REGULATORY MECHANISMS OF CELLULAR RESPIRATION

III. Enzyme Distribution in the Cell. Its Influence on the Metabolism of Pyruvic Acid by Bakers' Yeast*

By E. S. GUZMAN BARRON, MARIA ISABEL ARDAO, and MARION HEARON

(From the Chemical Division, Department of Medicine of The University of Chicago, Chicago)

(Received for publication, June 23, 1950)

In 1911 Neuberg and Karczag (1) discovered the presence in yeast cells of carboxylase, the enzyme which decarboxylates pyruvic acid into acetaldehyde, and the investigations of Meyerhof (2) and of Warburg (3) have since then elucidated the mechanism of glucose fermentation in yeast. In the sequence of reactions, pyruvic acid is decarboxylated to acetaldehyde, and acetaldehyde is reduced to alcohol. However, when pyruvate is added to yeast cells around neutrality and in the absence of oxygen there is no utilization (4–6), whereas it is metabolized in the presence of oxygen (7–9). The mechanism of this utilization of pyruvic acid has not been established, although Rünnstrom postulated direct oxidation to acetate as a possible pathway. Lack of pyruvate utilization in the absence of oxygen was attributed by Smithe to a decreased membrane permeability. The experiments presented in this paper demonstrate that the metabolism of pyruvic acid by bakers' yeast proceeds in the presence of oxygen by its initial oxidation to acetic acid, and subsequent oxidation through the tricarboxylic acid cycle of Krebs. In the absence of oxygen pyruvic acid is metabolized mainly by dismutation, and only when the concentration of undissociated acid is at least 15 per cent of the total pyruvate. The oxidative pathway of pyruvic acid, in a cell so rich in carboxylase as yeast, has been explained by postulating an arrangement of enzymes within the cell whereby carboxylase is distributed mainly at the center of the cell whereas pyruvic oxidase is located at the periphery.

EXPERIMENTAL

The yeast used in these experiments was Fleischmann's bakers' yeast, washed 4 times with distilled water and aerated with oxygen for 20 hours before use. In general, 10 to 20 mg. (dry weight) of this suspension was added to the buffer solutions used in the experiments. These buffers were glycine-HCl, glycine-H_3PO_4, hippurate,

* Aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

phosphate, and Teorell and Stenhagen's universal buffer (10). O_2 uptake and CO_2 measurements were made with the usual Warburg-Barcroft manometers and vessels at 28°. Chemical determinations were made according to the following methods: pyruvic acid, Friedemann and Haugen (11); acetic acid, Friedemann (12); lactic acid, Barker and Summerson (13); acetaldehyde, Stotz (14); succinic acid, by enzymatic oxidation with pigeon breast muscle succinoxidase, previous oxidation of malonic acid and ether extraction in the Kutscher-Steudel extraction apparatus.

Pyruvic acid was twice distilled in vacuum. From this sample of crystalline pyruvic acid, a 1 M solution was prepared. α-ketoglutaric and oxalacetic acids were prepared at the laboratory.

The Aerobic and Anaerobic Metabolism of Pyruvic Acid by Bakers' Yeast Cells.—The endogenous respiration of washed and aerated bakers' yeast cells is remarkably independent of the H^+ ion concentration of the solution from pH 1–10. This stability has made possible the measurement of pyruvate metabolism at all pH values within those limits. In the presence of oxygen, when the metabolism was measured by the O_2 uptake, the highest rate was obtained at pH 2.85, a value close to the pK value of pyruvic acid (pK, 2.49). From this peak, the rate of oxidation diminished rapidly on the acid side so that at pH 1.08 it was only 5 per cent of the optimum value. On the other side it diminished more gradually there being still some oxidation (3 per cent) at pH 8.3. Oxidation stopped at pH 9.3 (Fig. 1).

There was no pyruvate metabolism by yeast cells in the absence of oxygen down to pH 4. At pH 3.0 there was some CO_2 production, which increased as the pH value was lowered. In no case, however, did CO_2 production in the absence of oxygen approach the O_2 uptake in air as the gas phase (Table I). On the assumption that in air pyruvic acid is completely oxidized ($CH_3COCOOH + 2.5\ O_2 = 3\ CO_2 + 2\ H_2O$), and in the absence of oxygen it is either decarboxylated with formation of acetaldehyde ($CH_3COCOOH = CH_3COH + CO_2$) or is dismuted to lactic acid, acetic acid, and CO_2 ($2CH_3COCOOH + H_2O = CH_3CHOHCOOH + CH_3COOH + CO_2$), the yeast cells which at pH 2.0 gave an O_2 uptake of 406 c.mm. would give 178 c.mm. CO_2 if all the pyruvic acid had been decarboxylated, or 89 c.mm. CO_2 if the anaerobic metabolism had been a dismutation. As the average CO_2 production was 83 c.mm., this value would represent 47 per cent decarboxylation or 93 per cent dismutation. At pH 2.3, according to the same calculations, there would be an anaerobic utilization of 27 per cent if pyruvate was decarboxylated, or 53 per cent if it was utilized by dismutation.

At the lower pH values when there is no retention of CO_2 it is easy to follow simultaneously the rate of O_2 uptake and that of CO_2 formation. When both reactions were measured continuously there was in no case an initial increase in CO_2 formation, which would have taken place if the metabolism of added pyruvic acid had started with its decarboxylation to acetaldehyde and CO_2.

The respiratory quotient was always around 1, except in two experiments in which it reached values equal to the calculated values for complete oxidation (Table II).

To determine whether the anaerobic metabolism of pyruvic acid by washed bakers' yeast was due to decarboxylation or dismutation, analyses of pyruvic

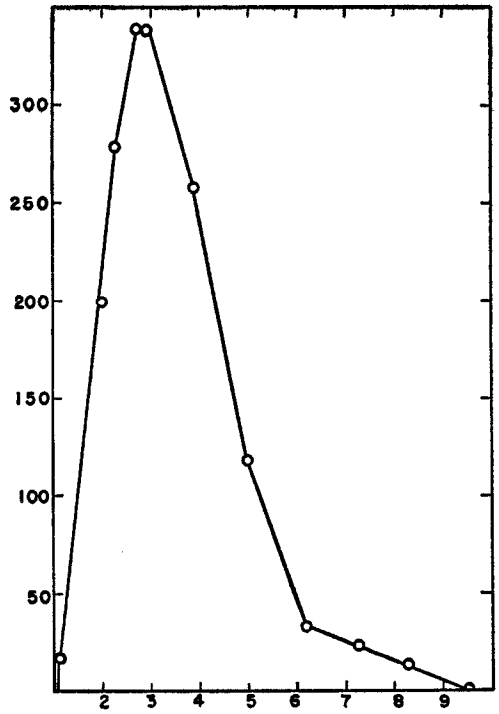

FIG. 1. Effect of pH on the oxidation of pyruvic acid by bakers' yeast (washed and O_2 bubbled for 20 hours). Buffer, Teorell's universal buffer. Pyruvic acid, adjusted to the same pH, 0.01 M. Temperature 28°. Duration of experiments, 90 minutes. Abscissa, pH; ordinate, O_2 uptake in c.mm. Values are blank-subtracted.

acid, of acetaldehyde, and of lactic acid were made at the end of the manometric experiments. In air all the pyruvic acid was completely utilized, but in the absence of oxygen, there was only 20 per cent utilization. If all the pyruvic acid were metabolized by dismutation (a reaction which is common in bacteria and in animal tissues (15, 16)), it would produce 91 c.mm. of lactic acid and of CO_2; 67 c.mm. lactic acid and 113 c.mm. CO_2 were found. If pyruvic acid had been decarboxylated, 183 c.mm. CO_2 and of acetaldehyde would have been produced; there were only 4.5 c.mm. of acetaldehyde. In conclusion, pyruvic acid added to bakers' yeast in an acid reaction, and in the absence of

TABLE I

Aerobic and Anaerobic Metabolism of Pyruvic Acid by Washed Bakers' Yeast (Fleischmann)

Buffer, Teorell's universal buffer. Pyruvic acid, 30 micromoles, adjusted to the pH value of the buffer. O_2 uptake with air as gas phase. CO_2 production with N_2 as gas phase. Temperature 28°. All figures blank-subtracted.

pH	Time	O_2 uptake	CO_2 production
	min.	*c.mm.*	*c.mm*
2.0	120	391	86
"	"	428	79
"	135	506	85
2.3	101	435	42
"	191	485	63
2.35	86	400	41
2.85	90	696	43
3.0	120	501	32
4.0	60	92	0
5.0	90	77	0
6.0	150	95	0

TABLE II

The Respiratory Quotient of the Oxidation of Pyruvic Acid by Washed Bakers' Yeast

Pyruvic acid, 30 micromoles for 3 cc. fluid volume. Temperature 28°. Figures are blank-subtracted.

Experiment	Time	pH	O_2 uptake	CO_2 production	R.Q.
	min.		*c.mm.*	*c.mm.*	
I	3	2.8	3.5	3.3	0.94
	6		19.2	18.7	0.95
	9		34.7	32.8	0.95
	12		54.2	49.5	0.91
	15		74.4	73.2	0.975
	24		142.1	134.5	0.95
	30		180.6	172.3	0.95
	45		302.8	296.5	0.98
	90		664.7	646.3	0.97
II	189	2.4	542	499.7	0.92
III	104	2.9	178.9	168	0.94
IV	75	2.35	105.8	102	0.96
V	160	3.5	510	625	1.23
VI	150	3.0	480	620	1.30

oxygen, is metabolized mostly by dismutation and only 4 per cent by decarboxylation to acetaldehyde (Table III).

The influence of the H^+ ion concentration can best be shown in experiments at pH 6 and pH 2. At the first pH there was a low oxidation rate and no anaerobic metabolism; at pH 2, there was a high oxidation rate and some anaerobic metabolism (Fig. 2).

It was postulated by Smithe that lack of pyruvic acid metabolism in the absence of oxygen may be due to an inhibition of permeability. However, when glucose was added to yeast cells at pH 2.3, in the absence of oxygen, there was immediate fermentation (as shown by the CO_2 formation) 17 times as high as the CO_2 formation on addition of pyruvate (Fig. 3).

TABLE III

Metabolism of Pyruvic Acid by Washed Bakers' Yeast

Buffer, Teorell's universal buffer, pH 2.8. Pyruvic acid adjusted to same pH, 41 micromoles. Yeast, 20 mg. dry weight in 5.0 cc., volume of fluid. Duration of experiments, 180 minutes. Temperature 28°. All figures are blank-subtracted.

Measurements	O_2 uptake Aerobic	CO_2 production Anaerobic
	c.mm.	*c.mm.*
O_2 uptake (aerobic) or CO_2 production	1514	113
Pyruvic acid utilization	918	183
Lactic acid formation		67.2
Acetaldehyde "		4.5

When the metabolism of pyruvic acid by bakers' yeast in acid solutions was followed until disappearance of the substrate, it was found that the O_2 uptake was equal to 65 per cent of the value for complete oxidation (Fig. 4). Similar results were found by Winzler (17) on measuring the oxidation of acetate by bakers' yeast. Whether the remaining 35 per cent of the pyruvic acid was used in synthesis reactions, as happens with acetate, was not determined.

Inhibition of Pyruvic Acid Oxidation by Malonic Acid.—Lynen (18) found that the oxidation of acetate by bakers' yeast was inhibited by malonic acid when the yeast cells were suspended in water and malonic acid was added as the free acid. Lack of inhibition at pH 7 was due to non-penetration through the cell membrane of the dissociated malonate ion. The oxidation of pyruvic acid was also almost completely inhibited on addition of malonic acid at a concentration of 0.03 M. When the concentration was diminished to 0.01 M the inhibition diminished with time (Fig. 5). This inhibition is due to accumulation of succinic acid, oxidation of which is inhibited by malonic acid. Kleinzeller (19) found no succinic acid formation from pyruvic acid in yeast. Our

experiments confirmed these findings. However, when pyruvic acid was oxidized by yeast in the presence of malonic acid there was succinic acid accumulation. The same phenomenon was observed during the metabolism of oxalacetic acid: there was pyruvic acid formation only when malonic acid was present. Succinic

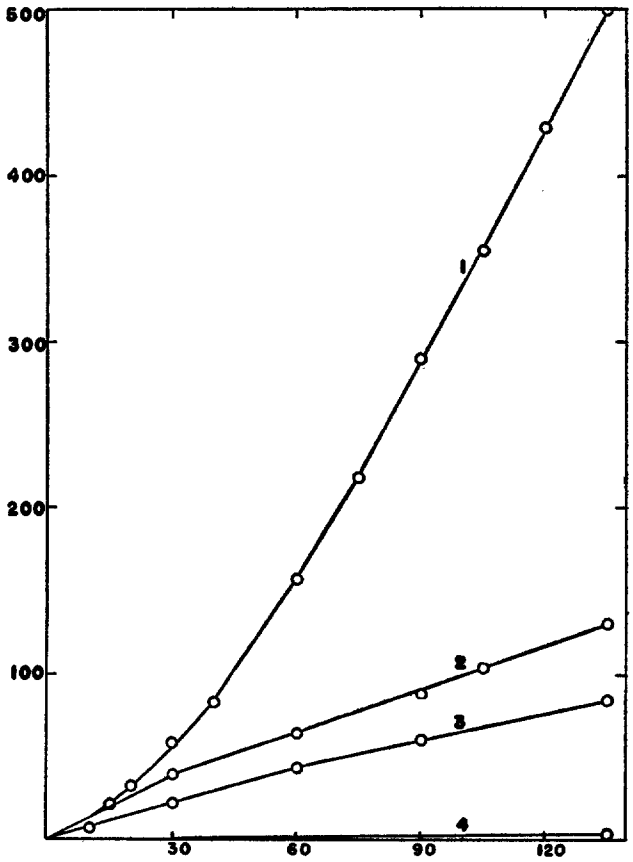

FIG. 2. Pyruvate metabolism at pH 2.0 and at pH 6.0. Buffer, Teorell's universal buffer. Pyruvate, 30 micromoles. Abscissa, time in minutes. Ordinate, O_2 uptake or CO_2 production in c.mm. *1*, O_2 uptake, pH 2.0. *2*, O_2 uptake, pH 6.0. *3*, CO_2 production (in N_2) pH 2.0. 4, CO_2 production (in N_2) pH 6.0. Yeast, 12.6 mg.

acid was also found on oxidation of pyruvic + oxalacetic acid, and of pyruvic + fumaric acid in the presence of malonic acid (Table IV).

Oxidation of the Acids of the Tricarboxylic Acid Cycle.—The experiments of Lynen (20), as well as those of Weinhouse and Millington (21), have shown that acetate oxidation in yeast starts with the formation of citric acid by the condensation of acetic acid with oxalacetic acid, and by the subsequent oxi-

dation *via* the tricarboxylic acid cycle of Krebs. The lack of oxidation of citric acid and succinic acid when added to yeast as the dissociated salts was attributed to the impermeability of the yeast membrane to the salts of dicarboxylic

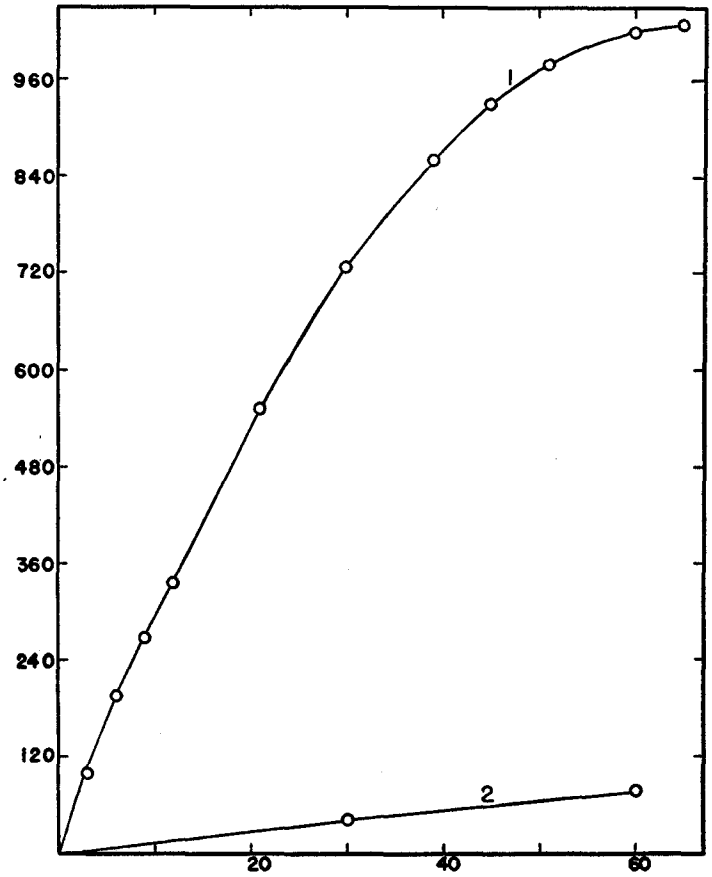

FIG. 3. CO_2 formation from glucose and from pyruvic acid in bakers' yeast anaerobically. Buffer, Teorell's universal buffer pH 2.3. Glucose, and pyruvic acid (brought to pH 2.3) 30 micromoles. Temperature 28°. Gas phase, N_2. Abscissa, time in minutes; ordinates, CO_2 production in c.mm. *1*, glucose; *2*, pyruvic acid.

and tricarboxylic acids. The yeast cell membrane obeys the rules formulated by Osterhout (22, 23) on penetration of weak acids and bases. Oxidation of succinic acid by yeast was observed by Lynen (18) on rupture of the cell membrane with liquid nitrogen and by reduction of methylene blue. The comparative rate of oxidation of the acids which belong to the tricarboxylic acid cycle was studied by suspending washed bakers' yeast cells in the following

buffers: HCl-NaCl (pH 1.08); glycine-HCl, and glycine-H_3PO_4 (pH's 2.27; 2.92); hippuric acid-NaOH (pH 3.90); K_2HPO_4-NaH_2PO_4 (pH's 5.0, 6.20, 7.28); and HBO_3-NaOH (pH's 8.25, 9.40). At pH 1.08, only pyruvic acid was oxidized. At pH 2.27, 2.92, and 3.90, pyruvic, acetic, citric, and succinic acids were oxidized. Oxidation of citric and succinic acids ceased at pH 5 (Table V). At this pH, the first carboxyl group of citric acid is still largely dissociated (99 per cent); the second carboxyl, 70 per cent dissociated; and the third, 70 per cent undissociated. Succinic acid at this pH value exists as 90 per cent

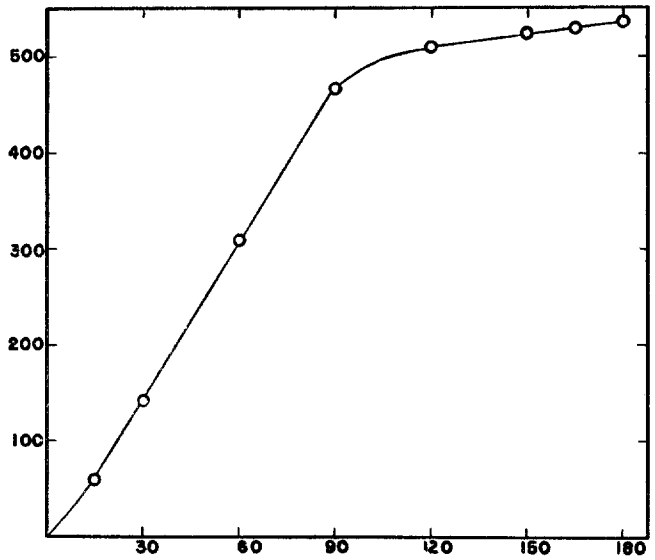

FIG. 4. Oxidation of pyruvic acid by bakers' yeast. Teorell's buffer, pH 2.35. Temperature 28°. 15 micromoles pyruvic acid per vessel. Abscissa, time in minutes; ordinate, O_2 uptake in c.mm. Figures given are blank-subtracted. O_2 uptake for complete oxidation = 840 c.mm.

dissociated succinate ion and 70 per cent as the undissociated acid in its second carboxyl group. The two other acids of the tricarboxylic acid cycle, malic and α-ketoglutaric, were not oxidized by bakers' yeast at pH 2.0 or at 6.0. Undoubtedly these two dicarboxylic acids do not penetrate the cell membrane. In the absence of oxygen there was no decarboxylation of α-ketoglutaric acid, and only a negligible amount of decarboxylation of oxalacetic acid, mostly accounted for as non-enzymatic decarboxylation. Oxalacetic acid decarboxylation by yeast gave only 9.3 c.mm. CO_2 per mg. dry weight per hour at pH 2.0 and none at pH 6.0.

The First Step of Aerobic Pyruvic Acid Metabolism.—The lack of decarboxylation of pyruvic acid in the absence of oxygen, as well as its rapid utilization

in the presence of oxygen with respiratory quotients close to one, is indication that pyruvic acid may be oxidized directly to acetic acid without previous decarboxylation to acetaldehyde. To demonstrate the presence of acetic acid during the oxidation of pyruvic acid, advantage was taken of two factors

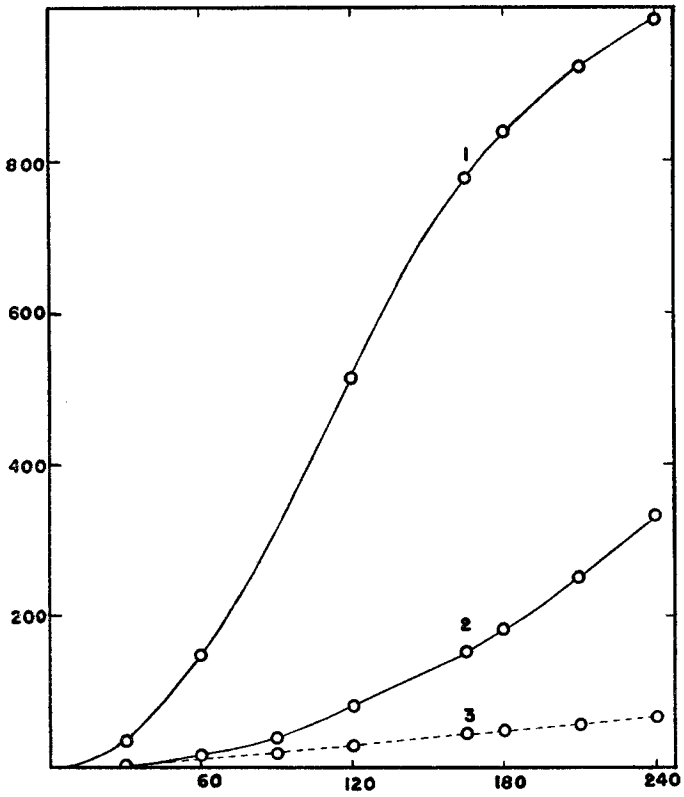

FIG. 5. Inhibition of pyruvic acid oxidation by malonic acid. Buffer, Teorell's universal buffer, pH 2.85. Pyruvic acid, 0.01 M. Temperature 28°. Abscissa, time in minutes; ordinate, O_2 uptake in c.mm. *1*, pyruvic acid. *2*, pyruvic acid + malonic acid, 0.01 M. *3*, pyruvic acid + malonic acid, 0.03 M (figures blank-subtracted).

which could contribute to the accumulation of acetic acids: (1) the greater speed of oxidation of pyruvic acid as compared with that of acetic acid at pH 2.0 or below; (2) inhibition of acetate oxidation by fluoroacetate. Fluoroacetic acid penetrates into the yeast cell as the undissociated acid so that at low pH values it can inhibit both pyruvic acid and acetic acid oxidation at very low concentrations. At pH 2.76, 1×10^{-6} M fluoroacetic acid inhibited the oxidation of acetic acid 78 per cent during the 1st hour, whereas the oxida-

tion of pyruvic acid was inhibited only 34 per cent. A concentration of fluoroacetic acid of 4×10^{-6} M inhibited acetate oxidation completely, while pyruvic acid oxidation was 80 per cent inhibited (Table VI). Accordingly, the yeast

TABLE IV

Formation of Succinate from Pyruvic and Oxalacetic Acids

Buffer, Teorell's universal buffer, pH 2.75. Pyruvic, oxalacetic, and malonic acids, adjusted to the same pH, 50 micromoles each. Washed bakers' yeast, 60 mg. dry weight. Volume of fluid, 5 cc. Temperature 28°. Duration of experiments, 150 minutes.

Additions	O_2 uptake	Succinate
	c.mm.	*c.mm.*
None	740	None
Malonic acid	585	"
Pyruvic acid	2197	"
" + malonic acid	1077	345
Oxalacetic acid	1965	None
" + malonic acid	1015	219
Pyruvic + oxalacetic acid	3044	None
" + " + malonic acid	1133	368
" + fumaric + malonic acid	1064	159.5

TABLE V

Oxidation of the Acids of the Tricarboxylic Acid Cycle by Washed Bakers' Yeast

Substrates, 30 micromoles adjusted to the pH of the buffers. Yeast, 8 mg. dry weight. Temperature 28°. All figures blank-subtracted. Time, 90 minutes.

pH	O_2 uptake, *c.mm.*			
	Pyruvate	Acetate	Citrate	Succinate
1.08	17	0	0	0
2.27	278.5	173	10	20
2.92	339	200	18	25
3.90	257	207	4	12
5.00	118	327	0	0
6.20	35	292	0	0
7.28	24	340	—	—
8.25	13	322	—	—
9.40	0	101	—	—

cells were suspended in glycine-HCl buffer, pH 1.91. At the end of the experiments, proteins were precipitated with tungstate, and pyruvic and acetic acid determinations were made. The steam distillation of acetic acid was performed in the presence of H_2SO_4 and $HgSO_4$ to avoid distillation of pyruvic acid. Although fluoroacetic acid inhibited the O_2 uptake by 30 per cent, there

was no inhibition in the utilization of pyruvic acid. In the presence of pyruvic acid there was formation of 246 c.mm. acetic acid (11 micromoles); and in the presence of fluoroacetic acid, 413 c.mm. (19.4 micromoles) (Table VII).

TABLE VI

Fluoroacetate Inhibition of Pyruvate and Acetate Oxidation by Bakers' Yeast

Acetate and pyruvate, 0.01 M. Fluoroacetate added 15 minutes before the addition of substrates. Temperature 28°.

pH	Time	Fluoroacetate	O_2 uptake, *c.mm.*		Inhibition	O_2 uptake, *c.mm.*		Inhibition
			Pyruvate	Pyruvate + Fluoro-acetate		Acetate	Acetate + Fluoro-acetate	
	min.	*M*						*per cent*
2.76	60	1×10^{-5}	349	229	34.5	357	79	78
	120		771	653	15.3	796	318	60
	180		1015	934	8	1016	687	32.4
2.80	60	5×10^{-5}	517	15	97	508	0	Complete
	120		822	89	89	948	0	"
2.76	"	"	800	113	86	789	0	"
2.76	90	4×10^{-6}	670	179	73	514	0	"

TABLE VII

Acetic Acid Formation from Pyruvic Acid Oxidation by Washed Bakers' Yeast (20 Hours O_2 Bubbling)

9.8 mg. (dry weight) of yeast suspended in 4.5 cc. of 0.05 M glycine-HCl buffer, pH 1.91. Pyruvic acid, 0.5 cc., 0.1 M. Fluoroacetic acid 0.06 cc., 1×10^{-4} M. Temperature 28°. Duration of experiments, 195 minutes.

Additions	O_2 uptake	Acetic acid formation	Pyruvic acid utilization
		c.mm.	*c.mm.*
None		None	
Pyruvic acid	1004	246	878
Pyruvic acid + fluoroacetic acid	702	413	878

DISCUSSION

The experiments presented in this paper, as well as those published previously (7–9), have shown that pyruvic acid added to bakers' yeast intact cells is not metabolized anaerobically in neutral reaction, whereas it is utilized in the presence of oxygen. In acid solutions, however, there was anaerobic pyruvic acid metabolism accompanied by a tremendous increase in utilization

in the presence of oxygen. Smithe (7) postulated that anaerobiosis may inhibit cell permeability, and that CO_2 formation in acid solutions may be due to damage of the cell by the acid reaction of the solution. The uniformly constant endogenous respiration of "starved" washed bakers' yeast cells, whether they are suspended in a solution at pH 1.08 or 6.0, speaks against a damage to the cell at low pH's. The yeast cell is remarkably resistant to changes in the pH value of the external environment.

The rate of utilization of pyruvic acid by bakers' yeast cells seems determined by the fraction present as the undissociated acid, the only form which penetrates the cell membrane. This property brought forth a pH optimum for the oxidation of pyruvic acid so low (2.6–2.9) that it cannot be taken as the pH optimum for enzymatic activity, but rather as an expression of the rate of penetration of pyruvic acid through the cell membrane. Pyruvic acid may be utilized in a variety of ways (24): by direct oxidation to acetic acid and CO_2; by decarboxylation to acetaldehyde and CO_2; by dismutation to lactic acid, acetic acid, and CO_2; by hydrolysis to acetic and formic acids. The obvious pathway to be followed in yeast cells would have been the second; *i.e.*, decarboxylation to acetaldehyde. Such a process is independent of the gas phase and proceeds equally well in the presence and in the absence of oxygen. In acid solution, when there was CO_2 formation on addition of pyruvic acid, CO_2 formation was mostly due to dismutation, as demonstrated by lactic acid formation. Acetaldehyde formation represented only a very small fraction of the pyruvic acid utilized anaerobically, 2.5 per cent. It may be concluded, therefore, that pyruvate utilization in bakers' yeast cells does not start by its initial decarboxylation to acetaldehyde. The validity of the hypothesis that pyruvic acid metabolism starts by its initial oxidation to acetic acid and subsequent oxidation through the tricarboxylic acid cycle was shown by three experiments: (1) accumulation of acetic acid on oxidation of pyruvic acid at a pH value where the rate of acetic acid oxidation was about half that of pyruvic acid; (2) accumulation of acetic acid by addition of fluoroacetic acid at a concentration which inhibits acetic acid oxidation without any effect on pyruvic acid oxidation; (3) accumulation of succinic acid on oxidation of pyruvic acid, pyruvic + oxalacetic acids, and pyruvic + fumaric acids, all in the presence of malonic acid.

When glucose was added to bakers' yeast in the absence of oxygen, at pH 2.3, it was rapidly fermented with the formation of CO_2 and alcohol. The glucose molecule on its path to the end products of fermentation, undoubtedly produced pyruvic acid, which was decarboxylated to acetaldehyde, which in its turn was reduced to alcohol. However, when pyruvic acid was added from the buffered solution, it was either directly oxidized or, in the absence of oxygen, was metabolized by dismutation and only very slightly by decarboxylation. This discrepancy can be explained by taking into consideration the

arrangement of the enzymes within the cell. If carboxylase exists only in the center of the cell, while pyruvic acid oxidase is distributed close to the periphery, the pyruvic acid formed during the fermentation of glucose *within* the cell will be rapidly decarboxylated by carboxylase, whereas pyruvic acid added from outside the cell will be oxidized by pyruvic acid oxidase.

The arrangement of the enzymes within the cell is, in our opinion, a factor of great importance in the regulation of cellular metabolism, since this localization will contribute to the determination of metabolic pathways, especially when various enzymes exist within the cell for the metabolism of one substrate. When the cell is broken, the usual arrangement is disturbed and the substrate will be metabolized in an entirely different manner. If all the enzymes are present, the extent and the metabolic pathway will be determined by the concentration of the enzymes; if some of the enzymes have become inactive during the process of cell destruction, the number of pathways will be correspondingly diminished. This happens to the yeast cells when they are dried. Destruction of pyruvic acid oxidase limits the metabolism of pyruvic acid to its decarboxylation, a process which is almost nil in the intact cell, and goes equally well in the presence as in the absence of oxygen.

SUMMARY

The rate of the aerobic metabolism of pyruvic acid by bakers' yeast cells is determined mainly by the amount of undissociated acid present. As a consequence, the greatest rate of oxidation was observed at pH 2.8. Oxidation, at a slow rate, started at pH 1.08; at pH 9.4 there was no oxidation at all. The anaerobic metabolism, only a fraction of the aerobic, was observed only in acid solutions. There was none at pH values higher than 3.

Pyruvic acid in the presence of oxygen was oxidized directly to acetic acid; in the absence of oxygen it was metabolized mainly by dismutation to lactic and acetic acids, and CO_2. Acetic acid formation was demonstrated on oxidation of pyruvic acid at pH 1.91, and on addition of fluoroacetic acid. Succinic acid formation was shown by addition of malonic acid. These metabolic pathways in a cell so rich in carboxylase may be explained by the arrangement of enzymes within the cell, so that carboxylase is at the center, while pyruvic acid oxidase is located at the periphery.

Succinic and citric acids were oxidized only in acid solutions up to pH 4. Malic and α-ketoglutaric acids were not oxidized, undoubtedly because of lack of penetration.

BIBLIOGRAPHY

1. Neuberg, C., and Karczag, L., *Biochem. Z.*, 1911, **36,** 68.
2. Meyerhof, O., *Ergebn. Physiol.*, 1937, **39,** 10.
3. Warburg, O., Wasserstoffübertragende Fermente, Werner Saenger, Berlin, 1948, 9.

4. Hagglund, E., and Augustsson, A. M., *Biochem. Z.*, 1926, **170,** 102.
5. Haehn, H., and Glaubitz, M., *Z. physiol. Chem.*, 1927, **168,** 233.
6. Neuberg, C., *Biochem. Z.*, 1927, **180,** 471.
7. Smithe, C. V., *J. Biol. Chem.*, 1938, **125,** 635.
8. Rünnstrom, J., Sperber, E., and Karlsson, E., *Ark. Kemi, Mineral. och Geol.*, 1932, **13B,** No. 10.
9. Rünnstrom, J., and Marcuse, R., *Ark. Kemi, Mineral. och Geol.*, 1944, **18A,** No. 14.
10. Teorell, T., and Stenhagen, E., *Biochem. Z.*, 1938, **299,** 416.
11. Friedemann, T. E., and Haugen, G. E., *J. Biol. Chem.*, 1943, **147,** 415.
12. Friedemann, T. E., *J. Biol. Chem.*, 1938, **123,** 161.
13. Barker, J. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138,** 535.
14. Stotz, E., *J. Biol. Chem.*, 1943, **148,** 585.
15. Krebs, H. A., *Biochem. J.*, 1937, **31,** 661.
16. Barron, E. S. G., and Lyman, C. M., *J. Biol. Chem.*, 1939, **127,** 143.
17. Winzler, R. J., *J. Cell. and Comp. Physiol.*, 1940, **15,** 341.
18. Lynen, F., *Ann. Chem.*, 1943, **554,** 40.
19. Kleinzeller, A., *Biochem. J.*, 1941, **35,** 495.
20. Lynen, F., *Ann. Chem.*, 1945, **558,** 47.
21. Weinhouse, S., and Millington, R. H., *J. Am. Chem. Soc.*, 1947, **69,** 3089.
22. Osterhout, W. J. V., *J. Gen. Physiol.*, 1925, **8,** 131.
23. Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1935, **21,** 125.
24. Barron, E. S. G., *Bol. soc. quim. Peru*, 1940, **6,** 7.