Biochemical Pharmacology, 1964, Vol. 13, pp. 755–765. Pergamon Press Ltd., Printed in Great Britain.

THE INDUCTION OF ALCOHOLIC FERMENTATION IN YEAST BY SOME ORGANIC ACIDS: IMPLICATIONS IN POTASSIUM TRANSPORT*

THOMAS G. SCHARFF†

Department of Pharmacology, School of Medicine, University of Louisville, Louisville, Ky., U.S.A.

(*Received* 25 *September* 1963; *accepted* 12 *December* 1963)

Abstract—To suspensions of nongrowing commercial baker's yeast were added various organic acids under anaerobic conditions and low pH. Acetic, propionic, and isobutyric acids elicited carbohydrate breakdown, fermentation, and potassium ion uptake. Formic, lactic, glycolic, succinic, glutaric, citric, and hydrochloric acids did not produce these effects nor did ethanol and acetaldehyde. Various monovalent cations had different inhibitory effects on the production of anaerobic CO_2 by acetic acid. Potassium was most inhibitory, and Na^+ was least inhibitory. Similarly, K^+ was most readily transported into the cells, whereas Na^+ was least readily taken up. The effects of the acids and the implications in K^+ transport are discussed.

THE EFFECTS of acetic acid on cells have been studied repeatedly under aerobic conditions, and many of the actions of this substance are attributed to its metabolism via the tricarboxylic acid cycle. Scattered reports show, however, that acetic acid affects cells under anaerobic conditions. Maesen and Lako[1] reported that acetic acid at low pH inhibits fermentation in yeast. Under proper conditions K^+ could abolish the inhibition. They suggested that K^+ does this by exchanging for intracellular H^+ produced by acetic acid. Samson *et al.*[2] demonstrated an inhibition of fermentation in yeast by several fatty acids. They proposed that fatty acid anion within the cell may inhibit metabolism by binding nonspecifically to protein, rather than by acidifying the cell interior.

Leggett *et al.*[3] have claimed that the movement of potassium ion into yeast is a passive process, depending primarily on a rate-limiting diffusion step followed by an exchange of potassium ion for an exchangeable cation. Previous to the report of Leggett *et al.* it had been accepted that potassium ion transport is an 'active' process requiring a supply of energy from the cell.

The present report deals with effects of penetrating acids on yeast cells under anaerobic conditions. It shows that such acids elicit a dissimilation of the carbohydrate stores of the cell. Certain monovalent cations, potassium ion in particular, can be transported into the cell with a resultant decrease in fermentation rate.

* Supported by Grant GB-377 of the National Science Foundation.

† This work was done during the tenure of an Established Investigatorship of the American Heart Association.

METHODS

All experiments were conducted at 30° under anaerobic conditions on nongrowing commercial baker's yeast prepared as described previously.[4] Gas production was measured in a nitrogen atmosphere in the Warburg apparatus. In experiments involving the uptake of monovalent cation, nitrogen gas was bubbled through the suspensions in order to assure anaerobiosis. K^+ and Na^+ were measured by use of internal-standard flame photometry. All substrates and reagents were of the highest purity obtainable commercially. Acetaldehyde was freshly redistilled prior to experiment. Inorganic phosphate was determined by use of a modified Fiske–Subbarrow[5] method. All yeast concentrations are expressed in terms of milligrams wet weight per milliliter suspension.

Separation and analysis of carbohydrates were based on the procedure described by Berke and Rothstein[6].

Radioactivity counts were made in a Tracerlab model LSC-10B liquid scintillation counter in the ^{14}C-labeled-acetate experiments. Determinations of radioactivity in the evolved CO_2 were made through the technique of Buhler.[7] Quantitative estimates of alcohol involved the use of a commercial (Determatube C-Alc: Worthington Biochemical Corp.) alcohol dehydrogenase–diaphorase preparation. Identification of alcohols produced in the experiments was established with the aid of a ChromAlyzer-100 gas chromatograph (Labline) and confirmed by infrared spectrophotometry on a Perkin–Elmer model 237 infrared spectrophotometer.*

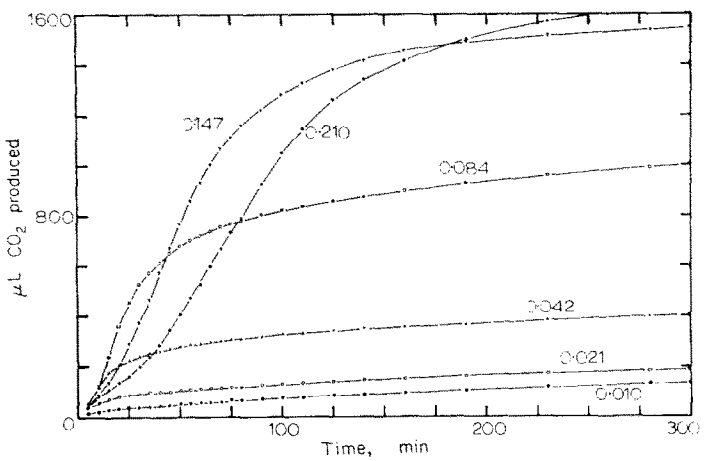

FIG. 1. Carbon dioxide production with time in presence of various concentrations of acetic acid. Yeast, 50 mg/ml in 3-ml volume; 5-hr experiment at pH 4·5. Numbers on graph represent molarity of acetic acid.

RESULTS

Induction of fermentation by certain organic acids

Addition of acetic acid to yeast suspensions under anaerobic conditions resulted in an evolution of CO_2. Figure 1 shows the evolution of CO_2 resulting from addition

* The author is indebted to Mr. Kenneth Bailey of this department and to Dr. Daniel Sweeney of the Department of Chemistry of Bellarmine College for performing and interpreting the i.r. analyses.

of varying concentrations of acetic acid at pH 4·5. After a short period of slow evolution a rapid CO_2 production was seen, followed by a diminution in rate (slope) as time progressed. At the end of 2 hr CO_2 production was very low in each case. In Fig. 2 the total CO_2 produced is plotted against acetic acid concentration. At the higher concentrations of acetic acid a plateau was approached. A comparison of the rates of CO_2 production (slopes of Fig. 1) at different times for each curve reveals that, with increasing acid concentration, a longer lag period occurred before the maximal rate was seen, and the maximal rate was highest at intermediate concentrations. However, even though maximal rates were not so great at higher concentrations, evolution was more prolonged, and total CO_2 evolution at the higher concentrations was greatest (Figs. 1, 2).

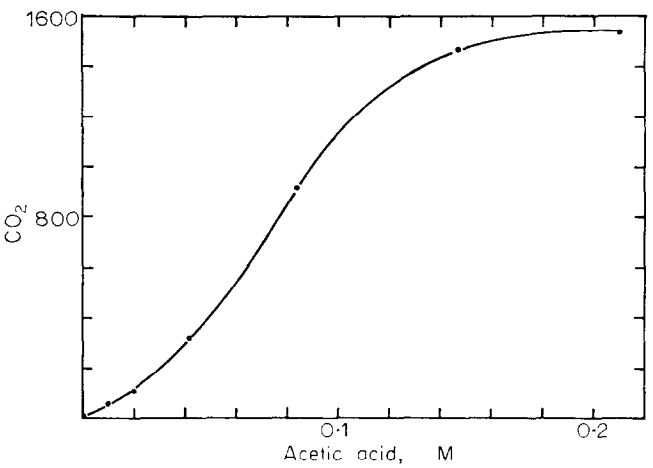

FIG. 2. Total production of carbon dioxide in yeast exposed anaerobically to various concentrations of acetic acid. Yeast, 50 mg/ml in 3-ml volume; acetic acid adjusted to pH 4·5. Values taken from Fig. 1.

The maximal rate of evolution of CO_2 by acetic acid (approximately 7·7 μl CO_2/mg per hr), was 11–12 times less than CO_2 production in the presence of glucose alone (86–90 μl CO_2/mg per hr with 0·1 M glucose). The rate of CO_2 evolution by yeast in the presence of glucose and acetic acid was equal to only one tenth or less of that for glucose alone. Hence the acetic acid-inhibited production of CO_2 from glucose approaches the CO_2 rate from acetic acid alone at high acetic acid concentration, and the rate of CO_2 production from acetic acid in the presence of glucose does not cancel the inhibitory effect of this acid on glucose fermentation.

The specificity of acetic acid in causing CO_2 production was next tested. The relative abilities of various acids to elicit CO_2 production are shown in Table 1. The pH of each suspension equalled or was lower than the p*K*a of the acid used. CO_2 produced from acetaldehyde, ethanol, and hydrochloric acid was negligible. Only acetic, proprionic, and isobutyric acids gave rise to large amounts of CO_2. The rate patterns of CO_2 evolution for the two higher-weight acids were similar to those of acetic acid described previously. Formic acid almost immediately produced CO_2 at a high rate, but the rate very rapidly fell off, possibly because of a specific toxicity of this acid on the cell. Glycolic acid showed a reverse pattern of production,

being very slow at first, but increasing to an appreciable value at the end of 2 hr. With the possible exception of glycolic acid, however, only the alkyl monocarboxylic acids, of the acids used, brought about significant CO_2 production.

The source of the CO_2 was next investigated. Penetration of acetic acid and reaction with bicarbonate ion was eliminated as an important CO_2 source because the 0·01 M bicarbonate concentration in yeast[8] could not account for the large amounts of CO_2 evolved in our experiments.

TABLE 1. ANAEROBIC PRODUCTION OF CO_2 BY VARIOUS SUBSTANCES

Yeast concentration 50 mg/ml for CO_2 experiments, 100 mg/ml for K^+ uptake experiments. Concentration of compound, 0·08 M, except for HCl, which was of concentration to give final pH of 2·7 in the suspension. Results are average of two experiments.

Compound	Initial pH of suspension	Concentration of compound (M)	CO_2* produced in 2 hr (μmoles/g yeast)	Net K^+† uptake in 2 hr (μmoles/g yeast)
Ethanol	4·5	0·08	0	
Acetaldehyde	4·5	0·2	0	2
Acetic acid	4·6	0·08	196	56
Propionic acid	4·4	0·08	175	32
Isobutyric acid	4·45	0·08	190	17
Formic acid	3·8	0·08	5	−2
Glycolic acid	3·8	0·08	45	3
Lactic acid	3·8	0·08	0	−3
Succinic acid	3·8	0·08	0	4
Glutaric acid	4·3	0·1	0	−3
Citric acid	3·17	0·08	0	−6
Hydrochloric acid	2·7	(pH 2·7)	3	−4
Hydrochloric acid + 0·1 M glucose	4·0			61

* No KCl added.
† 0·01 M KCl initially.

TABLE 2. LACK OF RADIOACTIVITY IN CO_2 FORMED ANAEROBICALLY IN PRESENCE OF 1- OR 2-^{14}C-LABELED ACETIC ACID

90 mg yeast (wet wt.) in 3-ml volume; acetic acid, 0·08 M at pH 4·5. Background 71 cpm.

Flask	CO_2 produced (μl)	In CO_2 trap (cpm)	Corrected for volatility of acid (cpm)	In medium (total cpm)	CO_2 or acid (cpm/μmole)
$CH_3{}^{14}COOH$ + yeast*	503	85	6		0·3
$CH_3{}^{14}COOH$, no yeast (counts from volatile acid trapped)		79			
$^{14}CH_3COOH$ + yeast*	518	79	0		0
$CH_3{}^{14}COOH$ (initial)				1610	67
$^{14}CH_3COOH$ (initial)				2310	96

* Average from two flasks.

The possibility that the CO_2 was coming from the acid itself through unknown reactions was ruled out in experiments in which 1-^{14}C or 2-^{14}C-acetic acid was added to suspensions and radioactivity determined in the CO_2. These results are shown in Table 2. It can be seen that essentially no radioactivity was detected in amounts

greater than the 79 cpm encountered when volatile acetic acid was trapped in KOH. It was concluded that the carbon atoms in the CO_2 did not come from acetic acid.

The carbohydrate stores of the cells were then considered as a possible source of the CO_2 evolved in the presence of acetic acid. If the carbohydrate stores were being broken down and fermented, ethanol should be produced in quantity equal to that of CO_2. Warburg flasks containing yeast with one or another of several acids were incubated, and CO_2 was determined. Immediately at the end of experiment, the media were separated from the cells in Millipore® filters (Millipore Filter Corp.). Alcohol was determined with the Determatube C-Alc preparation; this technique is not specific for ethanol. Results are shown in Table 3. In Part A it is seen that 50 μmoles

TABLE 3. ANAEROBIC ALCOHOL PRODUCTION AND CO_2 PRODUCTION FOR VARIOUS ACIDS

Warburg vessels contained 150 mg of yeast in total volume of 3 ml. Acid concentration in each case, 0·1 M, adjusted to pH approaching p*K*a value. Time of experiment, 2·5 hr. Acetic acid values are averages of five experiments, others represent single experiments. Gas phase: nitrogen.

Expt.	Acid	pH	Amounts produced in 2·5 hr (μmoles)	
			Alcohol	CO_2
A	None	4·5	1·1	1·7
	Acetic	4·5	50	49·8
B	*n*-Propionic	4·5	47	36
	Isobutyric	4·5	47	36·5
	Lactic	3·8	11	10·7
	Succinic	3·8	7	8·9
	Glutaric	4·3	3	5

of alcohol corresponded well with 49·8 μmoles of CO_2. In Part B, involving single determinations on supernates of yeast treated with other acids, a rough 1:1 correlation is seen for propionic and isobutyric acids. Smaller, roughly corresponding amounts of alcohol and CO_2 are seen for lactic, succinic, and glutaric acids. The latter three acids were previously shown in this paper to give rise to little CO_2 in a 2-hr period, probably because of their inability to penetrate readily into the cell. (Most of the CO_2 evolved in the lactic acid experiment appeared during the last 30 min; it is possible that continued incubation would give rise to ever increasing quantities of CO_2 and alcohol.) It was concluded that acid treatment resulted in equal production of CO_2 and an alcohol.

If the action of the acids resulted in a breakdown of carbohydrate stores and an ensuing fermentation, carbohydrate should be lost from the cell in amounts showing a ratio of 1 glucose molecule lost for 2 molecules each of alcohol and CO_2 produced. In Part A of Table 4 it is seen that 48 μmoles of CO_2 and 47 μmoles of alcohol were produced. The difference in loss of carbohydrate (in glucose equivalents) in the control and acid-treated suspensions was 21·6 μmoles, (31·5–9·9), an amount equivalent to 43·2 μmoles each of alcohol and CO_2. The experimental values for carbohydrate breakdown are in good agreement with the expected 2:1 ratio of alcohol and CO_2 to glucose lost. The high loss (9·9 μmoles) of carbohydrate in the control suspensions

is unexplained. From the table it is evident that such apparent loss was not accounted for by alcohol or CO_2 via fermentation. Glycogen is not normally fermented endogenously,[9] but in the absence of substrate over long periods it is depleted anaerobically.[10] In Part B of Table 4 results are shown for another experiment in which the carbohydrate stores were further fractionated in an effort to determine which fraction of the carbohydrate showed greatest depletion. The total carbohydrate lost was in a ratio slightly lower than 1:2 with alcohol or CO_2. It appears that greater errors in carbohydrate determinations were encountered with the added manipulation. However, it appears that the trehalose and alkali-soluble fractions showed the greatest loss in acetic acid-treated cells.

TABLE 4. ANAEROBIC BREAKDOWN OF CARBOHYDRATE STORES; CO_2 AND ALCOHOL FORMATION IN PRESENCE OF ACETIC ACID

Values obtained from 150 mg yeast in Warburg flasks; each value is an average of two determinations on separate suspensions. Acetic acid, 0·1 M, pH 4·5.

Expt.	Sample	Formed		Carbohydrate found						
		CO_2 (μmoles)	alc	Tre-halose	alkali sol.	Δ alk. sol.	alkali insol. (μmoles)	Δ alk insol.		Total change
A	Original yeast				49·4		31·8			
	H_2O-treated	2·2	3		45·6	−3·8	25·7	−6·1		−9·9
	HAc-treated	48	47		27·3	−22·1	22·4	−9·4		31·5
					Δ tre.	alcohol insol.	Δ alc. insol.	alkali insol.	Δ alk. insol.	
B	Original yeast			13·4		24·7		43·1		
	H_2O-treated	2·0	2·8	17·1	−3·7	27·5	−2·8	31·1	−12·0	−5·5
	HAc-treated	47·5	53	2·4	−11·0	22·9	−1·8	35·3	−7·8	−20·6

Berke and Rothstein[6] reported a loss of glucose from yeast induced into fermentation by addition of 2, 4-dinitrophenol. In our experiments no carbohydrate was detected in the medium of cells exposed to acetic acid. However, the conditions in our experiments were different (higher yeast concentration, higher pH) from those of Berke and Rothstein, and it remains to be seen whether or not free glucose is released by acids.

In view of the above findings, it seemed highly unlikely that reactions other than those of carbohydrate breakdown and fermentation were important in CO_2 production. However, in order to strengthen further the possibility of carbohydrate breakdown and fermentation, a procedure was used to identify *ethanol* positively in supernates of suspensions exposed to *any* of the three effective acids. Large (500-ml) suspensions of yeast were exposed to one or another of the three acids—acetic, 1-propanoic, and isobutyric. After 2·5 hr the supernates were separated by centrifugation, acidified with HCl, extracted twice with 100-ml portions of CCl_4, then made alkaline and distilled according to the procedure given by Rothstein and Berke.[11] The microrectification flask of Gettler *et al.*[12] was used in a final distillation.

The "ring of condensate" was introduced into a gas chromatograph. Results are seen in Table 5. The data represent only two peaks, water and ethanol, regardless of which acid was added to the yeast. The water peak resulted from failure to remove water completely from the distillates. The chromatographic results were confirmed

with infrared spectrophotometry. Hence ethanol alone was produced in significant amounts in all three cases, and it was concluded that acetic acid was not unique in its ability to cause carbohydrate dissimilation.

In the last distillation in the microrectification flask the distillation temperature was not known, but since water was also detected in the gas chromatograph, it

TABLE 5. GAS CHROMATOGRAPHY OF CCl_4-EXTRACTED, DISTILLED SUPERNATES OF ACID-TREATED YEAST

Helium flow, 25 ml/min; temp. 96°; 200 mA current; column, Silicone 550 (Labline) 8 ft, 3/16 in. Distillation procedure that of Rothstein and Berke[11]. Yeast, 100 mg/ml treated for 2·5 hr with 0·1 M acetic, *n*-propionic, or isobutyric acid at pH 4·5. No peaks other than those listed below were observed in 19 min.

Sample	Total sample size (μl)	Time of appearance of peak maximum (min)
Ethanol	2–4	3·8
n-Propanol	2	8·3
Isobutanol	2	12·6
Acetic acid-treated, distillate	5–10	2·8, 3·7
n-propionic acid-treated, distillate	5–10	2·9, 3·7
Isobutyric acid-treated, distillate	5–10	2·8, 3·8
Water	2	2·8
Ethanol, propanol, isobutanol mixture	6	3·9, 8·3, 12·6
Ethanol, propanol, isobutanol, H_2O	8	2·9, 3·9, 8·2, 12·6

seemed likely that alcohols such as *n*-propanol and isobutanol would appear if they were formed at all. Of the three alcohols that might be expected from treatment with the three acids, the highest-boiling azeotrope with water boils at 90°, a temperature below the boiling point of water itself. To rule out the possibility that other alcohols were formed but not distilled, another experiment was done in which the supernates were distilled after alkalinization but without extraction. Two-tenths ml of final distillates, boiling at 99°, were obtained from 400-ml supernates of suspensions treated with acetic, *n*-propionic, or isobutyric acids. No peaks other than those of water and ethanol were seen.

It has been reported that acetic acid inhibits fermentation and respiration in yeast.[1, 2] Inhibition of fermentation of glucose could be reversed by washing the cells.[2] We have confirmed these results at 0·1 M acetic acid and extended them to show the reversibility, through washing, of inhibition of respiration of glucose. In addition, neither phosphate leakage nor K^+ leakage was noted in cells treated with 0·1 M acetic acid. Appearance of either of these substances in the external medium has been taken to indicate breakdown of permeability barriers in the yeast cell membrane.[4, 13, 14] Finally, using a staining technique devised earlier[4] to determine membrane integrity, we found that no significant staining occurred in the acid-treated cells. It was concluded that the inhibitory effects on glucose utilization by acetic acid at concentrations below 0·1 M, at least, are reversible. Hence the liberation of CO_2 by acetic acid anaerobically is not related to any obvious irreversible effects on the membrane or on metabolism.

Rothstein and Demis[15] showed that various monovalent cations affected to different degrees the rate of excretion of hydrogen ions from fermenting yeast. Since K^+

exchanges for H^+ produced during fermentation,[16, 17] the ability of monovalent cations to lower external pH is a measure of the ability of the ions to exchange for H^+ produced during fermentation. We reasoned that CO_2 liberated by yeast anaerobically in the presence of acetic acid might also be brought about by increased

TABLE 6. EFFECTS OF VARIOUS MONOVALENT CATIONS ON ANAEROBIC PRODUCTION OF CO_2 BY ACETIC ACID

Yeast, 50 mg/ml; salts (as chlorides), 0·01 M; lactose 0·02 M; 0·08 M acetic acid, pH 4·5. Each value is average of two experiments.

Ion	CO_2 produced (μl/g yeast per 2 hr)	Inhibition (%)
Control	7,150	0
Lactose	7,150	0
Na^+	6,940	3
Cs^+	6,780	5
Li^+	6,400	10
Rb^+	5,560	22
K^+	5,090	29

intracellular H^+ concentration; then certain monovalent cations should inhibit CO_2 production. The data of Table 6 indeed show inhibitory effects of certain monovalent cations on CO_2 evolution, in the order $K^+ > Rb^+ > Li^+ > Cs^+ > Na^+$. This is

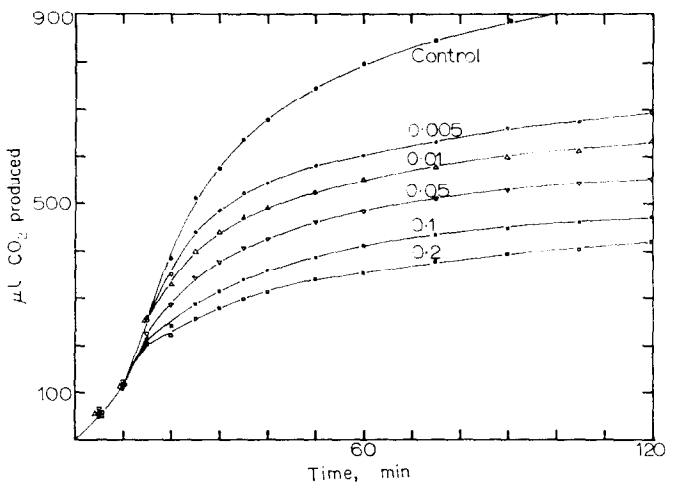

FIG. 3. Anaerobic carbon dioxide production in the presence of varying concentrations of potassium chloride. Yeast, 50 mg/ml in 3-ml volume. Acetic acid, 0·08 M, at pH 4·5. Numbers on curves refer to the molarity of added KCl.

essentially the order obtained by Rothstein and Demis[15] for H^+ exchange during fermentation of glucose, except that the relative positions of Na^+ and Li^+ are reversed. No data were presented by them for Cs^+.

In Fig. 3 the effects of the most effective inhibitor of CO_2 production, K^+, are seen for different K^+ concentrations. Increased K^+ resulted in increased inhibition of CO_2

production, but even at 0·2 M concentration K^+ did not completely abolish CO_2 production. In addition, although K^+ was added simultaneously with acetic acid, no concentration of K^+ used here affected the rate of CO_2 evolution for the first 10 min.

If inhibitory ability of a monovalent cation on CO_2 production from acid is related to the ease of uptake of the cation, one would expect Na^+ to be taken up less readily and K^+ more readily in acid-treated suspensions. Figure 4 shows that Na^+ uptake from

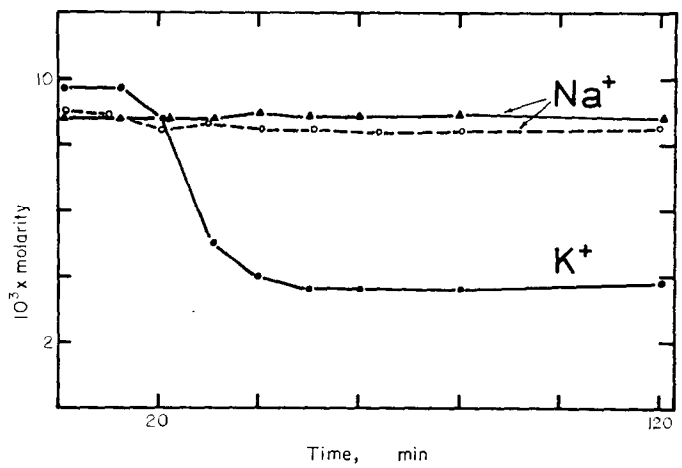

FIG. 4. Na^+ and K^+ concentration in medium of yeast treated with acetic acid anaerobically. Yeast, 100 mg/ml. NaCl or KCl about 0·01M initially. Acetic acid, 0·1 M. Broken line: Na^+ in absence of K^+. Solid lines: each ion in presence of other.

the medium in the absence of added K^+ (broken line) was only slight. In the presence of K^+ the net Na^+ uptake was insignificant, whereas K^+ was taken up rapidly after a 10- to 20-min lag period. A K^+–H^+ exchange was involved at least partially in the K^+ uptake, since the addition of K^+ to the medium of cells exposed to acetic acid resulted in a reduced buffering capacity of the medium after 90 min exposure to acetic acid (Table 7). In conclusion, it is seen that the same general uptake-exchange

TABLE 7. QUANTITY OF 0·02 M HCl REQUIRED TO LOWER pH OF SUPERNATES OF CELLS PREVIOUSLY TREATED FOR 90 min WITH ACETIC ACID IN THE PRESENCE OR ABSENCE OF POTASSIUM ION

Yeast, 200 mg/ml; acetic acid, 0·1 M; KCl, 0·1 M or no addition; pH of suspensions initially, 4·98. Figures are averages of two experiments.

	pH of supernate	Total of 0·02 M HCl required to titrate		
Supernate	before titration	pH 3·5	pH 2·5	pH 2·0
No added K	4·95	9·0	22·0	63·0
0·1 M KCl	4·75	7·75	18·0	58·0

characteristics of Na^+, K^+, H^+ transport are found in both glucose-fermenting yeast suspensions[15] and in acetic acid-treated suspensions. Furthermore, it is seen from Table 1 that acids other than acetic acid not only elicit carbohydrate dissimilation but K^+ uptake as well. One outstanding difference between the effects of K^+ on CO_2 production in the presence of glucose or acetic acid at low pH, is that K^+ stimulates the former[15] but inhibits the latter. It thus appears that K^+ inhibits dissimilation of carbohydrate stores by acids (this communication) but stimulates H^+-inhibited fermentation of glucose.[15]

DISCUSSION

There is ample evidence, of which only a few instances are cited, [18-20] that anions do not readily penetrate passively the yeast cell membrane into the interior. It is also known that the undissociated alkyl monocarboxylic acids penetrate readily into baker's yeast cells,[8, 21, 22] whereas hydroxyl-substituted and polycarboxylic acids penetrate very slowly. From our data it is seen that acids which bring about carbohydrate dissimilation and K^+ uptake are the acids that easily penetrate the cell; acids which do not easily penetrate the cell did not affect metabolism or effect a K^+ uptake. It is not difficult to conclude that penetration of acid is a necessary step in the production of metabolic and transport effects.

That increased intracellular acidity may be important in breakdown of carbohydrate was indicated from the following: (a) extracellular K^+ lowered CO_2 production (Fig. 3) and brought about a release of hydrogen ion from the cell (Table 7); (b) the effective acids share a common property-ability to dissociate—and no other obvious reactions of the acids are involved (no CO_2 from acetic acid itself, Table 2; in anaerobic experiments not reported in this paper, acetic acid concentration did not continue to decline in the medium but reached an equilibrium value); (c) other ions[15] which can exchange for H^+ also inhibited fermentation (Table 6); (d) acetaldehyde and alcohol under anaerobic conditions produced no effects (Table 1).

The mechanism by which carbohydrate is dissimilated by acids is not known. It has been reported earlier that 2, 4-dinitrophenol also elicits carbohydrate dissimilation,[6] but no comparison of the mechanisms has been made—if different mechanisms are involved.

Leggett *et al.*[3] have questioned the prevailing belief that K^+ transport in yeast involves an active process. They base their claim primarily on two observations which indicate a passive transport of K^+: (1) by pretreating cells with acid (HCl), the cells could be made to take up K^+; no substrate was needed. (2) The kinetics of K^+ transport which they found fit a postulated mechanism in which K^+ diffuses through a rate-limiting barrier and then exchanges for another cation (H^+). We were not able to repeat the results of these investigators when we pretreated *commercial* baker's yeast with HCl at pH 2·7 or 4·0. Furthermore, when we added HCl to yeast suspensions we could detect neither K^+ loss nor K^+ uptake. When glucose was added with the HCl (Table 1), a rapid uptake of K^+ ensued. The observation by Leggett *et al.* that kinetics of K^+ transport fit a proposed mechanism does not eliminate other *active* mechanisms. In the present study with acids under anaerobic conditions, K^+ transport occurred under conditions in which fermentation (metabolism) was taking place; it therefore appears premature to discard the concept of an active process in K^+ transport in yeast.

Studies of the effects of acids on yeast under aerobic conditions have not been done as yet in our laboratory.

Acknowledgement—The author gratefully acknowledges the competent technical assistance of Mrs. Ocia Montgomery in the performance of these experiments.

REFERENCES

1. T. J. M. Maesen and E. Lako, *Biochim. biophys. Acta* **9**, 106 (1952).
2. F. E. Samson, A. M. Katz and D. L. Harris, *Arch. Biochem.* **54**, 406 (1955).
3. J. E. Leggett, R. A. Olsen and B. D. Spangler, *Proc. Nat. Acad. Sci. (Wash.)* **48**, 1949 (1962).
4. T. G. Scharff, *Biochem. Pharmacol.* **5**, 79 (1960).
5. A. Rothstein, R. C. Meier and T. G. Scharff, *Amer. J. Physiol.* **173**, 41 (1953).
6. H. L. Berke and A. Rothstein, *Arch. Biochem.* **72**, 380 (1957).
7. D. R. Buhler, *Analyt. Chem.* **4**, 413 (1962).
8. E. J. Conway and M. Downey, *Biochem. J.* **47**, 355 (1950).
9. T. J. B. Stier and J. N. Stannard, *J. gen. Physiol.* **19**, 479 (1936).
10. G. T. Scott, M. A. Jacobson and M. E. Rice, *Arch. Biochem.* **30**, 282 (1951).
11. A. Rothstein and H. Berke, *Arch. Biochem.* **36**, 195 (1952).
12. A. O. Gettler, J. B. Niederl and A. Benedetti-Pichler, *J. Amer. chem. Soc.* **54**, 1476 (1932).
13. H. Passow, A. Rothstein and B. Loewenstein, *J. gen. Physiol.* **43**, 97 (1959).
14. R. D. Hotchkiss, *Ann. N.Y. Acad. Sci. (Wash.)* **46**, 479 (1946).
15. A. Rothstein and C. Demis, *Arch. Biochem.* **44**, 18 (1953).
16. A. Rothstein and L. Enns, *J. cell. comp. Physiol.* **28**, 231 (1946).
17. E. J. Conway and E. O'Malley, *Biochem. J.* **40**, 59 (1946).
18. E. J. Conway and M. Downey, *Biochem. J.* **47**, 347 (1950).
19. A. Rothstein, *UR Report*-382, p. 1. Atomic Energy Project, Univ. of Rochester (1955).
20. R. Collander, O. Turpeinen and E. Fabritius, *Protoplasma (Wien)* **13**, 348 (1931).
21. M. Malm, *Physiol. Plantarum* **3**, 376 (1950).
22. E. Oura, H. Suomalainen and R. Collander, *Physiol. Plantarum* **12**, 534 (1959).