The Role of Intracellular pH in the Regulation of Cation Exchanges in Yeast

By J. P. RYAN and H. RYAN

Department of Biochemistry, University College, Dublin, Irish Republic

(Received 17 January 1972)

1. When yeast oxidizes propan-2-ol in the presence of KCl no uptake of K^+ occurs. 2. When propionate is added to suspensions containing propan-2-ol, or if the suspensions are bubbled with CO_2 , a considerable uptake of K^+ occurs. 3. Maximum K^+ uptake occurs at a propionate concentration of 2 mM. 4. The addition of 20 mM-propionate to the suspension lowers the intracellular pH of the yeast from a resting value in the region of 6.2 to approx. 5.6. 5. When K^+ uptake is measured in the presence of 20 mM-propionate, progressive changes in the rate of K^+ uptake and intracellular pH occur. The optimum rate of K^+ uptake occurs at an intracellular pH of 5.70. 6. The effect of both intra- and extra-cellular pH on K^+ - K^+ exchange was studied and an optimum rate was found at an extracellular pH of 5.35, the corresponding intracellular pH being 6.44. 7. When a Na^+ -loaded yeast oxidizes propan-2-ol in the presence of KCl, a steady efflux of Na^+ and influx of K^+ occurs. The addition of 10 mM-propionate to the suspension markedly inhibited the Na^+ efflux but only slightly decreased the K^+ influx. 8. The effect of both extra- and intra-cellular pH on Na^+ efflux was studied with propan-2-ol and with glucose. The results can be best interpreted in terms of intracellular pH changes, and an optimum was obtained at approx. pH 6.40.

When yeast oxidizes ethanol in the presence of KCl, K^+ is taken up by the cells and H^+ is excreted into the suspending medium (Rothstein & Enns, 1946). In a previous paper (Ryan *et al.*, 1971) it was shown that when the suspensions were buffered at pH 4.75 the addition of either acetate or propionate to the suspension fluids increased the initial rate of K^+ uptake, the $\Delta K^+/\Delta O_2$ ratio, and the total amount of K^+ accumulated by the yeast. Bubbling the suspensions with CO_2 also markedly increased K^+ uptake. On the basis of these observations it was suggested that intracellular pH is a major factor in the control of the K^+ - H^+ -exchange system.

During the oxidation of ethanol large quantities of acetic acid are produced (Eaton & Klein, 1957; Ryan, 1967); thus any investigations of intracellular pH change with this substrate are necessarily complicated. With propan-2-ol as a substrate, however, the system is considerably simplified. The present paper is concerned largely with cation exchanges during the oxidation of propan-2-ol. The effect of intracellular pH on K^+ - H^+ exchange is further investigated, and the studies are extended to include the effects of both intracellular and extracellular pH on the K^+ - K^+ exchange that occurs in normal yeast, and the K^+ - Na^+ exchange that occurs in a Na^+ -loaded yeast.

Materials and Methods

Yeast

Fresh baker's yeast (*Saccharomyces cerevisiae*, strain RN; supplied by the Cork Yeast Co., Water-

course Rd., Cork, Eire) was used. Before use, twice-washed yeast was 'starved' by oxygenation for 24 h at 22°C of a 1:10 (w/v) suspension adjusted to pH 7.4 with 0.02 M-tris-HCl buffer. The yeast starved in this way had a resting intracellular pH of about 6.0–6.2.

Chemical determinations

Sodium and potassium. These were determined with a Beckman model DU flame spectrophotometer.

Propionic acid. This was determined by the micro-diffusion procedure described by Conway (1962).

Oxygen consumption. This was determined by the conventional manometric technique, with 3 ml samples of yeast/flask, shaken at 120 cycles/min at 22°C.

Experimental procedures

Aerobic suspensions. Samples of the 'starved' yeast were suspended (1:20, w/v) in the various media described below and bubbled with O_2 at 22°C. The yeast samples were harvested either by centrifugation for 10 min at 1000g, or on Millipore filters (SM, pore size 5 μ m). When the yeast was required for determination of propionic acid the samples were not washed, but allowance was made for propionic acid present in the interspace (Conway & Downey, 1950). For K^+ or Na^+ determinations, 2 ml samples were removed, the yeast was harvested on Millipore filters and washed twice with 4–5 ml of ice-cold glass-distilled water. The results of the various analyses

are expressed throughout per kg wet wt. of centrifuged yeast.

Intracellular pH. Two methods were used to determine the intracellular pH. (1) Freezing-and-thawing method. Samples of centrifuged yeast (2.5 g) were frozen with liquid N_2 and thawed rapidly to 22°C. The pH of the resulting mixture was determined with a Beckman model G pH-meter. A small correction (<0.1 pH unit) was made for extracellular buffer in the interspace fluid. (2) Propionic acid-distribution method. The method was the same as that described by Conway & Downey (1950) for acetic acid. In this case, the appropriate pK_a values used for propionic acid were 4.72 and 4.76, inside and outside the cell respectively.

Studies with radioactive potassium. The ^{42}KCl was supplied at an initial radioactivity of 1 mCi/0.225 g, by the Atomic Research Establishment, Harwell, Berks., U.K. The amount of tracer included in the final suspensions was 15–20 μCi . Samples (5 ml; 0.1 g of yeast) were removed and harvested on Millipore filters. The suspending fluid was also retained. The yeast samples were boiled and diluted to 10 ml. The samples were then centrifuged, and 5 ml portions were counted for radioactivity in a well-type crystal-scintillation counter (Nuclear-Chicago model 181 A), at about 20% efficiency. The suspending fluid samples were diluted (1:100) and 5 ml samples also counted for radioactivity. In this way approx. 2000–3000 c.p.m. were obtained. The uptake of ^{42}K , expressed as mequiv./kg wet wt. of centrifuged yeast, was calculated from the specific radioactivity of the supernatant solutions, suitable allowance being made for background and decay. Chemical analyses were also carried out to determine the total K^+ in each sample.

Preparation of Na^+ -loaded yeast. A twice-washed sample (about 50 g) of fresh yeast was fermented for 2 h in 10 vol. of 0.2 M-trisodium citrate containing 5% (w/v) glucose (Conway & Moore, 1954). The Na^+ -loaded yeast was then washed twice with 0.1 M-NaCl,

and 'starved' for 18 h before use in 0.1 M-NaCl, adjusted to pH 7.4 with 0.02 M-tris-HCl buffer. The yeast prepared in this way contained 60–70 mequiv. of Na^+ /kg wet wt. of centrifuged yeast.

Results

Uptake of K^+ during the oxidation of various alcohols

The uptake of K^+ during the oxidation of various alcohols was investigated. In each case the concentration of alcohol in the suspension was that which gave a maximum rate of oxidation under otherwise similar experimental conditions. The yeast samples were suspended in 0.02 M-citric acid (adjusted to pH 4.75 with 1 M-tris) and the appropriate concentration of alcohol. The suspensions were bubbled with O_2 for 5 min at 22°C and 1 M-KCl was added ('zero time') to give a final concentration of 0.01 M-KCl and a ratio of 1:20 (w/v) yeast to suspending fluid. Samples were removed immediately after the addition of KCl (0 min) and after 10 and 20 min. The yeast was analysed for potassium content.

Of the alcohols tested only propan-1-ol, propan-2-ol and butan-1-ol had appreciable rates of oxidation compared with ethanol. Table 1 shows that of these three alcohols only propan-1-ol supports an appreciable K^+ uptake. With propan-2-ol as a substrate practically no K^+ uptake occurs, although the rate of oxidation is some 60% of that of ethanol. A number of other alcohols, including methanol, 2-methylpropan-1-ol, butan-2-ol and 2-methylpropan-2-ol, failed to support K^+ uptake. Since all of these were oxidized at rates less than 20% of that of ethanol such findings were not considered significant.

Effect of added propionate or bubbling with CO_2 on K^+ uptake during the oxidation of propan-2-ol

When yeast oxidizes propan-2-ol a considerable uptake of K^+ occurs if propionate is added to the suspensions, or if the suspensions are bubbled with

Table 1. *Uptake of K^+ during the oxidation of various alcohols*

The yeast was suspended in 0.02 M-citric acid (adjusted to pH 4.75 with 1 M-tris) and various alcohols were added at the concentration stated. The suspensions were bubbled with O_2 at 22°C. After a period of 5 min 1 M-KCl was added (0 min) to give a final concentration of 0.01 M-KCl and a ratio of 1:20 (w/v) yeast to suspending fluid. Each value represents the average of at least three experiments.

Alcohol added	Concn. (% v/v)	Uptake of K^+ (mequiv./kg)		Consumption of O_2 (mmol/h per kg)
		10 min	20 min	
None	—	0.2	0.6	12
Ethanol	1.5	25.3	30.2	524
Propan-1-ol	3.0	8.9	13.5	379
Propan-2-ol	3.0	1.4	0.2	318
Butan-1-ol	1.5	2.5	1.7	277

CO₂+O₂ (30:70). In these studies the general procedure was the same as described in the preceding section. In the suspensions containing propionate,

1M-propionic acid (adjusted to pH4.75 with 1M-tris) was added to give a final concentration of 0.01M-tris-propionate. In studying the effect of carbon dioxide the suspensions were bubbled with CO₂+O₂ (30:70) at 22°C. The results are shown in Fig. 1.

Fig. 1. Effect of propionate and bubbling with CO₂ on K⁺ uptake

The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-0.01M-KCl, also containing: 3% (v/v) propan-2-ol and bubbled with O₂ (●); 10mm-propionic acid and bubbled with O₂ (▲); 3% (v/v) propan-2-ol and 10mm-propionic acid and bubbled with O₂ (○); 3% (v/v) propan-2-ol and bubbled with CO₂+O₂ (30:70) (Δ). Values are averages of two experiments.

Effect of various propionate concentrations on intracellular pH, K⁺ uptake and oxygen consumption

In determining the effect of various concentrations of propionate on intracellular pH and K⁺ uptake the yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-3% (v/v) propan-2-ol. Various amounts of 1M-propionic acid (also adjusted to pH4.75 with 1M-tris) were added and the suspensions were bubbled with O₂ at 22°C. After 10min samples were removed and the intracellular pH was determined by the freezing-and-thawing procedure (see the Materials and Methods section). To the remaining suspension 1M-KCl was added ("zero time") to give a final concentration of 0.01M. Samples were removed immediately after the addition of the KCl, and at intervals of 5 and 10min, and analysed for K⁺ content. Oxygen-consumption rates were determined, under similar conditions, by using 1:200 (w/v) yeast suspensions.

Table 2 shows that the greatest K⁺ uptake occurs in the presence of 2mm-propionate. Under these conditions the initial intracellular pH had a mean value of 5.87. Oxygen consumption is progressively inhibited as the concentration of propionate is increased.

Progressive changes in intracellular pH accompanying K⁺ uptake

It was shown in Table 2 that, in the presence of 20mm-propionate, although the O₂ consumption was

Table 2. Effect of various concentrations of propionate on intracellular pH, uptake of K⁺ and oxygen consumption

The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-3% (v/v) propan-2-ol. The suspensions contained various amounts of propionate and were bubbled with O₂ at 22°C. After 10min samples were removed for pH determination. To the remaining suspension 1M-KCl was added (0min) to give a final concentration of 0.01M. Oxygen consumption rates were determined by using 1:200 (w/v) suspensions. Each value represents the average of two experiments.

Concn. of propionate (mm)	Intracellular pH	Uptake of K ⁺ (mequiv./kg)		Consumption of O ₂ (mmol/h per kg)
		5 min	10 min	
0	6.24	1.7	1.4	420
1.0	6.02	10.2	10.5	387
2.0	5.87	15.4	19.0	352
5.0	5.74	8.8	15.7	273
10.0	5.64	5.6	11.5	105
20.0	5.61	2.1	5.2	35
50.0	5.53	0.3	1.3	4
100.0	5.47	0.0	0.4	0

greatly decreased a significant uptake of K^+ still occurred. Further experiments with this concentration of propionate showed that progressive changes occurred in both the intracellular pH and the rate of K^+ uptake. In these studies the general conditions were the same as described in Table 2. Duplicate samples were removed at 0 min and thereafter at 5 min intervals, and the yeast was analysed for K^+ and propionate content. The concentration of propionate in the suspending fluid was also determined at each time-interval. The intracellular pH was calculated from the distribution of propionate as described in the Materials and Methods section. The results are shown in Table 3. The rate of K^+ uptake, expressed as mmol/h per kg, was calculated for each 5 min interval. When these rates are plotted against the mean intracellular pH for the corresponding time-intervals, it may be seen (Fig. 2) that the optimum rate of K^+ uptake occurs at an intracellular pH of approx. 5.70.

Effect of intra- and extra-cellular pH on K^+ - K^+ exchange

Only small amounts of K^+ are taken up by the yeast during the oxidation of propan-2-ol. It was shown, however, when labelled K^+ was included in the medium, that a considerable exchange of K^+ takes place between the intra- and extra-cellular fluids (Fig. 3). To investigate the effect of intracellular pH on the K^+ - K^+ -exchange system it was necessary to raise the intracellular pH above its resting value of pH 6.0–6.2. Ideally it would be desirable to raise the intracellular pH to selected values at a constant extracellular pH. Attempts to achieve this by the addition of various amounts of NH_4^+ to the suspensions proved unsatisfactory. Waddell & Bates (1969) showed that a linear relationship holds between extracellular and intracellular pH for isolated rat diaphragm. A similar relationship was found for isolated perfused turtle heart, ascites-tumour cells and cat brain (for references see Waddell & Bates, 1969). Such a relationship also holds for yeast, as is shown in Fig. 4. In these experiments the yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to various pH values with 1M-tris)–3% (v/v) propan-2-ol. The suspensions were bubbled with O_2 for 20 min at 22°C. The intracellular pH values were determined by the freezing-and-thawing procedure (see the Materials and Methods section).

Experiments were then carried out in which the yeast was suspended at various pH values as described for Fig. 4. After 20 min bubbling with O_2 , 1M-KCl (labelled with ^{42}K) was added to the suspensions to give a final concentration of 0.01M-KCl. Samples were removed immediately after the addition of KCl ('zero time') and after 40 min. The total influx of K^+ was determined from the radioactivity (c.p.m.)

Table 3. *Progressive changes in intracellular pH accompanying uptake of K^+*

The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH 4.75 with 1M-tris)–3% (v/v) propan-2-ol–20mM-propionic acid (adjusted to pH 4.75 with 1M-tris). The suspensions were bubbled with O_2 at 22°C. After bubbling for 10 min, 1M-KCl was added (0 min) to give a final concentration of 0.01M. The pH was calculated from the distribution of propionate, as described in the Materials and Methods section. Each value represents the average from four experiments.

Time (min)	Intracellular pH	K^+ content (mequiv./kg of yeast)
0	5.55	112.2
5	5.61	115.5
10	5.66	120.9
15	5.73	127.2
20	5.79	132.9
25	5.83	136.9
30	5.88	139.8
35	5.91	142.0
40	5.95	143.7

Fig. 2. *Effect of intracellular pH on the K^+ - H^+ -exchange rate*

The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH 4.75 with 1M-tris)–3% (v/v) propan-2-ol–20mM-propionic acid and bubbled with O_2 at 22°C. After 10 min (zero time) 1M-KCl was added to give a final concentration of 0.01M-KCl. Values are averages of four experiments.

in the yeast samples, and the net uptake of K^+ was determined chemically. The difference between these two values gives the amount of K^+-K^+ exchange in the 40 min period. The results are shown in Table 4 at each extracellular pH value. The corresponding intracellular pH values are also shown; these were calculated from the relationship shown in Fig. 4(a).

Fig. 3. Exchange of intracellular and extracellular K^+ during propan-2-ol oxidation

The yeast was suspended (1:50, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-0.01M-KCl (labelled with ^{42}K)-3% (v/v) propan-2-ol and bubbled with O_2 at 22°C. ●, Total K^+ uptake as measured with ^{42}K ; ○, net K^+ uptake as measured chemically. Values are averages of two experiments.

Effect of propionate on Na^+ efflux during the oxidation of propan-2-ol

Na^+ -loaded yeast was prepared as described in the Materials and Methods section. The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-0.1M-NaCl-0.02M-KCl-3% (v/v) propan-2-ol. The suspensions contained either 0.01M-propionate or no propionate, and were bubbled with O_2 at 22°C. Fig. 5 shows that, in the absence of propionate, an efflux of Na^+ occurs which is balanced by an almost quantitative uptake of K^+ . The addition of 10mM-propionate to the suspension markedly inhibited the Na^+ efflux but only slightly decreases the K^+ influx.

Effect of intra- and extra-cellular pH on Na^+ efflux

The addition of propionate to the suspensions has the effects of both lowering intracellular pH (Table 2) and markedly decreasing Na^+ efflux (Fig. 5). To investigate further the dependence of Na^+ efflux on intracellular pH the experimental procedure adopted was similar to that used in the K^+-K^+ -exchange studies. The relationship between intra- and extra-cellular pH for Na^+ -loaded yeast was almost identical with that found with normal yeast with propan-2-ol as a substrate (see Fig. 4). However, with glucose as a substrate the linear relationship no longer holds, presumably owing to the accumulation of HCO_3^- and other anions within the cell. Na^+ efflux was studied at different extracellular pH values with propan-2-ol and glucose. The Na^+ -loaded yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to various pH values with 1M-tris)-0.1M-NaCl. The suspensions contained either 3% (v/v) propan-2-ol or 5% (w/v) glucose and were bubbled with O_2 at 22°C. After 20min 1M-KCl was added ('zero time') to give a final concentration of 0.02M-KCl. The

Table 4. K^+-K^+ exchange during oxidation of propan-2-ol

The yeast was suspended (1:50, w/v) in 0.02M-citric acid (adjusted to various pH values with 1M-tris)-3% (v/v) isopropanol and bubbled with O_2 at 22°C for 20min. Then 0.01M-KCl (labelled with ^{42}K) was added (0min). Total K^+ uptake was measured with ^{42}K . Net K^+ uptake was determined chemically. Initial K^+ content = 107.5 mequiv./kg.

pH		Uptake of K^+ (mequiv./40min per kg)		K^+-K^+ -exchange rate (mequiv./h per kg)
Extracellular	Intracellular	Total	Net	
2.80	5.77	12.1	1.9	15.3
4.50	6.21	16.8	0.6	24.3
5.35	6.44	19.4	1.1	27.5
6.50	6.74	16.5	2.3	21.3
7.00	6.87	14.3	2.3	18.0
8.00	7.13	11.2	2.6	12.9
9.00	7.39	9.7	3.3	9.6

Fig. 4. Effect of extracellular pH on intracellular pH in normal and in Na⁺-loaded yeast

The yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to various pH values with 1M-tris)-3% (v/v) propan-2-ol and bubbled with O₂ at 22°C. Suspensions of Na⁺-loaded yeast also contained 0.1M-NaCl. After 20min the intracellular pH was determined by the freezing-and-thawing procedure (see the Materials and Methods section). The lines were fitted by the method of least squares. (a) Normal yeast; (b) Na⁺-loaded yeast. For normal yeast (a), $pH_i = 5.04 + 0.26 pH_o$, and for Na⁺-loaded yeast (b), $pH_i = 5.07 + 0.25 pH_o$, where pH_i and pH_o are the intracellular and extracellular pH respectively.

increased KCl concentration, over that used in previous experiments, is necessary to overcome the competitive effect of Na⁺ ions for the K⁺ carrier (Conway *et al.*, 1954). Samples were removed immediately after the addition of KCl and at intervals of 10, 20, 30 and 40min and the yeast was analysed for Na⁺ content. These results showed that the rate of Na⁺ efflux was linear in all suspensions for at least a period of 20min. The rates of Na⁺ efflux (mequiv./h

Fig. 5. Effect of propionate on Na⁺ efflux and K⁺ uptake

The Na⁺-loaded yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to pH4.75 with 1M-tris)-0.1M-NaCl-0.02M-KCl-3% (v/v) propan-2-ol and bubbled with O₂ at 22°C. Without propionic acid: ▲, K⁺ uptake; ●, Na⁺ efflux. With 10mm-propionic acid: △, K⁺ uptake; ○, Na⁺ efflux. Values are averages of three experiments. Initial [K⁺], 45mequiv./kg; initial [Na⁺], 65mequiv./kg of yeast.

per kg) were calculated from the results up to 20min. The results are plotted on the basis of both extracellular and intracellular pH in Fig. 6. In the suspensions containing propan-2-ol the intracellular pH values were calculated from the relationship shown in Fig. 4(b). When glucose was the substrate the intracellular pH values were determined on duplicate samples removed at zero time, again by the freezing-and-thawing procedure. The rates of Na⁺ efflux obtained with propan-2-ol and with glucose correspond closely when plotted on the basis of intracellular pH. There is a distinct difference between the two sets of results when plotted on the basis of extracellular pH.

Discussion

When yeast oxidizes propan-2-ol or butan-1-ol no significant uptake of K⁺ occurs. Although Barron & Levine (1952) had reported that propan-2-ol was not oxidized by yeast, these workers used concentrations considerably below the optimum for oxidation, as found here. The first product of propan-2-ol oxidation is acetone, and further oxidation does not occur, since acetone (0.1-3.0%, v/v) added to yeast suspensions

Fig. 6. Effect of extracellular pH and intracellular pH on Na⁺ efflux

The Na⁺-loaded yeast was suspended (1:20, w/v) in 0.02M-citric acid (adjusted to various pH values with 1M-tris)-0.1M-NaCl-0.02M-KCl and bubbled with O₂ at 22°C. The substrates were 3% (v/v) propan-2-ol (●) and 5% (w/v) glucose (○). (a) Effect of extracellular pH; (b) effect of intracellular pH. Values are averages of three experiments. Initial [K⁺], 47 mequiv./kg; initial Na⁺, 65 mequiv./kg wet wt. of yeast.

failed to promote O₂ consumption. The oxidation of butan-1-ol is also limited in that it proceeds only to the aldehyde stage (Latzko, 1961; Pena *et al.*, 1969). That the K⁺-H⁺-exchange system does not operate during the oxidation of these alcohols may be attributed to the fact that they do not produce acid metabolically.

When propionate is added to suspensions oxidizing propan-2-ol the intracellular pH falls below the resting value and K⁺ now enters the cell. In the presence of 20 mM-propionate O₂ uptake is markedly reduced but a slow uptake of K⁺ still occurs. Under these conditions the rate of K⁺ uptake varies as the intra-

Fig. 7. Overall effect of intracellular pH on cation exchanges in yeast

The experimental conditions are described in the text. Δ, K⁺-H⁺ exchange; □, K⁺-K⁺ exchange; ○, K⁺-Na⁺ exchange.

cellular pH rises, and reaches an optimum rate at an intracellular pH value of approx. 5.70. The rate of O₂ consumption, measured under similar conditions, remained constant. This suggests that propionate, rather than lowered intracellular pH, is the cause of the reduced rate observed.

The rise in intracellular pH that occurs during K⁺-H⁺ exchange in yeast was first observed by Conway & Downey (1950) during glucose fermentation. An increase in intracellular pH also accompanies K⁺ uptake during glucose fermentation by *Neurospora crassa* (Slayman & Slayman, 1968), and by *Streptococcus faecalis* (Harold *et al.*, 1970), and may be common to H⁺-excreting micro-organisms in general.

When Na⁺-loaded yeast oxidizes propan-2-ol in a medium containing K⁺ there is a rapid efflux of Na⁺ in exchange for K⁺. The addition of propionate to the suspension markedly inhibits Na⁺ efflux but only slightly decreases K⁺ uptake. Under these conditions the lowered intracellular pH apparently favours the excretion of H⁺ rather than Na⁺ and suggests that there are two distinct systems for the efflux of cations.

The effect of extracellular and intracellular pH changes on the rate of Na⁺ efflux was investigated with both propan-2-ol and glucose as substrates. If the rate of Na⁺ efflux is dependent only on extracellular pH then variations in extracellular pH should produce the same effect with both substrates. Although the optimum pH is about 5.5 with either substrate, a considerable difference in the rate of efflux is observed on either side of the optimum. It is probable that this divergence is due to differences in

intracellular pH, for when the results are plotted on this basis the rates of Na^+ efflux at any intracellular pH value are almost identical with either substrate. The optimum intracellular pH for Na^+ efflux was approx. pH 6.40.

The K^+-K^+ exchange that occurs when normal yeast oxidizes propan-2-ol is similarly affected by intracellular pH changes, as is shown in Fig. 7. It is suggested therefore that both K^+-K^+ exchange in normal yeast and K^+-Na^+ exchange in Na^+ -loaded yeast occur on the same system. This system must be highly specific for Na^+ , however, since K^+-K^+ exchange does not occur when Na^+ is present in the cell (Conway *et al.*, 1954). The system that exchanges H^+ for K^+ appears to be specific for H^+ efflux. This system has an optimum intracellular pH of 5.70 and, as shown in Fig. 7, ceases to operate as the intracellular pH rises above 6.0.

Slayman & Slayman (1970) have studied the effect of extracellular pH on cation transport in *Neurospora crassa*. On the basis of their findings, they suggested that K^+ influx, H^+ efflux and Na^+ efflux could be accounted for in terms of a single pump. The effect of extracellular pH on Na^+ efflux in *Neurospora* was very similar to that found in yeast. However, the effect of intracellular pH changes was not considered.

It is probable that both extracellular and intracellular pH influence cation exchanges and hence it may be misleading to consider the effect of either independently of each other. With this reservation, it is considered that the present evidence supports

the view of Ryan *et al.* (1971), that intracellular pH is a major factor in the control of the K^+-H^+ -exchange system. It is further suggested that a second system involved in K^+-K^+ or K^+-Na^+ exchange is also directly influenced by intracellular pH changes.

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