Relationship of Potassium Ion Transport to Metabolism in Acid-Induced Fermentation in Yeast.* (29341)

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Until recently it has been thought that potassium ion transport into yeast involves an "active" process in which the cell must expend energy for the transport to occur. The "active" concept has been based on two types of evidence: (1) K+ transport occurs only in the presence of an added substrate (1,2); (2) K+ enters the cell against a concentration gradient(2).

Leggett *et al*(3) have attacked the concept of "active" K+ transport in yeast and have presented evidence that K+ transport is a "passive" process requiring no energy expenditure by the cell. Their conclusions were based primarily on the following: (1) K+ exchanged for intracellular H+ if the cells were exposed to hydrochloric acid prior to addition of K+; (2) the observed K+ transport kinetics fit a proposed passive process. We were not able to repeat in commercial yeast the results of Leggett *et al*(3).

Recently we have shown(4) that certain penetrating organic acids elicit a surge of ethyl alcohol and CO_2 production resulting from carbohydrate dissimilation. After 1-2 hours fermentation ceases, although acid is still present. Such a system therefore allows a study of K+ transport in the presence of intracellular acid but in the simultaneous presence or absence of metabolism (fermentation). Studies of the interrelationships of acid penetration, K+ transport and metabolism reported here lend support to the concept of "active" K+ transport.

Methods. Experiments were performed anaerobically under nitrogen on suspensions (5) of nongrowing baker's yeast at pH 4.5. Acetic acid was used to bring about fermentation and K+ uptake. In K+ uptake experiments nitrogen was bubbled through the suspensions to insure mixing as well as anaerobiosis. K+ was determined through internal-standard flame photometry.

Results. Temperature studies. Acetic acid was added to yeast suspensions at different temperatures. Periodic samples were withdrawn, filtered through Millipore (Millipore Filter Corp.) filters and the filtrates were titrated with base to yield the total acidity of the medium. In Fig. 1 it is seen that disappearance of acid from the medium (equilibration with cell) ceases essentially within 20 minutes of addition of the acetic acid at all of the temperatures tested. The rise in acidity in the 30° curve is unaccounted for, but was consistent from experiment to experiment. Since fermentation was occurring(4) (Fig. 2) it is possible that succinic acid was being secreted by the cell(6). Occurrence of quick penetration was substantiated by the fact that onset of fermentation at higher and lower temperatures occurred within 20 minutes after addition of acid (Fig. 2).

In Fig. 2 fermentation curves are seen for yeast treated with acetic acid at different temperatures. As could be expected, the CO_2 evolved was greater at the higher temperatures. At these higher temperatures the rate of CO_2 evolution declined with increasing time. In similar experiments in which K+ uptake was measured after addition of acetic acid and K+ (potassium chloride), net K+ uptake also depended on temperature (Fig. 3). A comparison of Fig. 2 and 3 reveals that maximal fermentation rates (slopes) and maximal K+ uptake rates (slopes) at a given temperature occur at roughly the same time after addition of acid. These maximal rates are plotted against each other in Fig. 4. Each point in the figure represents K+ uptake rate *vs* CO_2 rate for one temperature. The figure shows an essential linearity between K+ uptake rate and fermentation rate, one ion of K+ being taken up for every 3-4

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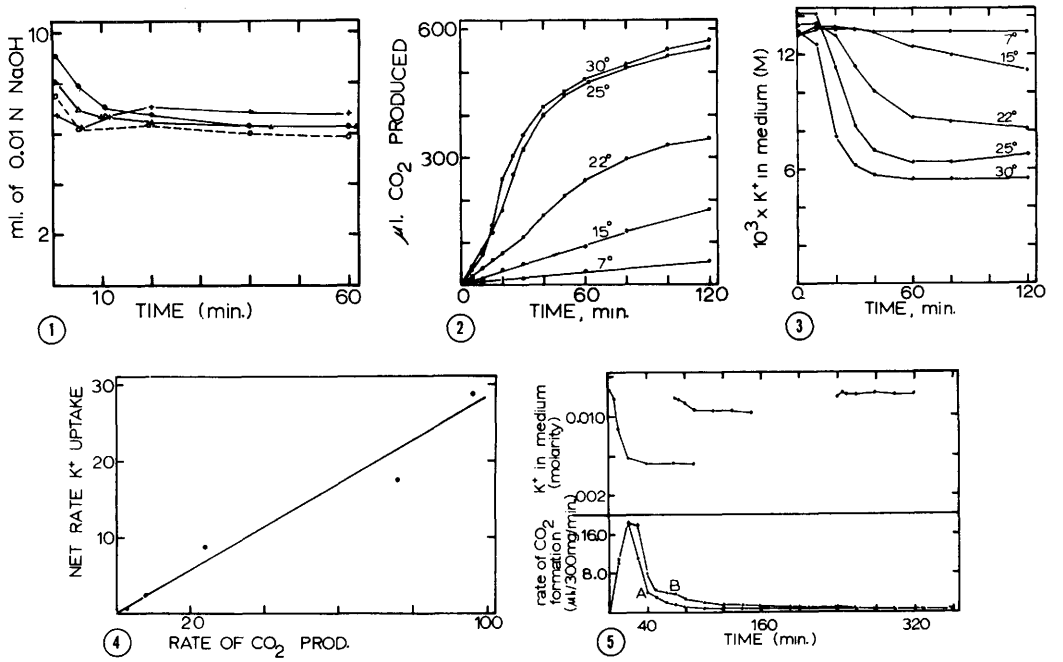

FIG. 1. Titrable acid in external medium of cell suspensions treated with acetic acid at different temperatures. Yeast concentration 360 mg/ml. Acetic acid about 0.1 M. Avg of 3 experiments. Curves: ●, 7°; △, 15°; ○, 22°; +, 30°.

FIG. 2. CO_2 produced vs time in cells treated with acetic acid. Yeast, 50 mg/ml yeast; acetic acid about 0.1 M; KCl, 0.01 M. Avg of 2 experiments.

FIG. 3. K+ uptake with time in cells treated with acetic acid. Yeast 100 mg/ml. Acetic acid, about 0.1 M; KCl, about 0.01 M. Avg of 3 experiments.

FIG. 4. Rate of K+ uptake vs CO_2 production rate. Values derived from Fig. 2 and 3. Rates expressed in micromoles per 100 mg yeast per hr.

FIG. 5. K+ uptake and CO_2 production with time. KCl added at different times after addition of 0.1 M acetic acid (see text). Initial KCl concentration about 0.012 M. Yeast washed 2 times immediately prior to experiment. Yeast concentrations: 50 mg/ml in CO_2 measurements, 100 mg/ml in K+ uptake. Temperature, 30°. Curve A—KCl added simultaneously with acid; Curve B—KCl added 60 or 240 min after acid.

(approximately) molecules of CO_2 produced.

Time-lag study. In a Warburg experiment (lower part of Fig. 5) done at 30°, acetic acid was added to yeast. Curve A represents *rate* of CO_2 production *vs* time where 0.012 M KCl was tipped simultaneously with the acid. Curve B represents rate of CO_2 production *vs* time where the same concentration of K+ was tipped 60 minutes or 240 minutes after addition of acid. As reported earlier(4) CO_2 production was lower in the presence of K+ (Curve A) than in its absence (Curve B). It is evident that an outburst and subsequent decline of CO_2 production occurred. After one hour the rate was low. After 3 hours the rate was negligible. These results have been found consistently. In the same experiment acetic acid was added to 3 other yeast suspensions. K+ ion (KCl) was added simultaneously with the acid in the first suspension, 70 minutes after addition of acid in the second suspension, and 240 minutes after addition of acid in the third suspension. After addition of K+, samples were taken periodically for K+ analysis of the medium. K+ concentrations are seen in the upper portion of Fig. 5. It will be noted that K+ disappearance from the medium occurred rapidly when fermentation was appreciable, but was slight when fermentation rate was low (70 minutes after addition of acid). Four hours after addition of the acid, both fermentation and K+ uptake were negligible. Hence, although acetic acid has long since penetrated into the cell (Fig. 1), K+ uptake occurred only when fermentation occurred.

Discussion. The experiments demonstrate

that no net uptake of K+ occurs in the presence of intracellular acid but in the absence of metabolism. Furthermore, a relatively linear relationship exists between fermentation rate and the dependent K+ uptake rate. Hence the acid appears to serve solely as a tool for inducing fermentation; fermentation in turn allows K+ uptake. These findings are in direct conflict with those of Leggett *et al*(3) whose claims are listed above. It is possible that Leggett *et al* were dealing with a single strain or mutant which *does* transport K+ passively. The possibility also exists that Leggett *et al* damaged the cells by pretreatment with acid; such damage might affect K+ transport.

Finally, an interesting direct relationship between K+ uptake and metabolism has been obtained. In previous studies of K+ uptake with sugar, metabolic rate was so high and K+ uptake so fast, that it would be difficult to measure the relative rates. The present findings, by virtue of the easily measured rates of both fermentation and K+ uptake, may present an approach to discovery of direct quantitative relationships between metabolism (energy expenditure by the cell) and transport. The technique must be improved in order to do so.

Summary. 1. In acid-induced fermentation in commercial baker's yeast, no direct relationship was seen between acid penetration and K+ uptake. A dependency of K+ uptake on metabolism was seen, however. 2. Evidence is presented in support of the concept of an "active" process in the transport of K+ into yeast. This evidence conflicts with recent contentions of a "passive" process in K+ transport in yeast. 3. A direct approach may be afforded to studies of energy expenditure by the yeast cell and K+ transport.

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Stress and Susceptibility to Viral Infections. III. Antibody Response and Viral Retention During Avoidance Learning Stress.* (29342)

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Mice subjected to emotional or nonspecific stress by an avoidance-learning situation in a shuttlebox, by confinement in mesh wire envelopes, or by high intensity sound are more susceptible than controls to the viruses of herpes simplex, Coxsackie B1, and vesicular stomatitis(1,2,3).

Two to four weeks of avoidance-learning stress were necessary before significant differences were noted in susceptibility of mice to herpes simplex and Coxsackie B1 viruses given by intraperitoneal inoculation(1,2). Sound stress(3) resulted in a very transient and diphasic susceptibility pattern in mice inoculated intranasally with vesicular stomatitis virus; these changes were present as early as the first day of stress. Furthermore, the periods or phases of increased susceptibility in sound-stressed mice were not dependent on adrenal functions. Leukocyte

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