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COMPETITION OF SUGARS FOR THE HEXOSE TRANSPORT SYSTEM IN YEAST

J. VAN STEVENINCK

Laboratory for Medical Chemistry, Wassenaarseweg 62, Leiden (The Netherlands)

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SUMMARY

1. Based on the model of transmembrane sugar transport, developed in previous papers, the kinetics of mutual inhibition between pairs of sugars were calculated. The equations derived take into consideration the existence of two modes of transport: facilitated diffusion and active transport, both utilizing the same carrier, as shown previously.

2. It was shown that the kinetics are more complicated than those of simple competitive inhibition, if at least one of the two competing sugars is transported actively.

3. The experimental results with various pairs of sugars appeared to be in good agreement with the theoretical equations derived for mutual transport inhibition.

4. The relationship between this transport model and data on sugar transport in recent literature is discussed briefly.

INTRODUCTION

Transport of sugars in yeast cells has been kinetically characterized as a carrier-mediated process[1–4]. Countertransport studies and demonstration of mutual inhibition of transport with pairs of sugars indicate that a number of sugars share a common carrier[3,5–7], according to the generally accepted criteria[8–11]. In some papers on this subject the term competitive inhibition has been utilized to describe the mutual transport inhibition of pairs of sugars. At first sight it seems self-evident that this would describe the situation sufficiently and many experimental data seem to be in accordance with this representation. The experimental conditions in these studies, however, were not always optimal to detect deviations of the kinetics of competitive inhibition. Moreover, some of these competition studies were performed with iodoacetate-poisoned yeast. As discussed previously[4,12] this will cloud the issue.

In previous papers experimental evidence has been presented for the existence of two sugar transport mechanisms in yeast, both proceeding *via* the same carrier[4,6,12]. As will be discussed in this paper, this situation should have its bearing on the kinetics of inhibition. In the present investigation the theoretical consequences of this transport model on the kinetics of inhibition will be compared with experimental results.

THEORETICAL

As discussed previously[4,6,12] two hexose transport mechanisms are operative in yeast: a passive, carrier-mediated, facilitated diffusion and an active, metabolically linked transport. In this active transport mechanism the same carrier is utilized as in facilitated diffusion; the binding of sugar to the carrier is catalyzed in this case by an enzyme (permease), and a phosphorylating reaction with polyphosphate as phosphate donor is involved in this binding. Facilitated diffusion was found *e.g.* for sorbose and for galactose in uninduced yeast. Active transport was found for galactose in induced yeast, glucose and fructose. This transport model can be outlined as follows:

Transport by facilitated diffusion:

$$S + C \rightleftharpoons (SC) \tag{1}$$

Active transport:

$$R + E \rightleftharpoons (RE) \tag{2}$$

$$(RE) + C + (KPO_3)_n \rightleftharpoons (R\text{–}E\text{–phosphate–}C) + (KPO_3)_{n-1} \tag{3}$$

$$(R\text{–}E\text{–phosphate–}C) \rightleftharpoons (R\text{–phosphate–}C) + E \tag{4}$$

where S = passively transported substrate; R = actively transported substrate; C = carrier; E = permease; $(KPO_3)_n$ = polyphosphate.

Subsequently the (SC) and $(R$–phosphate–$C)$ complexes will move across the membrane.

As shown previously, nickelous ions inhibit active transport at the level of reaction (3). Passive transport is not influenced by Ni^{2+} (ref. 5). So it seems possible that the presence of Ni^{2+} could change the equilibrium constant of reaction (3), without changing the equilibrium constant of reaction (2).

In iodoacetate-poisoned yeast active transport is abolished quickly, by depletion of the polyphosphate fraction involved in the active mechanism. Substrates, normally transported actively, are taken up *via* facilitated diffusion in iodoacetate-poisoned cells[12]. Experimentally a major shift of transport parameters is found. For this reason inhibition studies between pairs of sugars should not be performed with poisoned yeast if one or both of the sugars is transported actively.

The overall transport process shows Michaelis–Menten kinetics[1,12,13]. It is clear that the apparent Michaelis–Menten constant that can be determined from the relationship between substrate concentration and initial velocity of uptake (*e.g.* by the Lineweaver–Burk plot) has a quite different meaning in active and passive transport. In passive transport it represents the dissociation constant of the substrate–carrier complex (K_c and K_i in the following calculations); in active transport, however, the substrate–permease dissociation constant (K_e in the following calculations). These constants can be determined experimentally[5,6,12].

From (1) the normal Michaelis–Menten kinetics of transport can be derived:

$$K_c = \frac{[S]\,[C]}{[SC]} \tag{5}$$

$$C_t = [C] + [SC] \tag{6}$$

$$v = \frac{kC_t[S]}{K_e + [S]} \tag{7}$$

where C_t = total amount of carrier; v = velocity of transport; k = reaction constant.

If a second substrate, I, competes with S for the carrier, the conventional HUNTER AND DOWNS[14] equation for competitive inhibition can be derived:

$$\frac{\alpha}{1-\alpha}[I] = K_i + \frac{K_i}{K_e}[S] \tag{8}$$

where K_i = the carrier–I dissociation constant and α = the ratio of the transport velocity in the presence of inhibitor to the velocity without inhibitor, at the same substrate concentration.

If an actively transported substrate, R, inhibits transport of S, the situation is more complicated. In this case there will be a competition between S and (RE) (Eqns. 1 and 3) for the carrier. It is reasonable to assume that every particular (RE) complex is spatially associated with one particular carrier site. For this particular carrier site there will be a competition between S and (RE). There is, of course, no bulk (RE) concentration: only a "local concentration" with regard to C, which cannot be expressed in conventional units. The only thing that can be concluded is that (once a permease site has reacted with R) this local (RE) concentration is constant and presumably very high (assuming a close spatial relationship between permease and carrier). This local concentration with regard to C will be indicated by $[B]$ in the following calculations.

To evaluate the transport kinetics of S in the presence of R, it should be realized that one fraction involves S transport by RE-associated carriers (fraction A) and the other fraction involves S transport by the non-RE-associated carrier sites, $(C_t - A)$. First it will be assumed that each carrier site is associated with one permease, or:

$$E_t = C_t \tag{9}$$

From (2):

$$K_e = \frac{[R][E]}{[RE]} \tag{10}$$

$$E_t = [E] + [RE] \tag{11}$$

$$[RE] = \frac{[R]E_t}{K_e + [R]} = \frac{[R]C_t}{K_e + [R]} = A \tag{12}$$

Transport of S *via* the non-RE-associated carrier fraction, $(C_t - A)$, is competitively inhibited by free R, whereas S transport *via* the fraction A is competitively inhibited by (RE). If the transport velocity *via* fraction A is called v_1 and *via* $(C_t - A)$ v_2, the total velocity of inhibited transport is given by:

$$v_t = v_1 + v_2 \tag{13}$$

Transport employing the non-RE-associated fraction $(C_t - A)$ would obey Eqn. 14:

$$v_2 = \frac{k(C_t - A)[S]K_r}{K_r[S] + K_rK_e + K_e[R]} \tag{14}$$

where K_r = the carrier–R dissociation constant.

However, if K_c is of the same order of magnitude as K_r, and $[R] \ll [S]$, Eqn. 14 becomes:

$$v_2 = \frac{k(C_t - A)\,[S]}{[S] + K_c} \tag{15}$$

From (3):

$$K_i = \frac{[C']\,[B]\,[(KPO_3)_n]}{[REC']\,[(KPO_3)_{n-1}]} \tag{16}$$

where C' is the amount of free carrier within the fraction A. In the steady state $[(KPO_3)_n]/[(KPO_3)_{n-1}]$ can be considered as a constant, with a value close to one, as the transport phosphorylation given in Eqn. 3 implies the use of one phosphate monomer, decreasing n, but not changing the molecular concentration of the polyphosphate. So Eqn. 16 can be simplified to:

$$K_i = \frac{[C']\,[B]}{[REC']} \tag{17}$$

As there is a competition between S and (RE) for the carrier fraction A:

$$A = [C'] + [REC'] - [SC'] \tag{18}$$

From (5):

$$[C'] = \frac{K_c[SC']}{[S]} \tag{19}$$

From (17):

$$[REC'] = \frac{[C']\,[B]}{K_i} = \frac{K_c[SC']\,[B]}{[S]K_i} \tag{20}$$

Therefore:

$$A = \frac{K_c[SC']}{[S]} + \frac{K_c[SC']\,[B]}{[S]K_i} + [SC'] \tag{21}$$

or:

$$[SC'] = \frac{A[S]K_i}{K_cK_i + K_i[S] + K_c[B]} \tag{22}$$

and:

$$v_1 = \frac{kA[S]K_i}{K_i[S] + K_cK_i + K_c[B]} \tag{23}$$

By substituting Eqns. 15 and 23 into 13 one obtains:

$$v_t = \frac{kA[S]K_i}{K_i[S] + K_cK_i + K_c[B]} + \frac{k(C_t - A)\,[S]}{K_c + [S]} \tag{24}$$

From (7) and (24):

$$a = v_t/v = \frac{C_t - A}{C_t} + \frac{AK_i(K_c + [S])}{C_t\{K_i[S] + K_cK_i + K_c[B]\}} \tag{25}$$

By substituting Eqn. 12 into 25 one obtains:

$$\frac{a}{1-a}[R] = \frac{(K_e + [R])K_i[S]}{K_c[B]} + \frac{(K_e + [R])K_i}{[B]} + K_e \tag{26}$$

The value of the fraction $((K_e + [R])K_i)/[B]$ cannot be determined directly, but it is expected to be small, as $[B]$ has a very high value (see above). The value of the fraction will increase with increasing $[R]$, but is constant when $[R]$ is fixed. If the value of the fraction, at a fixed R concentration, is written as K_b, Eqn. 26 can be written as:

$$\frac{\alpha}{1-\alpha}[R] = K_e + K_b + \frac{K_b[S]}{K_e} \tag{27}$$

Under these conditions a plot of $\alpha[R]/(1-\alpha)$ against $[S]$ will yield a straight line. The analogy with the HUNTER AND DOWNS equation (Eqn. 8) is obvious.

If some carrier sites are not associated with a permease ($E_t < C_t$), the same calculations can be made. In this case, however, $E_t = \beta C_t$ ($\beta < 1$), and Eqn. 12 changes to:

$$[RE] = \frac{[R]E_t}{K_e + [R]} = \frac{[R]\beta C_t}{K_e + [R]} = A \tag{28}$$

and the final result will be:

$$\frac{\alpha}{1-\alpha}[R] = 1/\beta K_e + 1/\beta K_b + 1/\beta \frac{K_b[S]}{K_e} + (1/\beta - 1)[R] \tag{29}$$

The value of $\alpha[R]/(1-\alpha)$ is now dependent on $[R]$ in two ways: *via* the value of K_b, and *via* the term $(1/\beta - 1)[R]$. At a constant concentration of R, a plot of $\alpha[R]/(1-\alpha)$ against $[S]$ yields a straight line again. If $\beta = 1$, Eqn. 29 is identical to Eqn. 27.

From Eqn. 25 it follows that if $K_i \ll K_e$ and $[S]$ is relatively small, the expression reduces to $\alpha = (C_t - A)/C_t$ or, from Eqn. 28:

$$(1-\alpha) - \frac{A}{C_t} = \frac{\beta[R]}{K_e + [R]} \tag{30}$$

This means that under these circumstances the per cent inhibition of S transport is directly proportional to the per cent saturation of the permease with the inhibitor R, and when $\beta = 1$ ($E_t = C_t$) these are the kinetics of uncompetitive inhibition.

With two actively transported sugars, taken up *via* the same carrier, the competition for this carrier will be between (R_1E_1) and (R_2E_2), if different permeases are involved. Under the simplifying condition that the concentrations of R_1 and R_2 in the medium are high enough to virtually saturate the permeases, both (R_1E_1) and (R_2E_2) are maximal and constant, and independent of the free sugar concentrations $[R_1]$ and $[R_2]$. The ratio of the transport velocity of R_1 to the transport velocity of R_2 will under these conditions be determined by the relative affinities of (R_1E_1) and (R_2E_2) for the carrier. In other words: this ratio is determined by the K_i values (Eqn. 17) of the two substrates and is independent of the K_e values (Eqn. 10), which are the Michaelis–Menten constants of the overall transport mechanism of the substrates. As pointed out before, the K_i values may be influenced by the presence of nickelous ions, whereas the K_e values are not. Consequently the presence of nickelous ions may change the ratio of the transport velocities of R_1 and R_2.

Finally, if two actively transported sugars would be taken up *via* the same permease and the same carrier, an uncomplicated competitive inhibition should be expected. The degree of mutual inhibition will depend on the ratio of the K_e values of

the two substrates and on the ratio $[R_1]/[R_2]$, and will not be influenced by nickelous ions.

METHODS

Baker's yeast, strain Hansen C.B.S. 1172, was grown, harvested and starved as described before[15]. Adaptation to galactose was brought about as described previously[12]. Uptake of non-metabolizable substrates was measured according to the procedure described by CIRILLO[3]. Uptake of fermentable sugars was determined by measuring the disappearance of the sugars from the medium. The analytical methods utilized were: glucose, glucose oxidase method as modified by WASHKO AND RICE[16]; galactose, galactostat reagent (Worthington Biochemical Corp.); sorbose and fructose, method of DISCHE AND DEVI[17]. When ^{14}C-labelled sugars were used, the radioactivity was measured in a liquid scintillation counter, with the liquid scintillator described by BRAY[18].

RESULTS

Michaelis–Menten constants of sugar transport

In preliminary experiments the Michaelis–Menten constants of transport of the various sugars, utilized in these studies, were determined. The results appeared to be quite reproducible in many experiments and are summarized in Table I. The constants were calculated from the amounts of sugar, taken up during the first 10 min of incubation at 25°. In control experiments the velocity of uptake appeared to be virtually constant during at least 15 min, for all substrates tested. In the case of the non-metabolized substrates this relatively long interval of constant uptake velocity (with apparently negligible back-transport) is caused by the low V and high K_m values of these sugars.

Competition between two passively transported substrates

The inhibition of sorbose transport by galactose in uninduced cells shows the characteristics of a normal competitive inhibition, as given in Eqn. 8 (Fig. 1). Because of the high K_i value of galactose, high concentrations had to be used to get an appreciable inhibition of sorbose transport. Therefore the total sugar concentration in the medium in these experiments reached values up to 1000 mM. At these high concentrations an osmotic response of the cells is likely to occur[19], confusing the

TABLE I

MICHAELIS–MENTEN CONSTANTS (MEAN VALUE ± STANDARD ERROR) OF SUGAR TRANSPORT CALCULATED FROM SUGAR UPTAKE AT VARYING SUBSTRATE CONCENTRATIONS, AT 25°

Sugar	K_c (*mM*)	K_e (*mM*)
Sorbose	950 ± 61	
Galactose, uninduced	653 ± 59	
Galactose, induced		4.7 ± 0.4
Glucose		5.1 ± 0.3
Fructose		4.9 ± 0.3

results to some extent. The K_i of galactose, calculated from the slope of the line, is 527 mM; and 670 mM, as calculated from the intercept. The difference can be explained by the relatively low $\alpha[I]/(1-\alpha)$ values at higher sorbose concentrations (where an osmotic reaction of the cells can be expected) which tend to decrease the slope and to increase the intercept of the calculated line. These values should be compared with the K_c value of 653 mM of galactose transport in uninduced yeast cells (Table I).

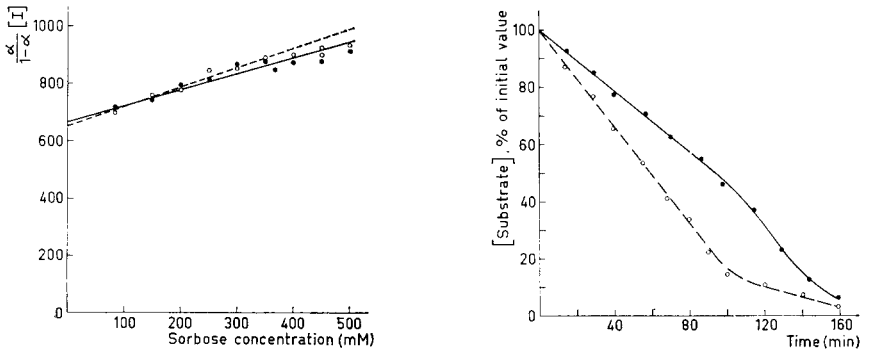

Fig. 1. Inhibition of sorbose transport by galactose, in uninduced cells, at 25°. ●—●, 500 mM galactose; ○—○, 300 mM galactose. The dotted line represents the theoretical relationship according to Eqn. 8, with $K_{c(\text{galactose})} = 653$ mM and $K_{c(\text{sorbose})} = 950$ mM.

Fig. 2. Competition for transport between fructose (●—●) and galactose (○— —○), measured from the disappearance of these sugars from the medium. The initial concentration in the medium of both sugars was 2%, yeast concentration: 5%, temperature: 25°. With a combination of glucose and galactose, similar results were obtained.

Competition between two actively transported substrates

In these experiments galactose-induced yeast cells were used. Two combinations were studied: glucose/galactose and fructose/galactose. The disappearance of these sugars from the medium was measured in the course of time, starting with a 1:1 ratio of the two substrates. As shown in Fig. 2 the sugars are taken up at a constant rate over a large time interval. Galactose is taken up faster than glucose or fructose, with a constant ratio galactose/glucose = 1.4 and galactose/fructose = 1.6, notwithstanding the fact that the concentration ratio in the medium changes from 1:1 at the beginning of the experiment to less than 1:4 after about 100 min. In other experiments, starting with different substrate concentrations, the same constant uptake ratios were found even with free sugar concentration ratios under 1:20. If the experiment is conducted in the presence of nickelous ions the transport ratio galactose/glucose changes from 1.4 to 2.2 and the transport ratio galactose/fructose from 1.6 to 2.8 (Fig. 3).

Competition between an actively and a passively transported substrate

These experiments were conducted with galactose-induced yeast. As actively transported inhibitors glucose and galactose were used. The passively transported substrate was sorbose.

The inhibition of sorbose transport by glucose and galactose is shown in Figs. 4 and 5. It is obvious that one is not dealing here with a simple competitive inhibition.

The kinetics of inhibition fit, however, Eqn. 27 with glucose, and Eqn. 29 with galactose as inhibitor of sorbose transport. With every 6 sets of experiments the parameters (± standard error) of Eqns. 27 and 29 could be equated to: $K_{e(\text{glucose})}$, 5.4 (± 0.5) mM; $K_{b(\text{glucose})}$, 1.3 (± 0.8) mM; ($\beta = 1$), with 20 mM glucose as inhibitor; $K_{e(\text{galactose})}$, 5.1 (± 0.4) mM; $K_{b(\text{galactose})}$, 0.9 (± 0.6) mM; $\beta = 0.67$ (± 0.04), with 20 mM galactose as inhibitor. At other inhibitor concentrations (10–50 mM), calculated parameters did not differ significantly from the above values.

The validity of Eqn. 30 was tested at relatively low sorbose concentration

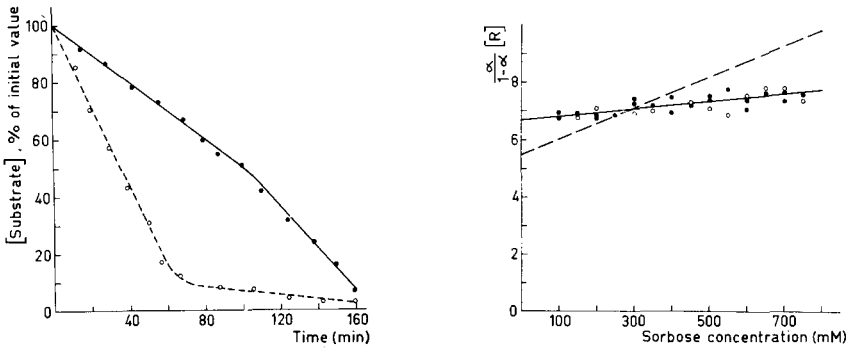

Fig. 3. Competition for transport between fructose (●—●) and galactose (○---○) in the presence of $5 \cdot 10^{-5}$ M Ni^{2+}. The initial concentration of both sugars was 2%. Yeast concentration: 15%, temperature: 25°. With a combination of glucose and galactose similar results were obtained (see text).

Fig. 4. The inhibition of sorbose transport by glucose in concentrations of 10 mM (●—●) and 20 mM (○—○). The dotted line represents the relationship of a simple competitive inhibition according to Eqn. 8, with $K_{i(\text{glucose})} = 5.4$ mM and $K_{e(\text{sorbose})} = 950$ mM.

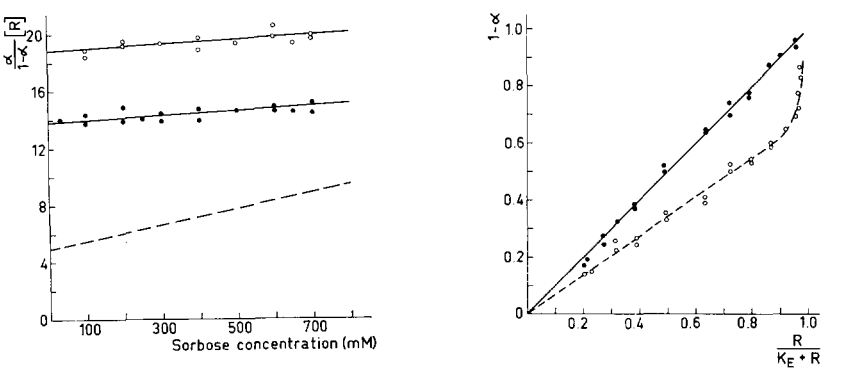

Fig. 5. The inhibition of sorbose transport by galactose, in concentrations of 10 mM (●—●) and 20 mM (○—○). The dotted line represents the relationship of a simple competitive inhibition according to Eqn. 8, with Michaelis–Menten constants of 5.1 mM for galactose and 950 mM for sorbose (see Table I).

Fig. 6. The inhibition of sorbose transport by galactose and glucose, at low sorbose concentration (60 mM). The value of $(1 - \alpha)$ for sorbose transport is plotted against the calculated saturation of the glucose and galactose permease, respectively, utilizing the K_e values summarized in Table I. ●—●, glucose; ○---○, galactose as inhibitor. With glucose: $\beta = 1$; with galactose: $\beta = 0.67$.

(60 mM). As shown in Fig. 6, the experimental results were in excellent agreement with the theoretical expectation. The deviation of the straight line at high galactose concentrations will be discussed below.

DISCUSSION

The hypothesis of sugar transport in yeast, postulated in previous papers[4,6,12] and summarized in the theoretical section of the present communication, implies certain consequences for the kinetics of mutual inhibition between pairs of sugars, competing for the common carrier. The kinetics of inhibition will be different according to the mode of transport of the competing sugars. A number of possibilities were considered theoretically and verified experimentally.

(1) Two substrates, both transported *via* facilitated diffusion (bypassing the permease step), should show competitive inhibition (Eqn. 8, Fig. 1). Notwithstanding the high experimental error (see RESULTS) the experiments on sorbose transport inhibition by galactose in uninduced cells must be interpreted as a simple competitive inhibition.

(2) Two substrates, both transported actively (utilizing different permeases but the same carrier) should give a mutual inhibition of transport of which the following characteristics can be derived theoretically: (a) The inhibition should be independent of the free sugar concentration in the medium and of the ratio between the sugar concentrations, as long as both permeases are near saturation ($[R] \gg K_e$). This is confirmed by the experimental results over a wide concentration range.

(b) The degree of mutual inhibition (in other words: the ratio velocity R_1 transport/velocity R_2 transport) is determined by the ratio of the affinities of the substrate–permease complexes (R_1E_1 and R_1E_2) for the carrier. These affinities, however, cannot be determined directly. The inhibition studies concerning actively transported sugars and sorbose (see below) indicate that the affinity of the substrate–permease complex is very high, both with glucose and with galactose as inhibitor. In both cases the K_b values are of the order of magnitude of 1 mM, but the experimental error is much too large to evaluate the ratio of the K_b values from these studies.

(c) As the transfer step of substrate from the permease to the carrier (Eqn. 3) is influenced by nickelous ions, a shift of the equilibration constant of this reaction may be expected in the presence of Ni^{2+}. It should be stressed that Ni^{2+} does not cause a change of the K_e values[5]. Consequently, the ratio of the relative affinities of the substrate–permease complexes to the carrier may alter in the presence of nickelous ions. The change of the transport ratios glucose/galactose and fructose/galactose after addition of nickelous ions is in excellent agreement with this expectation (Figs. 2 and 3).

(3) Inhibition of a passively transported substrate by an actively transported inhibitor gives a complicated situation. In the theoretical section the possibility that the passively transported, non-metabolized substrate would have an affinity for the permease was ignored. This is not *a priori* justified. CIRILLO[20] *e.g.* has shown that galactose induction of yeast results in a considerable increase of the velocity of facilitated diffusion of some non-metabolized sugars. The possibility of the following additional steps of the transport model should therefore be considered:

$$S + E \rightleftharpoons (SE) \quad \text{(a)}$$

$$(SE) + C \rightleftharpoons (SEC) \quad \text{(b)}$$

$$(SEC) \rightleftharpoons (SC) + E \quad \text{(c)}$$

This will, of course, complicate the inhibition kinetics. The yeast used in the present experiments did not show any change in the parameters of sorbose transport, however, after galactose induction. Moreover, no data were found in the present studies indicating any appreciable affinity of passively transported substrates for a permease. Apparently, the steps a, b and c may exist in some yeast strains with regard to certain sugars, but this does not represent a general feature.

In many experiments with varying sorbose and inhibitor concentrations, the validity of Eqn. 29 was proved (Figs. 4 and 5), the parameters being constant within the experimental error. In the case of glucose $E_t = C_t$ ($\beta = 1$), whereas for galactose $E_t < C_t$ ($\beta =$ approx. 0.67).

As shown in the theoretical section, $(1 - \alpha)$ should be directly proportional to the permease saturation, at low sorbose concentrations (Eqn. 30). This is shown to be true in Fig. 6. Theoretically it might be expected that $(1 - \alpha)$ should reach a maximal value of 0.67, at extremely high galactose concentrations. Fig. 6 shows, however, that at very high galactose concentrations there is a deviation from the originally perfectly straight line: the inhibition increases more than would be expected. This deviation becomes obvious at galactose concentrations over 300 mM, where the galactose permease is virtually saturated. The explanation of this phenomenon is that galactose can be transported both actively and passively[6]. At high galactose concentrations the permease is saturated, yielding a saturation of 67 % of the carrier sites. For the remaining 33 % of the carrier sites a simple competitive inhibition between sorbose and free galactose may be expected. Adopting a K_c value of 653 mM for passive galactose transport (as found in uninduced cells, see Table I), this competitive inhibition can be evaluated as follows. If the affinities of two substrates for the same carrier are of the same order of magnitude and $[S] \ll [R]$, the HUNTER AND DOWNS equation for competitive inhibition can be reduced to:

$$(1 - \alpha) = \frac{[R]}{K_{c(R)} + [R]}$$

The total inhibition of sorbose transport by active *plus* passive galactose transport at high galactose concentrations thus becomes:

$$(1 - \alpha) = \beta \frac{[R]}{K_c + [R]} + (1 - \beta) \frac{[R]}{K_{c(R)} + [R]}$$

The inhibition actually found experimentally corresponds very closely to the inhibition calculated from this equation.

The good agreement between the experimental results and the theoretically calculated kinetics can be considered as valuable support for the permease–carrier transport model, from which the theoretical considerations were derived. This model may be compared with the KEPES permease–carrier model of transmembrane transport[21,22]. In recent literature other examples of the existence of more than one mode of transmembrane sugar transport are given[23,24]. How far the kinetics, discussed in this paper, will contribute to diauxie phenomena, cannot yet be established.

A few experimentally found deviations from the expected kinetics in competition studies have been reported in recent literature (see *e.g.* ref. 25). It seems probable that these deviations can be explained according to the lines indicated in this communication.

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REFERENCES

1 T. G. SCHARFF AND E. H. KREMER, *Arch. Biochem. Biophys.*, 97 (1962) 192.
2 A. KOTYK, *Biochim. Biophys. Acta*, 135 (1967) 112.
3 V. P. CIRILLO, *Trans. N.Y. Acad. Sci.*, 23 (1961) 725.
4 A. ROTHSTEIN AND J. VAN STEVENINCK, *Ann. N.Y. Acad. Sci.*, 137 (1966) 606.
5 J. VAN STEVENINCK, *Biochim. Biophys. Acta*, 126 (1966) 154.
6 J. VAN STEVENINCK AND E. C. DAWSON, *Biochim. Biophys. Acta*, 150 (1968) 47.
7 V. P. CIRILLO, *J. Bacteriol.*, 84 (1962) 485.
8 W. WILBRANDT AND T. ROSENBERG, *Pharmacol. Rev.*, 13 (1961) 109.
9 T. Z. CSÁKY, *Ann. Rev. Physiol.*, 27 (1965) 415.
10 W. WILBRANDT, in P. KARLSON, *Funktionelle und Morphologische Organisation der Zelle*, Springer-Verlag, Berlin, 1963, p. 219.
11 M. J. HENDERSON, *Can. J. Biochem.*, 42 (1964) 933.
12 J. VAN STEVENINCK AND A. ROTHSTEIN, *J. Gen. Physiol.*, 49 (1965) 235.
13 L. HURWITZ AND A. ROTHSTEIN, *J. Cellular Comp. Physiol.*, 38 (1951) 437.
14 A. HUNTER AND C. E. DOWNS, *J. Biol. Chem.*, 157 (1945) 427.
15 J. VAN STEVENINCK AND H. L. BOOIJ, *J. Gen. Physiol.*, 48 (1964) 43.
16 M. E. WASHKO AND E. W. RICE, *Clin. Chem.*, 7 (1961) 542.
17 Z. DISCHE AND A. DEVI, *Biochim. Biophys. Acta*, 39 (1960) 140.
18 G. A. BRAY, *Anal. Biochem.*, 1 (1960) 279.
19 J. VAN STEVENINCK, *De Invloed van Metaalionen op de Glucoseopname door Gistcellen*, Thesis, 1962, Leiden.
20 V. P. CIRILLO, *Bacteriol. Proc.*, 105 (1966) 209.
21 A. KEPES, in N. E. GIBBONS, *8th Intern. Congr. Microbiol., Montreal, 1962*, University of Toronto Press, 1963, p. 38.
22 J. B. EGAN AND M. L. MORSE, *Biochim. Biophys. Acta*, 109 (1965) 172.
23 H. OKADA AND H. O. HALVORSON, *Biochim. Biophys. Acta*, 82 (1964) 538.
24 J. SAHA AND E. L. COE, *Biochem. Biophys. Res. Commun.*, 26 (1967) 441.
25 M. J. HENDERSON, *Can. J. Biochem.*, 42 (1964) 936.