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INTRACELLULAR pH AND THE KINETICS OF Rb^+ UPTAKE BY YEAST NON-CARRIER VERSUS MOBILE CARRIER-MEDIATED UPTAKE

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Summary

The effect of changes in the intracellular pH upon the concentration dependence of the Rb^+ uptake by yeast is investigated. It is shown, that the uptake of Rb^+ can be described by a mechanism in which the total concentration of primary binding sites at the outer side of the membrane is independent of the intracellular ligand composition and of the membrane potential, and the influx rate constants depend upon the intracellular pH and/or upon the membrane potential. It is argued that the involvement of a mobile carrier mechanism is not likely.

Introduction

Rosenberg and Wilbrandt [1] and Borst-Pauwels [2] showed in a theoretical study of the kinetics of mobile carrier-mediated transport of species across cellular membranes, that the V and the K_m of the translocation process are not independent kinetical parameters, but that they are interrelated. In addition, both kinetical parameters depend upon the intracellular concentration of those species which have an affinity for the carrier involved. In other words, if the maximum rate of transport (V) changes due to variations only in intracellular concentration of species, concomitant changes in the apparent affinity constant (K_m) are predicted when a mobile carried is involved.

Recently, Borst-Pauwels and Peters [3] showed that besides the V also the K_m of the phosphate uptake by yeast cells is markedly affected by changes in the intracellular pH (pH_i). A linear relationship between K_m and V was found. It was therefore considered, that phosphate uptake by yeast cells might be a mobile carrier-mediated process, and that either intracellular protons or intracellular hydroxyl anions have an affinity for the carrier.

Armstrong and Rothstein [4] showed, that protons have affinity to the monovalent cation uptake mechanism in yeast cells. In addition, Ryan and

Ryan [5] found that the uptake of monovalent cations by yeast cells is also markedly affected by changes in pH_i. Their data do not, however, allow a specification of kinetical parameters which are affected by changes in pH_i. In this paper we examined the effect of changes in pH_i upon the concentration dependence of the Rb^+ uptake by yeast cells. In this way, the kinetical parameters can be evaluated as a function of pH_i. It is examined whether changes in pH_i also affect the maximum rate of uptake and the apparent affinity constants of Rb^+ uptake by yeast cells, just as was found for phosphate uptake.

Methods

Yeast cells, *Saccharomyces cerevisiae*, strain Delft II, were starved under aeration over night. After starvation, the cells (2%, w/v) were incubated in 45 mM Tris/succinate of the desired pH, provided with 3% (w/v) glucose, 1% (v/v) ethanol or propanol at 25° C, respectively. Air of nitrogen was bubbled through the suspension. The uptake of Rb^+ (applied as chloride salt), using $^{86}Rb^+$ as a tracer, was studied according to the method of Borst-Pauwels et al. [6] as modified by Theuvenet and Borst-Pauwels [7]. Initial rates of uptake were determined from the slopes of the tangents to the uptake curves at zero time. In parallel experiments, at zero time of the Rb^+ uptake, pH_i was determined after freezing and boiling the cells as described by Borst-Pauwels and Dobbelmann [8].

Cells with different pH_i were prepared by the following methods: (1) Variation of the length of the anaerobic preincubation with glucose at pH 4.5 from 5 to 60 min. (2) Preincubation of the cells with glucose or ethanol for 60 min at pH 4.5 under aerobic conditions. (3) Preincubation of the cells with glucose for 60 min anaerobically at pH 4.5, and the addition of various concentrations of butyric acid (adjusted to pH 4.5 with Tris) after 54 min of preincubation. In the controls corresponding Tris concentrations were added, which had been adjusted to pH 4.5 with HCl. (4) Variation of the length of the aerobic preincubation with propanol at pH 4.5 from 5 to 60 min. (5) Preincubation of the cells with glucose, anaerobically, for 60 min at various pH values (range 3.5—7.6).

By these methods cells could be prepared with a pH_i ranging from 6.16 to 7.22. Cells prepared by Method 5 were only used to provide additional data on the effect of pH_i on the maximum rate of uptake.

Results

During anaerobic preincubation, in the presence of glucose, marked changes in pH_i occur. After a small, but significant drop, pH_i rises steadily (Fig. 1). Uptake of Rb^+ does not occur immediately after the addition of glucose, but only after a lag time of about 2 min. During this period pH_i decreases and there is no net proton efflux. The latter phenomenon has also been observed by Riemersma and Alsbach [9]. In view of this phenomenon, the shortest preincubation period was taken 5 min.

The kinetics of the Rb^+ uptake can be markedly affected by variation of the preincubation time. This is illustrated in Fig. 2 for the case of the Rb^+ uptake under anaerobic conditions with glucose as a metabolic substrate. The concen-

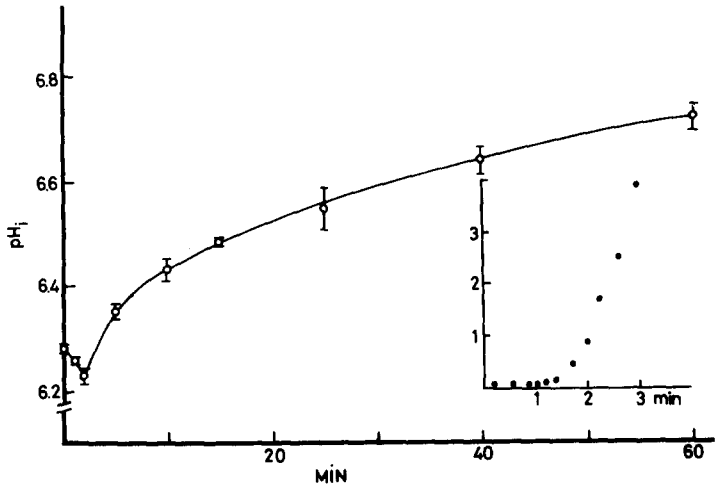

Fig. 1. Dependence of pH_i upon the period of anaerobic preincubation in the presence of 3% glucose at pH 4.5. Inset: time course of the uptake of carrier-free $^{86}Rb^+$ at pH 4.5. $^{86}Rb^+$ was added together with glucose at zero time. On the ordinate the radioactivity in the cells is presented in arbitrary units.

tration dependence of the uptake rates is represented graphically according to Hofstee [10]. For uptake described by Michaelis-Menten kinetics a straight line is expected. However, for the Rb^+ uptake convex curves are found. We have excluded that this is due to adsorption of Rb^+ to non-transporting sites with a

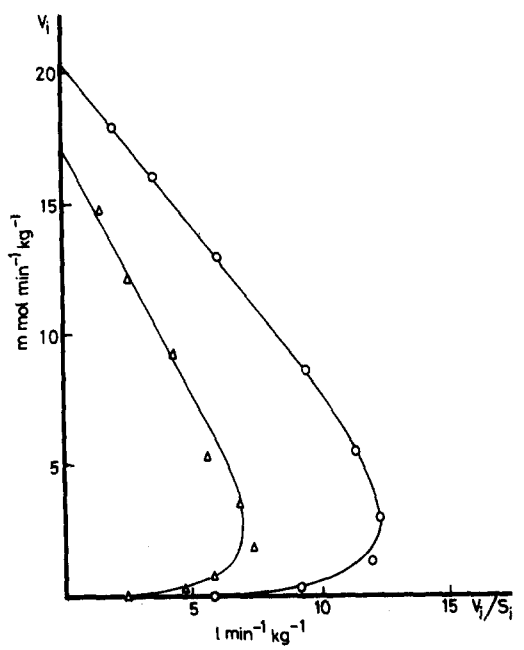

Fig. 2. Effect of the period of anaerobic preincubation in the presence of 3% glucose upon the concentration dependence of the Rb^+ uptake rate at pH 4.5. The initial rate of Rb^+ uptake is plotted against the quotient of this rate and the Rb^+ concentration (mM). (○) 10 min and (△) 60 min preincubation. Each point represents the mean value of triplicate experiments.

high affinity for Rb^+ by which the free Rb^+ concentration may be lowered relatively more at low Rb^+ concentration than at higher ones. It appeared that at 10^{-3} mM Rb^+, the lowest Rb^+ concentration applied, less than 1% of radioactive Rb^+ is bound to the yeast, whereas binding of about 70% is required to explain the data in Fig. 2. In addition, if the deviation from Michaelis-Menten kinetics is due to adsorption, the quantitative reduction in the free Rb^+ concentration would decrease on reducing the percentage of yeast with which the experiment is performed. Consequently, the observed deviation from Michaelis-Menten kinetics would diminish on reducing the percentage of yeast. However, an identical Hofstee plot is found when the percentage of yeast is decreased from 2 to 0.1%. It is thus clear that adsorption of Rb^+ to the yeast cannot account for the observed concentration dependence of the Rb^+ uptake.

Convex curves in the Hofstee plot may also occur in case of uptake via a mechanism with two binding sites [10,11]. As a matter of fact, Armstrong and Rothstein [12] and Borst-Pauwels et al. [13] presented experimental data which show that the uptake of monovalent cations by yeast is compatible with transport via a two-site mechanism. The isotherm for the Rb^+ uptake by yeast cells is therefore not described by a single Michaelis-Menten relation but by an equation of the form of Eqn. 1:

$$v_i = \frac{A_i s_i + B_i s_i^2}{C_i + D_i s_i + s_i^2} \tag{1}$$

where v_i represents the initial rate of the uptake of Rb^+ of a concentration s_i.

A_i—D_i are the kinetical coefficients of the uptake process and are independent of s_i [11]. With a curve-fitting program and the use of a digital computer we have computed the coefficients A_i—D_i of this rate equation, describing the isotherm of the Rb^+ uptake by yeast cells under the various experimental conditions applied. Coefficient B_i, representing the maximum rate of the Rb^+ uptake appeared to decrease when pH_i raises. A single relationship between coefficient B_i and pH_i is found, independent of the way by which pH_i is changed (Fig. 3).

The effect of variation of the preincubation time upon the kinetical parameters A_i—D_i and upon pH_i is shown for the anaerobic preincubation with glucose in Fig. 4 and for the aerobic preincubation with propanol in Fig. 5. These two figures show, that pH_i increases after 2 min under anaerobic conditions, and decreases under aerobic conditions during 10 min, after which a slight increase is observed. Both the coefficients A_i and B_i appeared to be closely correlated with pH_i; an increase in pH_i is accompanied by a decrease in A_i and B_i and a decrease in pH_i by an increase in A_i and B_i. This is not true for the coefficients C_i and D_i (Table I).

In the experiments in which butyric acid is used, the values of A_i, C_i and D_i have been corrected for a slight competitive inhibition by the extra amount of Tris added in order to adjust the pH to 4.5. We are aware of the fact that pH_i is not the only variable during anaerobic preincubation as the yeast cells also accumulate Tris ions. Furthermore, cells which have been metabolizing with ethanol or propanol as a substrate have taken up more Tris than cells which have been metabolizing on glucose. It was therefore investigated, whether the intracellular Tris concentration influenced the kinetic coefficients. Cells with

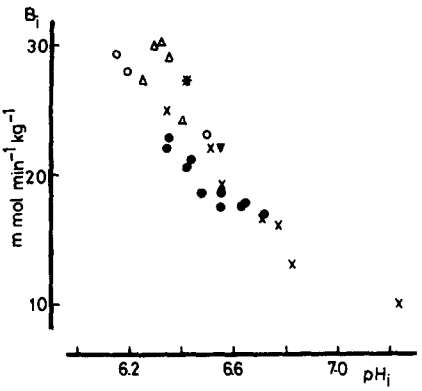

Fig. 3. Dependence of coefficient B_i upon pH$_i$. ○, cells prepared by Method 3; ●, Method 1; △, Method 4; X, Method 5; ▼, Method 2, glucose; *, Method 2, ethanol. For data see Table I and Figs. 4 and 5.

a different intracellular Tris concentration were prepared by preincubating the yeast anaerobically at pH 4.5 with glucose in the presence of various concentrations of Tris. Uptake of Rb^+ was studied at 45 mM Tris anaerobically in the presence of 3% glucose. It was found that the intracellular Tris concentration neither affected pH$_i$ nor any of the kinetic coefficients of the Rb^+ uptake significantly (not shown).

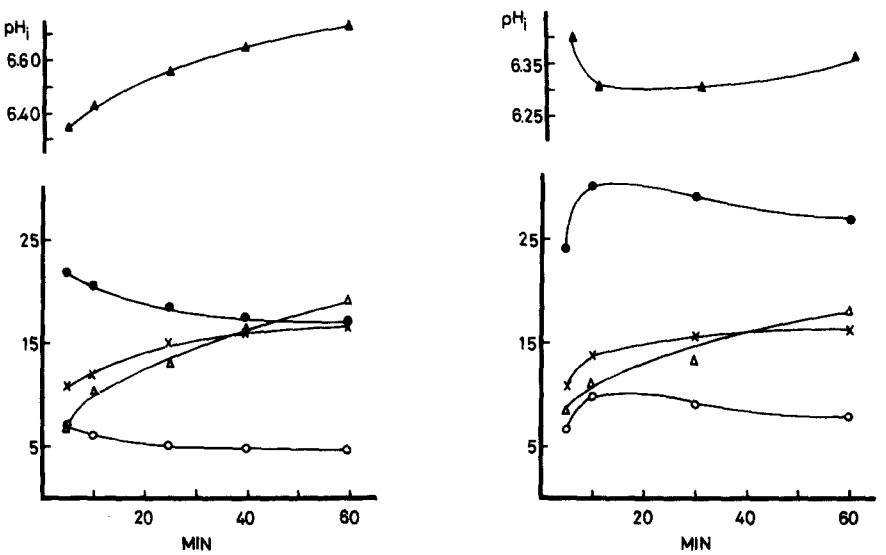

Fig. 4. Dependence of the coefficients A_i—D_i and pH$_i$ upon the period of the anaerobic preincubation in the presence of 3% glucose at pH 4.5. ○, $10A_i$; ●, B_i; △, $100C_i$; X, $10D_i$; and ▲, pH$_i$. Dimensions of the coefficients: A_i (mmol^2 · min^{-1} · kg^{-1} · l^{-1}), B_i (mmol · min^{-1} · kg^{-1}), C_i (mmol^2 · l^{-2}), D_i (mmol · l^{-1}).

Fig. 5. Dependence of the coefficients A_i—D_i and pH$_i$ upon the period of aerobic preincubation in the presence of 1% propanol at pH 4.5; see also legend to Fig. 4.

TABLE I

pH_i AND THE COEFFICIENTS A_i–D_i FOR CELLS WHICH HAD BEEN PREINCUBATED UNDER VARIOUS EXPERIMENTAL CONDITIONS FOR 60 MIN

S.D. is the standard deviation of three observations. The numbers between parentheses refer to the experimental conditions described under Methods.

Condition	pH_i	A_i ± S.D.	B_i ± S.D.	C_i ± S.D.	D_i ± S.D.
Glucose, N_2 (1)	6.72	0.54 ± 0.04	16.2 ± 1.1	0.19 ± 0.02	1.55 ± 0.15
Glucose, air (2)	6.55	0.53 ± 0.13	20.9 ± 1.2	0.19 ± 0.04	1.71 ± 0.25
Ethanol, air (2)	6.41	0.83 ± 0.16	27.4 ± 1.7	0.18 ± 0.03	1.62 ± 0.22
Propanol, air (2)	6.36	0.82 ± 0.07	27.0 ± 1.8	0.15 ± 0.06	1.60 ± 0.14
1 mM butyric acid (3)	6.51	0.72 ± 0.13	23.0 ± 0.8	0.23 ± 0.03	1.80 ± 0.15
4 mM butyric acid (3)	6.30	0.84 ± 0.10	27.2 ± 1.0	0.19 ± 0.03	1.60 ± 0.14
8 mM butyric acid (3)	6.16	0.90 ± 0.12	29.4 ± 1.1	0.21 ± 0.03	1.50 ± 0.20

Discussion

Comparison with phosphate uptake

The present results show, in accordance with the earlier observations of Ryan and Ryan [5], that the initial rate of the Rb^+ uptake is markedly affected by changes in pH_i. In addition it is shown, that only part of the kinetic parameters depend on pH_i. The coefficients in the numerator of the rate equation (Eqn. 1), A_i and B_i depend upon pH_i (Figs. 3—5), whereas the coefficients in the denominator, C_i and D_i, are not significantly affected by changes in pH_i (Table I). With respect to the dependence of the kinetic parameters upon pH_i, the Rb^+ uptake by yeast cells differs from the phosphate uptake. In the latter case, all the kinetic parameters depend upon pH_i. In addition, the pH optimum for the Rb^+ uptake appeared to be <6.0 (Fig. 3), whereas that for the phosphate uptake is 6.8 [3]. It is therefore unlikely that phosphate and Rb^+ are taken up by yeast cells by a common uptake mechanism and that a common pH-sensitive factor is involved in the two translocation processes.

Non-carrier versus mobile carrier-mediated uptake

As pointed out by Borst-Pauwels [14] Eqn. 1 may be common to various two-site mechanisms of uptake, both mobile carrier and non-carrier mechanisms of uptake. We have examined whether the present data allow a choice between the two possible types of mechanisms. We will first consider translocation via a mobile carrier.

In the appendix specific interrelationships between the coefficients A_i, B_i, and D_i are predicted when only uncharged carrier complexes are translocated across the membrane. On plotting A_i or $D_i - 2A_i/B_i$ against B_i straight lines through the origin are predicted (Eqns. A5 and A6). Fig. 6 shows that in the case of the Rb^+ uptake by yeast cells $D_i - 2A_i/B_i$ is independent of B_i. Consequently, the experimental data allow the rejection of a mobile carrier transport model in which only uncharged carrier complexes are able to cross the membrane.

As changes in pH_i may be accompanied by changes in the membrane poten-

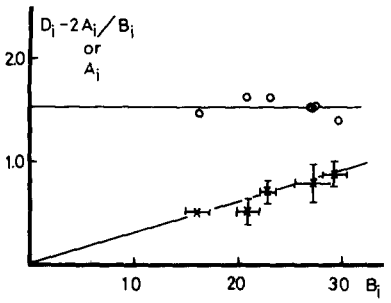

Fig. 6. Plots of $D_i - 2A_i/B_i$ and of A_i against B_i (data from Table I). ○, $D_i - 2A_i/B_i$ and ×, A_i. See also legend to Fig. 4.

tial (E) [15] we also considered the possibility that the uptake process may be affected by changes in the membrane potential. This will be the case when charged carrier complexes are translocated across the membrane. In that case the rate constants for both influx and efflux will depend upon E. However, as is shown in Appendix, the finding that $D_i - 2A_i/B_i$ is independent of B_i is very well compatible with a non-carrier mechanism, and this independency is in general not expected when ions are translocated across the membrane via charged carrier complexes.

The conclusion is reached, that a mobile carrier mechanism is probably not involved in the Rb^+ uptake by yeast cells. This is consistent with the finding of Rothstein [16] that the apparent affinity constant of the Rb^+ uptake (corresponding to coefficient D_i) is hardly affected when the intracellular Na^+ concentration is varied.

General features of models which may serve to explain the present data are, that the total concentration and affinity of the binding sites of the mechanism at the extracellular side of the membrane are independent of the intracellular ligand composition and of the membrane potential. Then namely, the coefficients C_i and D_i are only related to the affinity constants of the extracellular binding sites of the mechanism and are therefore independent of variations in pH_i and/or in E. On the other hand, the coefficients A_i and B_i are related to the influx rate constants which may depend upon pH_i and/or E (see Appendix, Eqns. A1—A4, taking $W_{II} = 0$). In fact, the ion-translocation process can be described by enzyme kinetics [11]. The rate constants of translocation across the cellular membrane are equivalent with rate constants of conversion of substrate to product. The dependence of A_i and B_i upon pH_i may reflect a direct coupling between cation influx and proton efflux which is expected when Rb^+ translocation across the membrane proceeds via a Rb^+—H^+ exchange reaction in the interior of the membrane (see for example the non-carrier tetramere model developed by Lieb and Stein [17]), or it may be due to an increase in the negative membrane potential caused by a more effective proton pump generating an electrogenic potential.

The preincubation effect

Figs. 4 and 5 show that independently of the metabolic activity of the cells

(anaerobic or aerobic metabolism) the kinetic coefficients C_i and D_i are increased with the length of the preincubation. This phenomenon ('preincubation effect') appeared to be independent of pH_i. This might be ascribed to a change in affinity of the external binding sites for Rb^+ during preincubation. We would like to point, however, to an other possible explanation. We recently found, that the addition of polyvalent cations to the suspending medium also increased the values of the kinetic coefficients C_i and D_i and did not affect the values of the kinetic coefficients A_i and B_i. This was explained by a reduction in the surface potential by the polyvalent cations [7]. Similarly the 'preincubation effect' might be due to a progressive reduction in the surface potential during the period of preincubation.

Appendix

A rate equation for transport via a mobile carrier mechanism in which two substrate binding sites are involved, has been derived by Borst-Pauwels [2]. Taking $n = 2$ in Eqn. 26 from that paper, an equation is obtained of the form of Eqn. 1. The coefficients A_i—D_i are functions of kinetic parameters of the uptake mechanism, intracellular and extracellular concentrations of species having affinity for the substrate binding sites and the metabolic state of the cells. In a condensed form these functions are represented by Eqns. A1—A4:

$$A_i = \frac{r'_1 C_t}{x'_2 + r'_2 W_{II}} \tag{A1}$$

$$B_i = \frac{2r'_2 C_t}{x'_2 + r'_2 W_{II}} \tag{A2}$$

$$C_i = \frac{x'_0 + r'_0 W_{II}}{x'_2 + r'_2 W_{II}} \tag{A3}$$

$$D_i = \frac{x'_1 + r'_1 W_{II}}{x'_2 + r'_2 W_{II}} \tag{A4}$$

where C_t is the total concentration of the carrier, r' with appropriate subscript is a complex function of influx rate constants, affinity constants and the extracellular ligand composition, and x' with appropriate subscript is a complex function of affinity constants and the extracellular ligand composition. W_{II} is a factor which is a function of efflux rate constants, affinity constants, the intracellular ligand composition and the metabolic state of the cells. All the coefficients in the Eqns. A1—A4 are $\geqslant 0$. By eliminating the factor W_{II} specific interrelationships between the coefficients A_i, B_i and D_i are easily found:

$$A_i = \frac{r'_1}{2r'_2} B_i = \alpha_i B_i \tag{A5}$$

and

$$D_i - 2A_i/B_i = \frac{x'_1 - 2\alpha_i x'_2}{2r'_2 C_t} B_i = \beta_i B_i \tag{A6}$$

When only uncharged carried complexes are translocated across the membrane, the coefficients α_i and β_i are constants. On plotting A_i or $D_i - 2A_i/B_i$ against B_i straight lines through the origin are predicted.

When charged carrier complexes are translocated across the membrane, the coefficients α_i and β_i may depend upon pH_i, namely when changes in pH_i are accompanied by changes in E. Then the functions r'_1 and r'_2 depend upon E, because the influx rate constants are no real constants any more but will depend on E.

Experimentally it is found, that α_i and the product $\beta_i B_i$ are independent of pH_i (Fig. 6). From Eqns. A2 and A6 it can be derived that

$$\beta_i B_i = \frac{x'_1 - 2\alpha_i x'_2}{x'_2 + r'_2 W_{II}} \tag{A7}$$

Therefore either $r'_2 W_{II}$ is independent of pH_i or $r'_2 W_{II} << x'_2$. According to Eqn. 29 in the paper of Borst-Pauwels [2], and realizing that the rate constants of influx increase when E becomes more negative and those of efflux decrease, it can be deduced that $r'_2 W_{II}$ will increase, as well. Consequently, the product $\beta_i B_i$ cannot be independent of pH_i (E), unless $r'_2 W_{II} << x'_2$. This will be true when $W_{II} = 0$. In that case the rate equation is similar to that for an enzymic process [11]. In other words, the finding that α_i and $\beta_i B_i$ is independent of pH_i, is very well compatible with a two-site non-carrier transport model.

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References

1 Rosenberg, T. and Wilbrandt, W. (1963) J. Theor. Biol. 56, 191—204
2 Borst-Pauwels, C.W.F.H. (1974) J. Theor. Biol. 48, 183—195
3 Borst-Pauwels, G.W.F.H. and Peters, P.H.J. (1977) Biochim. Biophys. Acta 466, 488—495
4 Armstrong, W.Mc.D. and Rothstein, A. (1964) J. Gen. Physiol. 48, 61—71
5 Ryan, J.P. and Ryan, H. (1972) Biochem. J. 128, 139—146
6 Borst-Pauwels, G.W.F.H., Schnetkamp, P. and Van Well, P. (1973) Biochim. Biophys. Acta 291, 274—279
7 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Biochim. Biophys. Acta 426, 745—756
8 Borst-Pauwels, G.W.F.H. and Dobbelmann, J. (1972) Acta Bot. Neerl. 21, 149—154
9 Riemersma, J.C. and Alsbach, E.J.J. (1974) Biochim. Biophys. Acta 339, 274—284
10 Hofstee, B.J. (1952) Science 116, 329—331
11 Borst-Pauwels, G.W.F.H. (1973) J. Theor. Biol. 40, 19—31
12 Armstrong, W.McD. and Rothstein, A. (1967) J. Gen. Physiol. 50, 967—988
13 Borst-Pauwels, G.W.F.H., Wolters, G.H.J. and Hendricks, J.J.G. (1971) Biochim. Biophys. Acta 225, 269—276
14 Borst-Pauwels, G.W.F.H. (1976) J. Theor. Biol. 56, 191—204
15 Pena, A. (1975) Arch. Biochem. Biophys. 167, 397—409
16 Rothstein, A. (1974) J. Gen. Physiol. 64, 608—621
17 Lieb, W.R. and Stein, W.D. (1970) Biophys. J. 10, 585—609