose than with fructose. At any rate, the addition of glucose led to an increase of ATP level in the mutant. One other feature of these experiments was that in the wild type the rate of glucose utilization was faster than the rate of fructose utilization, whereas the reverse was the case with the mutant.

Results in Fig. 5 further illustrate the differences in the profile of the early glycolytic intermediates during glucose metabolism by the mutant and the wild type. So that the early kinetics of glucose utilization in the mutant was not limited by the low ATP level of these cells, we have preincubated the cell suspension aerobically for 10 min in presence of 20 mM ethanol. Separate experiments indicated that this pretreatment increased ATP level without affecting the levels of glycolytic intermediates measured in this experiment. In the wild-type parent the concentrations of glucose-1-phosphate and fructose-6-phosphate bore a fixed relationship to those of glucose-6-phosphate within limits of experimental error, whereas in the mutant this constant ratio was maintained only between glucose-1-phosphate and glucose-6-phosphate. Despite the marked rise in the level of glucose-6-phosphate, that of fructose-6-phosphate was nearly zero throughout the course of incubation. The metabolism of glucose in the mutant was further characterized by the feature that the overshoot in the concentration of glucose-6-phosphate, seen in the wild type, was absent. One other feature of these and related experiments was that the rate of glucose utilization in the mutant was constant in the face of a marked rise in the intracellular concentration of glucose-6-phosphate. Nevertheless, the rate of glucose utilization in the mutant was never more than a third of that of the wild-type *S. cerevisiae*. Results in Fig. 6 showing the accu-

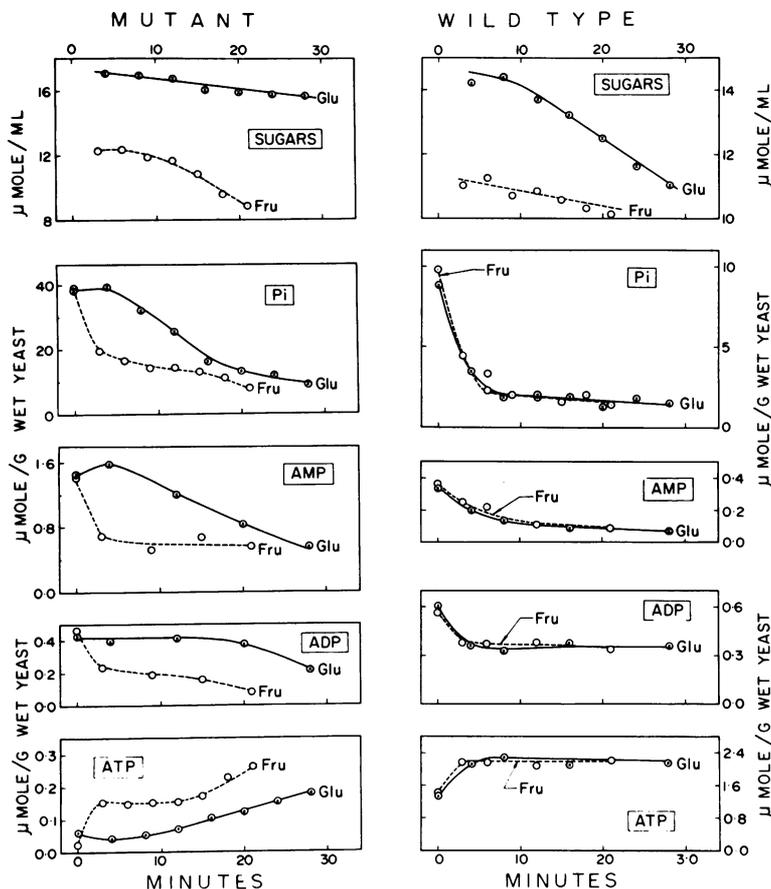


FIG. 4. Time-course of changes in the concentrations of adenine nucleotides and P_i during glucose and fructose metabolism by the phosphoglucosomeraseless mutant and the wild-type parent. Washed cells from overnight cultures from yeast extract-peptone-fructose were suspended in 0.1 M triethanolamine buffer, pH 7.3 and kept aerated by passing a stream of oxygen containing 5% carbon dioxide. Samples were withdrawn at indicated intervals in 0.7 N perchloric acid and the neutralized supernatants analyzed. Glucose was measured by glucose oxidase and fructose by a fluorometric procedure involving the use of hexokinase, phosphoglucosomerase and glucose-6-phosphate dehydrogenase. For the assay of adenosine triphosphate in glucose-treated mutant samples, the fluorometric procedure with the 3-phosphoglycerate kinase-glyceraldehyde-3-phosphate dehydrogenase couple was used. All other procedures were the same as in Materials and Methods. Solid lines indicate experiments with glucose (Glu) and broken lines with fructose (Fru). Figures on the left hand column refer to experiments with the mutant and those on the right to the wild-type parent. Note that in the figures showing sugar disappearance the ordinates do not begin at 0 and that the sugar concentrations are expressed as micromoles per milliliter of the suspension. Addition of glucose or fructose was made at time 0. Concentration of cells in the incubation mixture in milligrams of cells (wet weight) per ml were as: wild type—glucose 21; fructose 19; mutant—glucose 21.5; fructose 17.5.

mulation of the aldolase metabolites in the mutant during glucose and fructose metabolism also suggested the decreased flow of glucose carbon along the glycolytic pathway. Surprisingly, no gluconate-6-phosphate could be detected in the acid extracts when the mutant accumulated glucose-6-phosphate; the estimated level was less than 5 nmoles per g.

When the release of carbon dioxide from specifically labeled glucose was examined, the mutant was found to contribute a much larger share

of glucose carbon-1 than glucose carbon-6 in the expired gas. These results are shown in Table 2. This experiment indicated that, compared to the wild type, the loss of phosphoglucosomerase in the mutant had caused the major bulk of glucose catabolism to be channeled through the oxidative pentose phosphate pathway. However, a substantial part of glucose-6-phosphate was conserved as shown by the accumulation of radioactivity in the cell during metabolism of glucose- $1-^{14}C$. We have not followed the fate of the con-

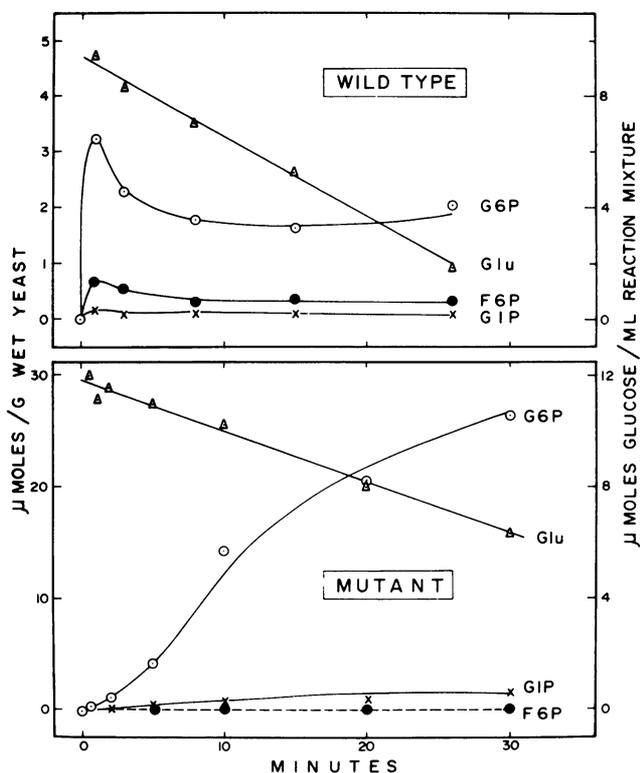


FIG. 5. Kinetics of hexose monophosphates during aerobic glucose metabolism by the phosphoglucosomerase-deficient mutant and the wild-type *S. cerevisiae*. Stationary phase cultures from yeast extract-peptone-fructose were washed and resuspended in 50 mM triethanolamine buffer containing 5 mM magnesium chloride at pH 7.4. The suspensions were incubated aerobically with 20 mM ethanol 10 min before 10 mM glucose (Glu) addition at time 0. Other details were as in Fig. 4. G1P, G6P, and F6P indicate, respectively, glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate. The incubation mixtures for the mutant and wild type contained, respectively, 46 and 15 mg (wet weight) of cells per ml.

served glucose. When washed cells were incubated with glucose or fructose in phosphate buffer, no appreciable difference could be found between the effect of these two sugars on the content of glucose in cell hydrolysates.

Synthesis of macromolecules during growth inhibition by glucose. We have indicated elsewhere (20) that the mutant 9520*b* synthesized glycolytic enzymes at a faster rate in presence of small concentrations (2 to 5 mM) of glucose than in its absence, although growth continued to be inhibited. At higher concentrations, however, addition of glucose brought about net decay of basal synthesis. Figure 7 shows the results of such an experiment. The steady state rate of differential synthesis of phosphoglycerate kinase was not only inhibited by the addition of 50 mM glucose, but there ensued a drop of the total enzyme activity. The differential rate of this decay seemed to increase with time. It has not been determined whether the decrease of enzyme activity in the cells was due to its release into the medium. Not

every glycolytic enzyme behaved in this manner. Hexokinase activity, for example, continued to increase after glucose addition at a rate slightly lower than the basal rate. Incubation of resting cultures in buffered-sugar solutions indicated that the rate of release of 260 nm-absorbing material into the medium was only marginally higher in the mutant in presence of glucose than in presence of fructose.

Results in Fig. 8 describe experiments that suggest a possible clue to the mechanism of growth inhibition by glucose. The synthesis of ribonucleic acid (RNA) during growth of the mutant was studied by following the incorporation of ^{14}C -uracil into trichloroacetic acid-insoluble material (3). The results indicated that fructose stimulated RNA synthesis after a lag period, whereas glucose brought about an inhibition. Higher concentrations of glucose stopped RNA synthesis completely within 3 min. Separate experiments, not shown here, suggested that this inhibition was reversible. After removal of

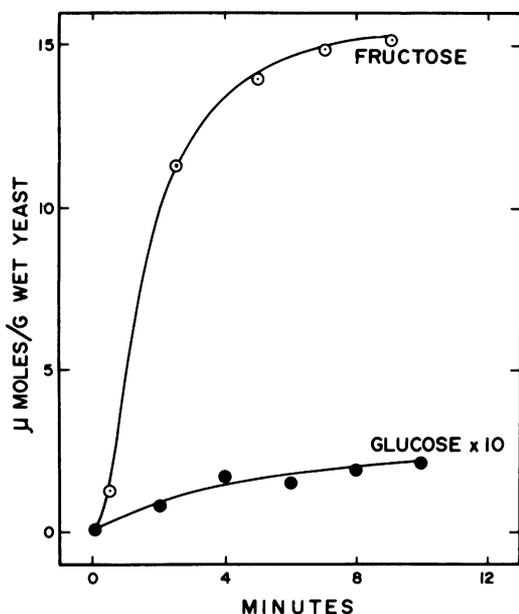


FIG. 6. Accumulation of aldolase metabolites in the phosphoglucosomeraseless mutant during aerobic metabolism of glucose and fructose. Two separate cultures in the stationary phase were used in 50 mM potassium phosphate buffer, pH 7.4. Fructose-1, 6-diphosphate, and triose phosphates were measured together and the results were expressed in units of total triose phosphates; those of glucose were multiplied by 10. Cell concentrations for fructose and glucose were, respectively, 26.5 and 192 mg (wet weight) of yeast per ml. All other details were the same as in Fig. 5.

glucose, incorporation of ^{14}C -uracil into RNA resumed at the uninhibited rate.

DISCUSSION

The partial loss of respiratory activity in the mutant clone 9520*b* appeared to be due to a genetic lesion additional to the one causing phosphoglucosomerase deficiency. This was suggested by the observation that in 9520*s*, a derivative of 9520*b*, respiratory activity was further reduced without much alteration of the phosphoglucosomerase activity. The reversion of both these clones on glucose led to reappearance of only phosphoglucosomerase, leaving respiratory activity unchanged. No revertant from 9520*s* could be isolated that grew on acetate. We believe the loss of respiration in 9520*s* was caused by genetic deletion of some cytoplasmic respiratory determinant (24). Although we have not mapped the phosphoglucosomerase mutation, the instability of the mutant and revertant enzymes to heat suggested a structural defect. Furthermore, the occurrence of a mutant lacking more than 99% of the wild-type activity in a

single-step isolation procedure indicated a single gene specifying the structure of phosphoglucosomerase. This was also consistent with the monophasic heat-inactivation kinetics of the wild-type enzyme.

Since the studies reported here have been carried out with a mutant continuously segregating into respiratory-deficient clones, the possibility remains that some of the biochemical properties of the mutant derive from its respiratory lesion. Two lines of evidence, however, suggest this to be unlikely. A partial loss of respiration has little effect on the metabolism of fructose in the mutant as shown by the similarity in its features of fructose metabolism with those of the wild type metabolizing either glucose or fructose (Fig. 3 and 4). The features of glucose metabolism in the mutant, however, are distinctly different. Secondly, the profiles of glycolytic intermediates and nucleotides during metabolism of either

TABLE 2. Release of radioactive carbon dioxide from glucose-1- ^{14}C and glucose-6- ^{14}C by cultures of the phosphoglucosomeraseless mutant and its wild-type parent^a

Yeast	Labeled glucose	Total ^{14}C in CO_2 (counts per min)	^{14}C -glucose/ ^{14}C -glucose $\times 100$	$^{14}\text{CO}_2$ from glucose-1- ^{14}C / $^{14}\text{CO}_2$ from glucose-6- ^{14}C
Mutant 9520 <i>b</i>	1- ^{14}C	10,000	0.42	14.0
	6- ^{14}C	863	0.03	
Wild type	1- ^{14}C	9,500	0.40	2.5
	6- ^{14}C	3,940	0.16	

^a Stationary phase cultures from yeast extract-peptone medium containing glucose (wild type) or fructose (mutant) were washed and diluted freshly in yeast extract-peptone and incubated for 3 hr at 30 C. The experiment was conducted aerobically in Warburg flasks containing the following: 2 ml of the diluted culture in yeast extract-peptone; 0.15 ml of 20% potassium hydroxide in the central cup; 0.1 ml of 10 N sulfuric acid in a side arm; 0.2 ml of 100 mM radioactive glucose containing 2 μCi (2.4×10^6 counts per min for glucose-1- ^{14}C and 2.5×10^6 counts per min for glucose-6- ^{14}C) in the other side arm. After 30 min of equilibration in the bath at 30 C, glucose was tipped in and incubation was continued for 15 min. Shaking was continued throughout. At the end of incubation, the acid was tipped in from the side arm and a shaking period of 1 hr was allowed for absorption of carbon dioxide by the alkali. Samples corresponding to 25 μliters of the alkali in the central cup were counted in Bray's scintillation fluid (4). An identical sample of alkali, not containing any added radioactivity, served as the control. The concentration of cells in the reaction mixture was such as to give the following extinction values at 650 nm: wild type, 5.2; mutant, 10.2.

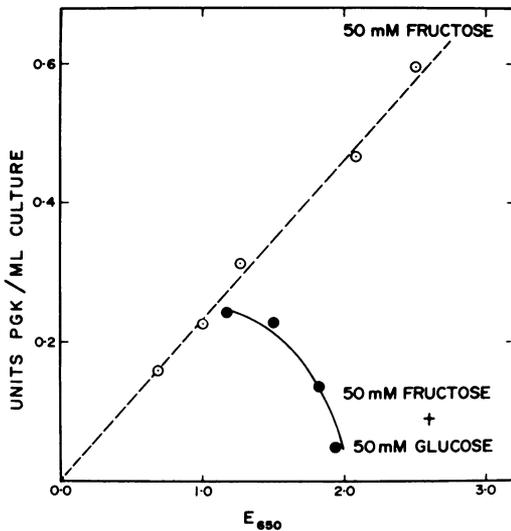


FIG. 7. Loss of phosphoglycerate kinase (PGK) in the mutant 9520b caused by addition of glucose. Overnight cultures from yeast extract-peptone-fructose were diluted in fresh medium. Samples of 10 ml were withdrawn for enzyme assay (20) after resumption of exponential growth. At the time the second sample was taken out, 50 mM glucose was added to one-half of the culture. E_{650} refers to the optical density of the culture at 650 nm.

sugar by 9520b were indistinguishable from those by 9520s, the petite variant. It is reasonable, therefore, to conclude that we are studying here primarily the effects of the loss of phosphoglucoisomerase rather than those due to the partial loss of respiratory activity.

One of the major differences in the properties of phosphoglucoisomerase-negative mutants described so far in bacteria and the one described here lies in their growth response on glucose (12, 13). The yeast mutant turned out to be exclusively glucose-negative in growth unlike the bacterial mutants. At first sight this appeared to be due to the limited capacity of the pentose-phosphate pathway to produce fructose-6-phosphate. However, the ability of maltose and galactose to support growth, although slowly, suggested that this pathway could indeed sustain the required flux of carbon. The inability of glucose in this regard was therefore not due to questions of insufficient rate of carbon flow or changes in cellular ATP or P_i pools (Fig. 4). Results in Table 1 on the levels of glucose-6-phosphate during growth indicated that unlike glucose, both galactose and maltose allowed accumulation of reasonably small levels of this intermediate. The rate of maltose fermentation by intact yeast cells is known to be comparable to that of glucose fermentation and is limited by the activity of

maltose permease rather than by maltase (8). The maintenance of controlled levels of glucose-6-phosphate in the mutant during growth on maltose indicated to us operation of a feedback regulation of maltose transport. We had observed that the ability of a maltose-grown cell suspension of the mutant in buffer to oxidize maltose fell off very rapidly. This, together with the earlier observations on the preferential inactivation by glucose of maltose permease rather than of α -glucosidase activity in *S. cerevisiae* (14, 22), suggested that glucose-6-phosphate might be the signal responsible for inactivation. Any rise of glucose-6-phosphate concentration as a result of the block in phosphoglucoisomerase would prevent further formation of glucose-6-phosphate controlled by the rate-limiting maltose permease. Maltose metabolism in the mutant, therefore, would be a delicately poised steady state between the synthesis or activation of maltose permease and the rate of glucose-6-phos-

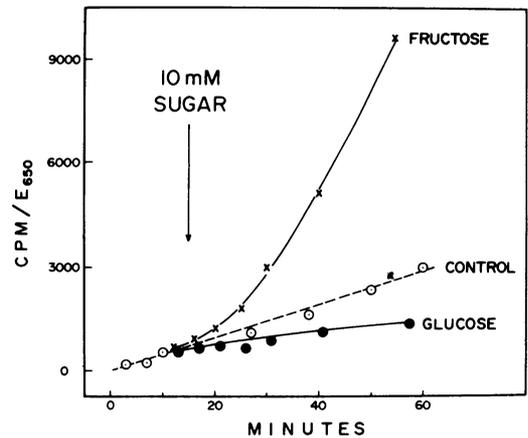


FIG. 8. Stimulation of RNA synthesis in the mutant 9520b by fructose and its inhibition by glucose. A 96-hr culture from yeast extract-peptone-acetate was diluted in fresh medium and incubated for an hour. At time 0, $1 \mu\text{Ci}$ carrier-free ^{14}C -uracil was added and periodic samples of 0.5 ml were added to a final concentration of 10% trichloroacetic acid at 0 C. At the instant denoted by the arrowhead, 10 mM glucose or fructose was added to two separate samples from the same culture. Sampling was continued as indicated. After 1 hr the cells were filtered on membrane filters and washed with 25 ml of 10% cold trichloroacetic acid. Filters were dried before counting on a gas-flow counter. The optical density of all the cultures at 650 nm (E_{650}) was 1.3 throughout except that containing fructose. At the end of the incubation the suspension containing fructose recorded an extinction of 1.5. At intermediate points the specific activity was calculated by assuming exponential growth throughout. The curve labeled control refers to the culture containing only acetate as the extra carbon source.

phate expenditure. Although no such control of galactose metabolism is known at the level of galactose transport, the low glucose-6-phosphate level during growth of the mutant on galactose suggests similar feedback controls.

The inhibition of growth of the mutant by glucose differed from the effect of 2-deoxyglucose on the growth of *Schizosaccharomyces pombe* (21) in that glucose did not alter the titer of viable cells (Fig. 2). Since glucose inhibited growth of the mutant on fructose as well, inhibition of fructose-1,6-diphosphatase reaction by glucose-6-phosphate (11) could not be the mechanism of growth inhibition; we have observed the presence of significant amounts of fructose-6-phosphate inside the cell under conditions indicated in Fig. 2. Although high concentrations of glucose-6-phosphate have been found to be an inhibitor of *E. coli* fructose-1,6-diphosphatase (11), it is perhaps easier to visualize that the loss of phosphate potential (5) caused by the abortive formation of glucose-6-phosphate in the double mutant would lead to accumulation of AMP, which would keep this gluconeogenic enzyme in an inhibited state. In the mutant described here, our experiments suggested (Fig. 8) inhibition of RNA synthesis as one of the expressions of the inhibitory effect of glucose. High concentrations of glucose-6-phosphate might inhibit some steps in RNA synthesis by competing, for example, with certain phosphorylated-nucleotide precursors. However, we have not determined whether the synthesis of other macromolecules is also inhibited by glucose. Addition of glucose brought about a decay of total activity of phosphoglycerate kinase, although hexokinase was not so affected. Release of 260 nm-absorbing materials also was not particularly marked. It appears, therefore, that if some permeability changes were involved in the growth inhibition, such effects were of a specific nature.

Intact cells of the mutant did not produce any detectable amounts of either fructose-6-phosphate or glucose-6-phosphate when treated with glucose and fructose, respectively, indicating that phosphoglucosomerase activity was not significant in vivo. The catabolism of glucose therefore took place, as results in Table 2 indicated, mainly via the pentose-phosphate pathway. This restricted the rate of formation of glycolytic intermediates as also ATP synthesis from glucose. In the experiment shown in Fig. 6, the rate of formation of alcohol in the mutant with glucose as the substrate was 1.0 μ mole per min per g (wet weight) of yeast, whereas with fructose this was nearly 10 times as much. In a wild-type yeast harvested in the exponential phase of growth, such as to have respiratory activity com-

parable to that of the mutant 9520b, the rate of aerobic alcohol formation from glucose was nearly 15 μ moles per min per g (wet weight) of yeast. The contribution of the pentose-phosphate pathway in glucose catabolism under these conditions in *S. cerevisiae* was, therefore, around 7% of the total glycolytic flux. The reduced flow through glycolysis in the mutant was also reflected in the difference between glucose and fructose in the initial kinetics of changes in cellular ADP, AMP, P_i , and ATP levels (Fig. 4). In absence of sugars the ATP-ADP ratio of the mutant was very much lower compared to the wild type; the level of P_i also was nearly four times as much.

The maximum rate of intracellular consumption of glucose-6-phosphate in the mutant, found by first incubating a cell suspension with glucose and then quickly removing the extracellular glucose by filtration and washing, was 3 μ moles per g (wet weight) of cells per min. A substantial part of glucose-6-phosphate metabolized was therefore diverted to extraglycolytic products, perhaps polysaccharides. The synthesis of such compounds was expected to be stimulated by the accumulated glucose-6-phosphate (23). However, we have not investigated this. The sustained accumulation of glucose-1-phosphate in Fig. 5 indicated that the rate of polysaccharide synthesis was slower than those of hexokinase and phosphoglucosomase. The yeast mutant differed from the *Escherichia coli* mutant (12) in one other feature; growth of the latter in absence of glucose yielded cells in which hydrolyzable glucose was very much reduced compared to cells grown on glucose. With the yeast mutant, however, cells grown on fructose plus small amounts of glucose had 112 and those grown on fructose alone had 87 μ moles of hydrolyzable glucose residue per g (wet weight). Whether the synthesis of glucose from fructose in the mutant was through a leaky phosphoglucosomase or through any other unknown reaction has not been determined.

The accumulation of glucose-6-phosphate during the metabolism of glucose in this mutant afforded an opportunity of studying the effect of glucose-6-phosphate on the rate of glucose utilization. The results in Fig. 5 on the kinetics of glucose-6-phosphate accumulation during glucose utilization showed that the rate of glucose utilization was independent of the rising concentration of glucose-6-phosphate in the range of 0 to 27 mM (μ moles per g (wet weight) of cells). The lack of kinetic correlation between the glucose-6-phosphate level and rate of glucose consumption suggested strongly that other determinants were responsible for the control of sugar utilization in yeast cells.

ACKNOWLEDGMENTS

I thank Zita Lobo for her excellent assistance.

LITERATURE CITED

1. Bernheim, N. J., and W. J. Dobrogosz. 1970. Amino sugar sensitivity in *Escherichia coli* mutants unable to grow on *N*-acetylglucosamine. *J. Bacteriol.* **101**:384-391.
2. Böck, A., and F. C. Neidhardt. 1966. Properties of a mutant of *Escherichia coli* with a temperature-sensitive fructose-1, 6-diphosphate aldolase. *J. Bacteriol.* **92**:470-476.
3. Bollum, F. J. 1966. Filter paper disk techniques for assaying radioactive macromolecules, p. 296-300. In G. L. Cantoni and D. R. Davies (ed.), *Procedures in nucleic acid research*. Harper and Row, New York.
4. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**:279-285.
5. Chance, B. 1961. Energy-linked cytochrome oxidation in mitochondria. *Nature (London)* **189**:719-725.
6. Cozzarelli, N. R., J. P. Koch, S. Hayashi, and E. C. C. Lin. 1965. Growth stasis by accumulated L- α -glycerophosphate in *Escherichia coli*. *J. Bacteriol.* **90**:1325-1329.
7. Davies, P. W. 1962. The oxygen cathode, p. 137-179. In W. L. Nastuk (ed.), *Physical techniques in biological research*, vol. 4. Academic Press Inc., New York.
8. De La Fuente, G., and A. Sols. 1962. Transport of sugars in yeasts. II. Mechanisms of utilization of disaccharides and related glycosides. *Biochim. Biophys. Acta* **56**:49-62.
9. Estabrook, R. W., and P. K. Maitra. 1962. A fluorimetric method for the quantitative microanalysis of adenine and pyridine nucleotides. *Anal. Biochem.* **3**:369-382.
10. Fiske, C. H., and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375-400.
11. Fraenkel, D. G. 1968. The accumulation of glucose 6-phosphate from glucose and its effect in an *Escherichia coli* mutant lacking phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. *J. Biol. Chem.* **243**:6451-6457.
12. Fraenkel, D. G., and S. R. Levisohn. 1967. Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase. *J. Bacteriol.* **93**:1571-1578.
13. Fraenkel, D., M. J. Osborn, B. L. Horecker, and S. M. Smith. 1963. Metabolism and cell wall structure of a mutant of *Salmonella typhimurium* deficient in phosphoglucose isomerase. *Biochem. Biophys. Res. Commun.* **11**:423-428.
14. Görts, C. P. M. 1969. Effect of glucose on the activity and the kinetics of the maltose uptake system and of α -glucosidase in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **184**:299-305.
15. Hattman, S. 1964. The functioning of T-even phages with unglucosylated DNA in restricting *Escherichia coli* host cells. *Virology* **24**:333-348.
16. Heredia, C. F., G. De La Fuente, and A. Sols. 1964. Metabolic studies with 2-deoxyhexoses I. Mechanism of inhibition of growth and fermentation in baker's yeast. *Biochim. Biophys. Acta* **86**:216-223.
17. Lederberg, J., and E. M. Lederberg. 1952. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63**:399-406.
18. Maitra, P. K. 1970. A glucokinase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **245**:2423-2431.
19. Maitra, P. K., and Z. Lobo. 1971. A kinetic study of glycolytic enzyme synthesis in yeast. *J. Biol. Chem.* **246**:475-488.
20. Maitra, P. K., and Z. Lobo. 1971. Control of glycolytic enzyme synthesis in yeast by products of the hexokinase reaction. *J. Biol. Chem.* **246**:489-499.
21. Megnet, R. 1965. Effect of 2-deoxyglucose on *Schizosaccharomyces pombe*. *J. Bacteriol.* **90**:1032-1035.
22. Robertson, J. J., and H. O. Halvorson. 1957. The components of maltozymase in yeast, and their behavior during deadaptation. *J. Bacteriol.* **73**:186-198.
23. Rothman, L. B., and E. Cabib. 1969. Regulation of glycogen synthesis in the intact yeast cell. *Biochemistry* **8**:3332-3341.
24. Winge, Ö., and C. Roberts. 1958. Yeast genetics, p. 128-156. In A. H. Cook (ed.), *The chemistry and biology of yeasts*. Academic Press Inc., New York.
25. Yarmolinsky, M. B., H. Wiesmeyer, H. M. Kalckar, and E. Jordan. 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. II. Galactose-induced sensitivity. *Proc. Nat. Acad. Sci. U.S.A.* **45**:1786-1791.