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COMPARATIVE PHYSIOLOGICAL STUDIES ON THE RESPIRATION OF VARIOUS YEASTS

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INTRODUCTION

In recent years, many comparative-physiological studies on respiration of yeasts have been carried out by numerous workers. Such projects are being carried on with the view of introducing some physiological methods in the study of phylogenetic relations of the yeasts.

There are two important characteristics used in the classification of yeasts. These are morphological and physiological properties. The former includes the taxonomic characters of vegetative and reproductive phase of cells as follows : (1) the existenence of the spore formation, (2) type of spore formation, (3) shape of spores, (4) mode of budding, (5) form of vegetative cells, (6) and other factors.[1-2] These define the main lines in yeast taxonomy. However, for the differentiation into species physiological characters can not be neglected. The physiological properties are fermentation and assimilation of carbohydrates, assimilation of nitrate, occurrence of carotinoid pigments, splitting of arbutin, production of acid, splitting of fat, vitamin requirements and other properties.[3] Therefore, it is quite important to study these physiological characters comparatively in order to analyze the phylogenetic relations of yeasts. The presently described investigations dealt with this problem.

In comparative physiological studies of yeasts, Wickerham reported comprehensive studies about comparison of ability of carbohydrate assimilation.[4] Recently, Barnett and his coworkers have described the oxidation of polyhydric alcohol and the tricarboxylic acid (TCA) cycle intermediates.[5,6]

As the means for acquiring energy in organisms, the physiological signification of the hexosemonophosphate (HMP) cycle has come recently to attract investigaters' attention. Then studies on the corelation between the Embden-Meyerhof-Parnas (EMP) pathway and the HMP cycle became important for elucidating the respiratory pattern of various organisms. The early works of Warburg and his associates established the fact that glucose-6-phosphate could be enzymically oxidized by yeast preparations to 6-phosphogluconic acid in the presence of triphosphopyridine nucleotide as a coenzyme.[7,8] Later, Cori and Lipmann,[9] Brodie and Lipmann,[10] Horecker and Smyruiotis,[11] Glaser and

Brown,[12] Kornberg,[13] Lipmann,[14] Dickens,[15] Cohen and McNair Scott[16] and many other workers have investigated the details of the HMP cycle.[17-29] Recently, investigation has been made to determine which is the major pathway in glucose catabolism in various organisms, that is, whether the EMP pathway or the HMP cycle.[30-33]

On the other hand, it is well known that there are specific inhibitors of the EMP pathway. The inhibition mechanism of monoiodoacetic acid has been studied by Lundsgaar,[34,35] Boysen Jensen,[36] Runström and Alm,[37] Adler, Euler and Günther,[38] Ankel and Szulmajester,[39] Stoppani, Actis, Deferrari and Gonzalez[40] and numerous other workers. On the inhibitiion mechanism of sodium fluoride, Chaix and Fromageot,[41] Sakaguchi and Baba,[42] Pickett and Clifton,[43] Massart and Noortgate,[44] Reiner[45] and other workers have reported in various organisms. By the recent report of Hoskin[23] it was made clear that arsenite inhibits the EMP pathway more effectively than the HMP cycle in guinea-pig brain.

The object of the studies reported here, in which nine strains of yeast with various respiratory quotient (RQ) values of glucose respiration were used, was to show from the point of view of comparative physiology some interrelations between the values of RQ and the following metabolic characteristics of yeasts : (1) oxidation of glucose, gluconate, pyruvate, ethanol and the TCA cycle intermediates; (2) inhibition by monoiodoacetic acid, sodium fluoride and arsenite on glucose oxidation; and (3) cytochrome components.

EXPERIMENTAL METHODS

Materials

Nine strains of yeasts were employed through the studies. They are *Candida utilis, Mycoderma cerevisiae, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces sake, Rhodotorula glutinis, Torula candida* and *Pichia membranaefaciens.*

Candida utilis, Mycoderma cerevisiae and *Schizosaccharomyces pombe* were received from the Institute for Fermentation, Osaka. *Saccharomyces cerevisiae* was isolated from pressed baker's yeast produced by Nippon Beet Sugar Co. *Saccharomyces carlsbergensis* was kindly presented by Prof. T. Sasaki of the Faculty of Agriculture of Hokkaido University. *Rhodotorula glutinis* and *Torula candida* were given by Dr. M. Yoneyama of Hiroshima University. *Saccharomyces sake* was obtained by isolation from "moromi" of sake. *Pichia membranaefaciens* was kindly furnished by Prof. I. Yokozuka of Yamanashi University. These organisms have been maintained in the writer's laboratory on agar slants containing "koji" juice.

Culture Methods

Modified Hennеberg solution was used for culture of yeasts tested. This modified solution contains 100 g cane sugar, 5 g peptone, 5 g KH_2PO_4, 2 g $MgSO_4$, 0.5 per cent yeast extract and 1000 ml. water. Inoculum was incubated in 300 ml. Erlenmeyer flasks containing 50 ml. of the solution at 30°C for 16 hrs. For the culture of yeasts a reciprocating shaker was used.

Preparation of Cell Suspension

The organisms grown in the media above described were harvested by centrifugation, washed with deionized water three times, and suspended in deionized water.

Preparation of Cell-Free Extract

After the disruption of washed cells by grinding with sea sand, homogenate was centrifuged at 3,000 r. p. m. for 5 minutes to remove sea sand powder and intact cells. Then an opalescent supernatant was obtained.

Manometric Determination

The rate of uptaken oxygen in air was determined by the conventional techniques of a Warburg respirometer[46]. The materials and their final molar concentrations in each reaction chamber were as follows : cell suspension, 0.5 ml.; substrate, from 10^{-3} M to 5×10^{-2} M ; phosphate buffer and acid potassium phtalate–sodium hydroxide buffer, pH 5.9 and 4.8 respectively, 2.5×10^{-2} M; inhibitor, from 5×10^{-4} M to 5×10^{-3} M. The volume of solution in the reaction chamber was adjusted with deionized water to make 2 ml. in total. Respiratory inhibitor in side arm was poured into reaction chamber at the following times after the beginning of measurement : monoiodoacetic acid, after twenty minutes; sodium fluoride, thirty minutes; arsenite, forty minutes. The center well contained 0.5 ml. of 20 per cent KOH and a fluted filter paper strip. The gas phase was air. The temperature was 30°C.

Substrates and Respiratory Inhibitors

As substrates, glucose, arabinose, gluconate, pyruvate, acetate, acetaldehyde, ethanol and the TCA cycle intermediates were employed. Oxidation of the TCA cycle intermediates was tested at pH 5.9. The other seven substrates were tested at pH 4.8. Phospate buffer and acid potassium phtalate–sodium hydroxide buffer were used at pH 5.9 and 4.8 respectively. As respiratory inhibitor, use was made of monoiodoacetic acid, sodium fluoride and arsenite.

Spectrophotometric Determinations

For estimation of cytochrome system of cell-suspensions, absorption spectra in wave lengths ranging from 450 to 620 mμ were observed under reduced condition of cytochromes with SHIMAZU's spectrophotometer Type QB-50. Sodium dithionite was used as a reductant.

Micro-Spectroscopic Determinations

For estimation of cytochrome system of cell-suspensions, a micro-spectroscope was also employed. Sodium dithionite was used as a reductant. These determinations were made under anaerobical condition.

Determination of Protein

Protein in cell-suspensions used in the investigations by spectrophotometer was estimated by the method of Lubochinsky and Zalta[47].

EXPERIMENTAL RESULTS

I. *OXIDATIVE ACTIVITIES OF VARIOUS YEASTS ON VARIOUS SUBSTRATES.*

(1) Oxidation of Glucose, Arabinose, Ethanol, Pyruvate and Gluconate.

Figures 1−8 and Table I show the oxidation of substrates by eight strains of yeasts. Out of five kinds of substrates both ethanol and glucose were comparatively activ ely oxidized by all the yeasts tested. Especially, glucose among the substrates examined was most intensively oxidized by *Rhodotorula glutinis, Candida utilis, Schizosaccharomyces pombe* and *Mycoderma cerevisiae*; these organisms have comparatively low RQ values of glucose oxidation. However, *Torula candida* and three other kinds of yeasts which have comparatively high RQ values of glucose oxidation, *Saccharomyces sake, Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*, utilized ethanol most actively. Three species of yeasts, *Rhodotorula glutinis, Candida utilis* and *Mycoderma cerevisiae* which utilize glucose preferably in comparison with other yeasts, oxidized gluconate rather actively. On the contrary, four other species of yeasts scarcely utilized gluconate. Pyruvate was fairly oxidized by eight strains of yeasts. Arabinose was slightly oxidized only by *Rhodotorula glutinis* and *Torula candida.*

Table I *Rates of respiration on glucose, arabinose, ethanol, pyruvate and gluconate as substrates**

Yeast	Q_{O_2} : Uptake of Oxygen (μl./mg dry wt. of yeast/hr.)						RQ
	endog.	glucose	arabinose	ethanol	pyruvate	gluconate	glucose
Rhodotorula glutinis	27	75	24	55	22	7	1.2
Candida utilis	14	92	0	88	75	9	1.1
Mycoderma cerevisiae	10	146	0	151	22	8	1.1
Schizosaccharomyces pombe	2.4	42	0	21	11	2.5	3.5
Saccharomyces sake	10	48	0	51	43	0	2.0
Saccharomyces cerevisiae	11	65	0	79	13	0	3.1
Saccharomyces carlsbergensis	2	13	0	13	10	0.6	6.5
Torula candida	14	8	7	37	11	4	1.3

* Manometer flasks contained washed yeast cells in 0.5 ml. of 10^{-1} M acid potassium phthalate-sodium hydroxide butfer pH 4.8, in the main compartments ; 2.5×10^{-2} M of substrate in 0.5 ml. of water in the side arms ; and 0.5 ml. of 20 per cent KOH with filter paper in each center well. Incubated in air at 30°C. The figures for Q_{O_2} have been corrected for endogenous respiration. 16 hrs. cultures were used. Abbreviations : endog. = no substrate added.

(2) Oxidation of the TCA Cycle Intermediates.

Oxidation of the TCA cycle intermediates was tested on eight strains of yeasts. Table II shows the oxidative activities of yeasts on the TCA cycle intermediate. Succinate was utilized by all tested organisms, though the intensities of the oxidation were not similar. Citrate was oxidized by only three kinds of yeasts. In general, strains which have higher RQ values showed relatively weak oxidative activities on the TCA cycle intermedi-

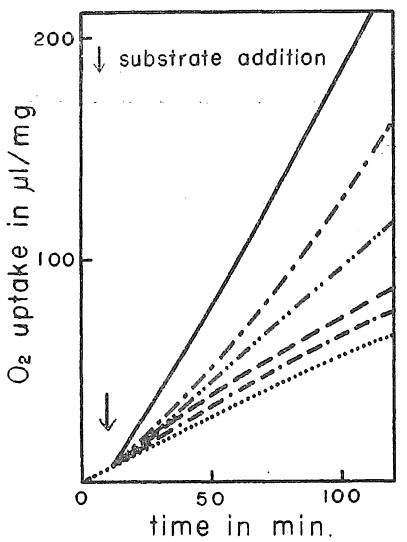

Fig. 1. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Rhodotorula glutinis*. The reaction mixtures contained 0.5 ml. of cell suspension in a final volume of 2.0 ml. The reactions were started at time indicated by arrow by tipping in 2.5×10^{-2} M of substrate from the side arm.

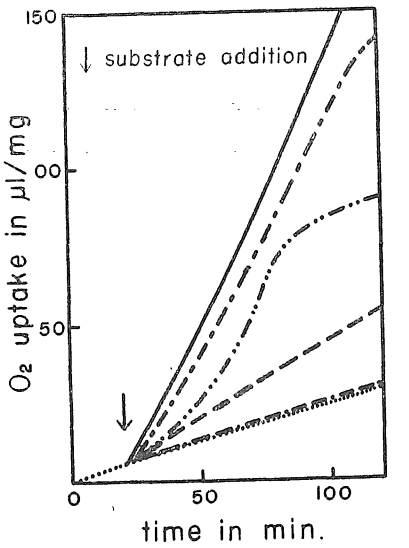

Fig. 2. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Candida utilis*. The reaction conditions were the same as those for Fig. 1.

Indication of added substrates :

glucose	ethanol
pyruvate	gluconate
arabinose	endogenous

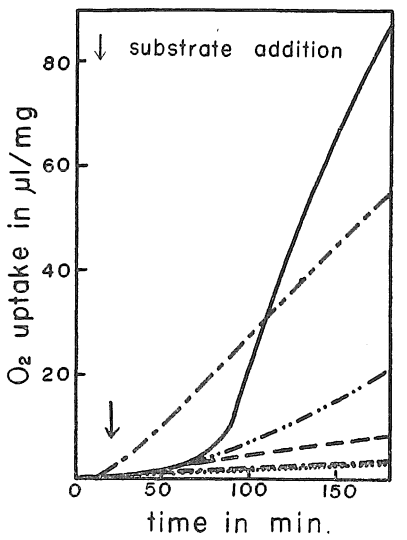

Fig. 3. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Schizosaccharomyces pombe*. The reaction conditions were the same as those for Fig. 1.

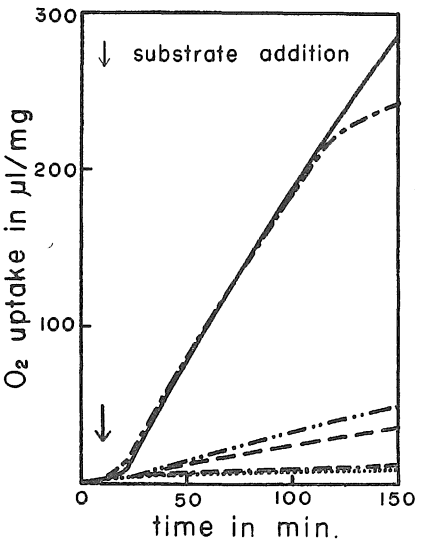

Fig. 4. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Mycoderma cerevisiae*. The reaction conditions were the same as those for Fig. 1.

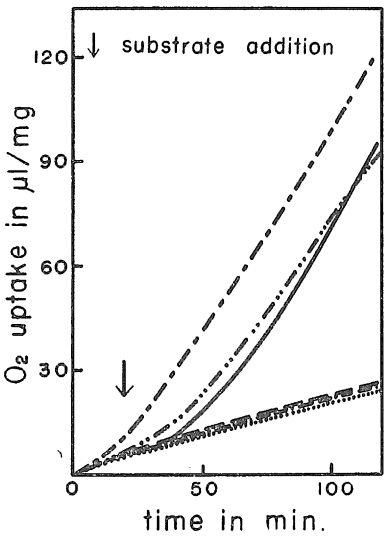

Fig. 5. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Saccharomyces sake*. The reaction conditions were the same as those for Fig. 1.

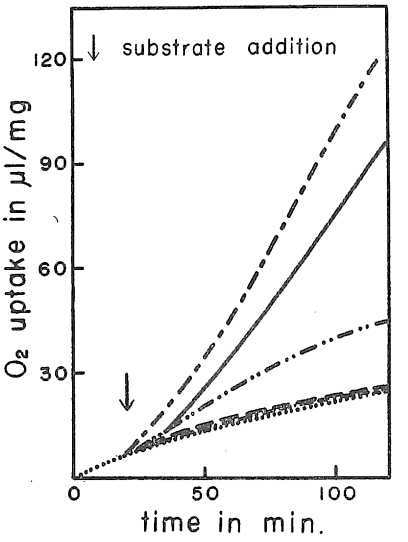

Fig. 6. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Saccharomyces cerevisiae*. The reaction conditions were the same as those for Fig. 1.

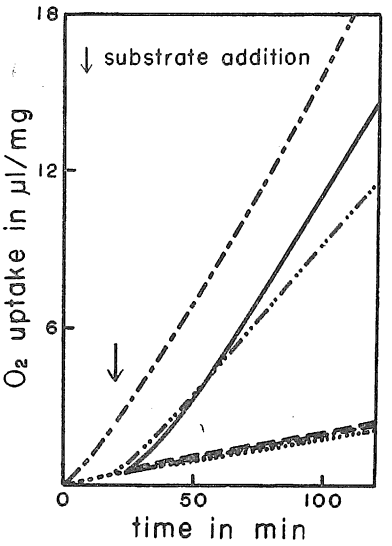

Fig. 7. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Saccharomyces carlsbergensis*. The reaction conditions were the same as those for Fig. 1.

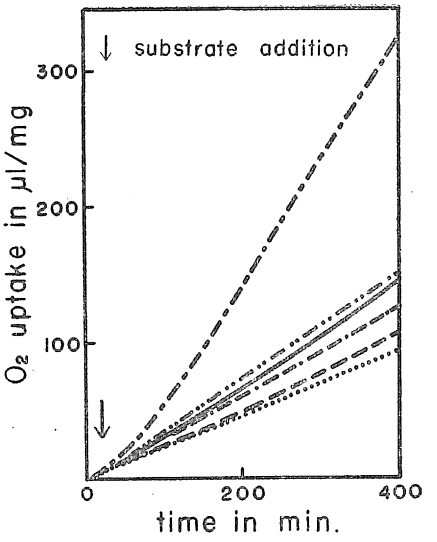

Fig. 8. Oxidation of glucose, arabinose, gluconate, pyruvate and ethanol by washed resting cells of *Torula candida*. The reaction conditions were the same as those for Fig. 1.

Table II *Rates of respiration on the TCA cycle intermediates as substrates**

Yeast	Q_{O_2} : Uptake of oxygen (μl./mg dry wt. of yeast/hr.)					RQ
	endog.	citrate	malate	α-KG	succinate	glucose
Candida utilis	14	44	35	17	7	1.1
Mycoderma cerevisiae	10	0	26	15	12	1.1
Pichia membranaefaciens	5.6	0	27	20	3	0.94
Rhodotorula glutinis	27	0	4	3	9	1.2
Schizosaccharomyces pombe	2.4	0.9	1.9	2.3	1.4	3.5
Saccharomyces sake	10	0	0	0	1	2.0
Saccharomyces cerevisiae	11	0	1	0	1	3.1
Saccharomyces carlsbergensis	2	3	3	9	3	6.5

* Compositions of contents of manometer flasks were as in Table I.
Abbreviations : endog. = no substrate added.
α-KG = α-Keto-glutaric acid.

ates. On the test by cell-free extract of *Saccharomyces cerevisiae* oxygen uptake in the presence of citrate was more than twofold of endogenous, whereas intact cells of the organism could not oxidize citrate (Table III).

Table III Rates of oxidation of the TCA cycle intermediates by cell-free extracts of Saccharomyces cerevisiae*

Yeast	Q_{O_2} : Uptake of oxygen (μl./mg dry wt. of yeast/ hr.)				
	endog.	citrate	malate	α-KG	succinate
Saccharomyces cerevisiae	0.35	0.85	0.1	0.75	0.3

* Manometer flasks contained cell-free extract in 0.5ml. of 10^{-1} M phosphate buffer pH 5.9, in the main compartments. Other compositions were as in Table II.

(3) Oxidation of the Tricarboxyric Acid Cycle Intermediates in Various Concentrations.

Candida utilis was used in the experiments, for it oxidized most actively the TCA cycle intermediates among the eight strains employed. As substrate, citrate, malate, α-keto-glutarate and succinate were employed. As a control, oxidation of glucose in various concentrations was examined. In general, to maintain active respiration this organism required comparatively high concentrations of those substrates. The oxidation of glucose was maximal at 6.7×10^{-3} M of concentration. Citrate was oxidized most actively at 1.3×10^{-2} M of concentration. The Michaelis constants of oxidations of both glucose and citrate were 2×10^{-3} M. On the other hand, in case of oxidation of malate and α-keto-glutarate, gradual increase of the oxidative rate was seen in the tested range of concenrtation from 10^{-3} to 5×10^{-2} M. The oxidation of succinate increased slightly in the range of concentration from 2×10^{-3} to 5×10^{-2} M (Figures 9–10).

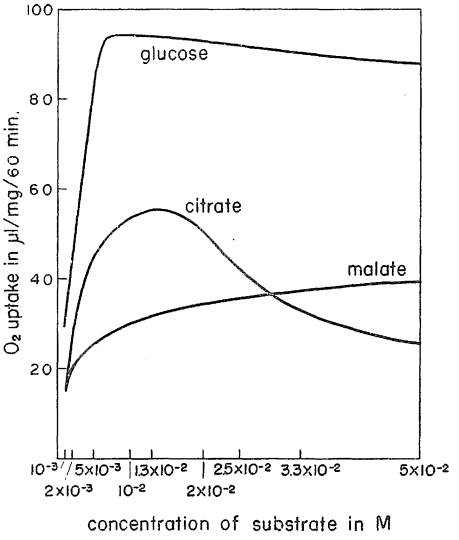

Fig. 9. Oxidation of glucose, citrate and malate in various concentrations by washed resting cells of *Candida utilis*. The reaction mixtures contained 0.5 ml. of cell suspension in a final volume of 2.0 ml.

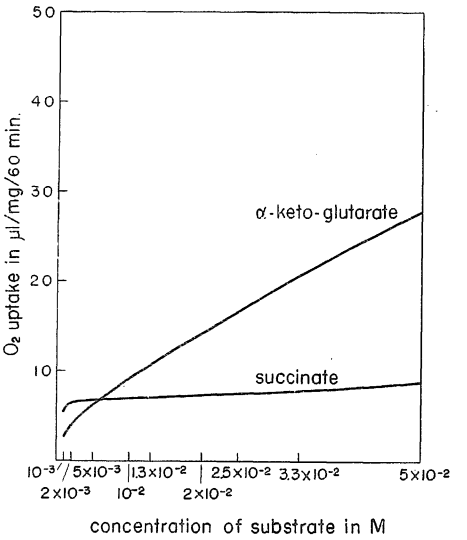

Fig. 10. Oxidation of α-keto-glutarate and succinate in various concentrations by washed resting cells of *Candida utilis*. The reaction mixtures contained 0.5 ml. of suspension in a final volume of 2.0 ml.

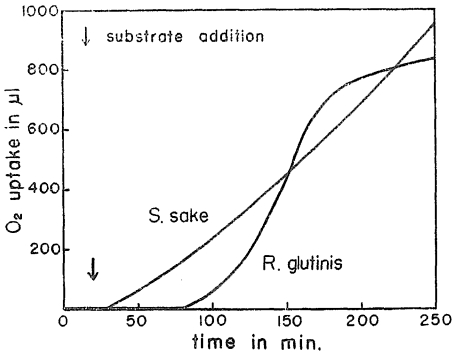

Fig. 11. Comparison of oxidation processes of ethanol by *Saccharomyces sake* and *Rhodotorula glutinis*. The reaction mixtures contained 0.5 ml. of cell suspension in a final volume of 2.0 ml. The reactions were started at time indicated by arrow by tipping in 2.5×10^{-2} M of ethanol from the side arm.

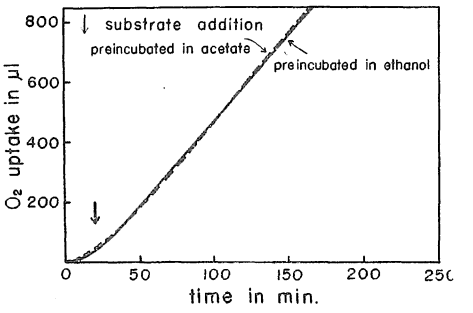

Fig. 12. Comparison of preincubation effects on ethanol oxidizing activity of washed resting cells of *Rhodotorula glutinis*. The organisms were preincubated in 2.5×10^{-2} M of ethanol or acetate for 4 hrs. The reaction conditions were the same as those for Fig. 11.

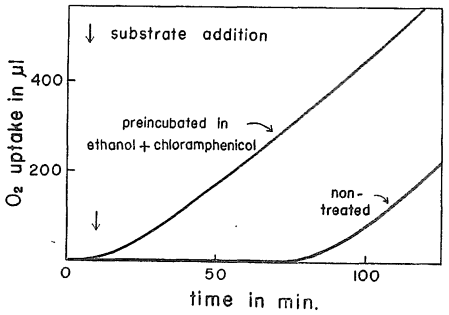

Fig. 13. Effect of chloramphenicol on preincubation in ethanol. Washed resting cells of *Rhodotorula glutinis* were preincubated in the mixed solution of 2.5×10^{-2} M ethanol and 200 γ/ml. of chloramphenicol for 4 hrs. The reaction conditions were the same as those for Fig. 11.

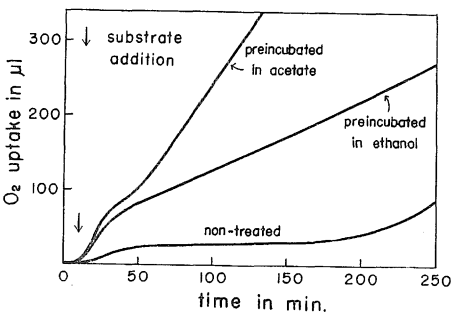

Fig. 14. Comparison of preincubation effects on acetate oxidizing activity by washed resting cells of *Rhodotorula glutinis*. The organisms were preincubated in 2.5×10^{-2} M of ethanol or acetate for 4 hrs. The reaction conditions were the same as those for Fig. 11, except that 2.5×10^{-2} M of acetate was employed as substrate.

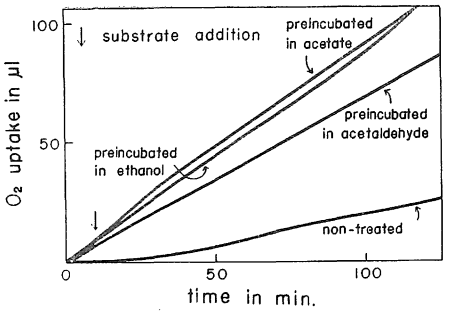

Fig. 15. Comparison of preincubation effects on acetaldehyde oxidizing activity by washed resting cells of *Rhodotorula glutinis*. The organisms were preincubated in 2.5×10^{-2} M of acetate, ethanol and acetaldehyde for 4 hrs. The reaction conditions were the same as those for Fig. 11, except that 2.5×10^{-2} M of acetaldehyde was employed as substrate.

II. EFFECTS OF PREINCUBATION IN ETHANOL AND ACETATE.

(1) Ethanol and Acetate Oxidizing Activity.

An adaptation phenomenon was seen in the case of ethanol oxidation by *Rhodotorula glutinis* which has relatively low RQ values, whereas *Saccharomyces sake* exhibited active oxidation as soon as ethanol was added (Figure 11). The maximum rate of ethanol oxidation by *Rhodotorula glutinis* arose about 130 minutes after the addition of substrate. Figure 12 shows the effects of preincubation in ethanol or acetate. By means of preincubation for four hours in 2.5×10^{-2} M of ethanol, *Rhodotorula glutinis* acquired an ability to oxidize ethanol without any lag phase. That is to say, the oxidation of ethanol proceeded analogously like that by *Saccharomyces sake*. Similarly, this organism by preincubation with acetate obtained the capacity to oxidize ethanol instantaneously (Figure 12). The acquisition of this ability was not disturbed by 200 γ/ml. of chloramphenicol (Figure 13). Untreated strain of this organism has only weak ability to oxidize acetate. After about three hours of contact with acetate, gradual increase of activity of acetate oxidation was observed. Four hours of preincubation in 2.5×10^{-2} M of acetate gave vigorous ability of acetate oxidation. Such acquired ability to oxidize acetate was seen also on the strain which had been preincubated in 2.5×10^{-2} M of ethanol (Figure 14).

(2) Acetaldehyde Oxidizing Activity.

Figure 15 exhibits the effect of preincubation on the acetaldehyde oxidizing activity of *Rhodotorula glutinis*. This organism, *Rhodotorula glutinis*, has only weak ability of oxidation of acetaldehyde. However, the procedure of preincubation either on ethanol, acetaldehyde or acetate gave active ability to oxidize acetaldehyde (Figure 15).

III. EFFECTS OF INHIBITORS ON RESPIRATION OF VARIOUS YEASTS.

(1) Inhibition by Monoiodoacetic Acid.

Rhodotorula glutinis.

Monoiodoacetic acid in a concentration of 5×10^{-4} M exerted only a slight inhibition upon oxidation of glucose. Even by 5×10^{-3} M of monoiodoacetic acid this organism exhibited fair respirattory activity. This organism was most insensitive to monoiodoacetic acid among eight kinds of yeasts tested (Figure 16). On CO_2 output also, monoiodoacetic acid showed extremely weak inhibition effect (Figure 17).

Candida utilis.

Monoiodoacetic acid of 5×10^{-4} M exerted little inhibition upon oxidation of glucose. However, this organism suffered remarkable inhibition by 5×10^{-3} M monoiodoacetic acid, namely, its respiratory activity was completely inhibited at 60 minutes after addition of the inhibitor (Figure 18). The CO_2 evolution suffered conspicuous inhibition by 5×10^{-4} M of monoiodoacetic acid. But perfect inhibition even by 5×10^{-3} M of monoiodoacetic acid (Figure 19) was not seen. The endogenous CO_2 evolution was scarcely inhibited by 3×10^{-3} M. In general, the endogenous respiration of wild yeasts such as *Rhodotorula* and *Candida* is more stable against monoiodoacetate inhibition than the respiration of

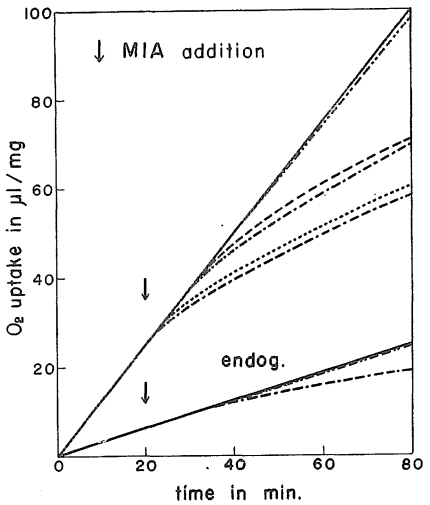

Fig. 16. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Rhodotorula glutinis*. The reaction mixtures contanined 0.5 ml. of 10^{-1} M buffer pH 4.8 and 0.5 ml. of 10^{-1} M glucose in a final volume of 2.0ml. The reactions were started at time indicated by arrow by tipping in various concentrations of monoiodoacetic acid from the side arms.

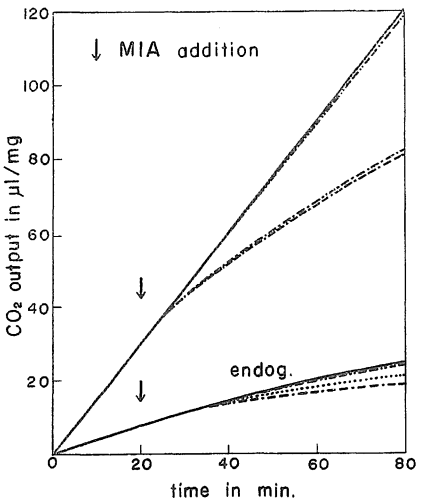

Fig. 17. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Rhodotorula glutinis*. The reaction conditions were the same as those for Fig. 16, except that 0.5 ml. of deionized water was placed instead of KOH solution in center well.

Concentration of added monoiodoacetic acid (from Fig. 16 to Fig. 31) :

———	non	·····	3×10^{-3}
—·—·—	5×10^{-4}	—·—·—	4×10^{-3}
– – –	1×10^{-3}	—·—·—	5×10^{-3}
—·—·—	2×10^{-3}		

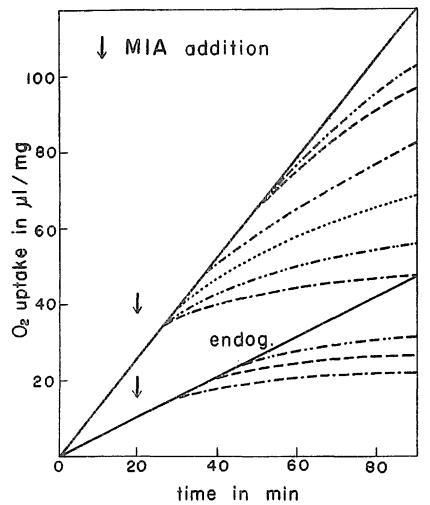

Fig. 18. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Candida utilis*. The reaction conditions were the same as those for Fig. 16.

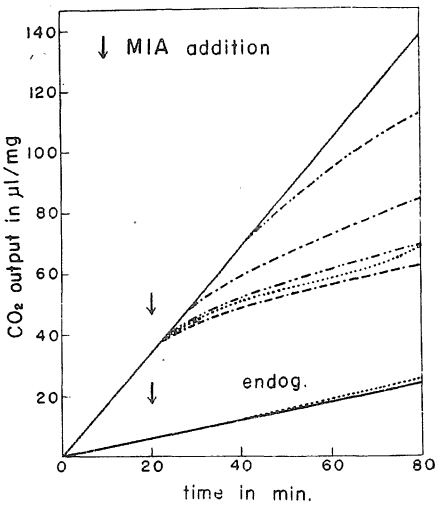

Fig. 19. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Candida utilis*. The reaction conditions were the same as those for Fig. 17.

exogenously supplied substrate.

Schizosaccharomyces pombe.

Upon the oxidation of glucose, 5×10^{-4} M of monoiodoacetic acid exerted only a little inhibition. However, the respiration was inhibited completely at 5×10^{-3} M (Figure 20). The endogenous respiratory activity was extremely weak. The activity of CO_2 evolution was inhibited at 5×10^{-4} M rather severely. That activity was inhibited perfectly at 4×10^{-3} M (Figure 21).

Saccharomyces cerevisiae.

Upon the oxidation of glucose, 5×10^{-4} M of monoiodoacetic acid exerted some conspicuous inhibition. The respiration was inhibited almost perfectly at 3×10^{-3} M. As to CO_2 output, intense inhibition was seen at 3×10^{-3} M (Figures 22, 23).

Saccharomyces sake.

Upon the oxidation of glucose, 5×10^{-4} M of monoiodoacetic acid exhibited fairly severe inhibition. The respiration was inhibited almost perfectly at 3×10^{-3} M (Fgigure 24). Similar inhibition was seen of CO_2 evolution (Figure 25).

Saccharomyces carlsbergensis.

Upon the oxidation of glucose, 5×10^{-4} M of monoiodoacetic acid exerted severe inhibition (Figure 26). As to CO_2 evolution, monoiodoacetic acid inhibited it perfectly at 5×10^{-4} M (Figure 27).

Mycoderma cerevisiae.

Upon the oxidation of glucose, 5×10^{-4} M of monoiodoacetic acid has an extremely severe inhibition effect. The respiration was inhibited perfectly at 1×10^{-3} M. Endogenous respiration was also inhibited perfectly by 1×10^{-3} M (Figure 28). CO_2 evolution was extremely inhibited at 5×10^{-4} M (Figure 29).

Torula candida.

Monoiodoacetic acid of 5×10^{-4} M exerted serious inhibition upon both O_2 uptake and CO_2 output (Figures 30, 31).

(2) Inhibition by Sodium Fluoride.

Among the eight kinds of yeasts mentioned above, *Rhodotorula glutinis* which was most insensitive to monoiodoacetic acid and *Saccharomyces cerevisiae* which was most sensitive to monoiodoacetic acid were investigated for sodium fluoride inhibition on their O_2 uptake.

Rhodotorula glutinis.

Respiration on exogenously supplied glucose was inhibited a little by 1×10^{-2} M of sodium fluoride. The respiratory activity was left practically intact even by 2×10^{-2} M of sodium fluoride (Figure 32).

Saccharomyces cerevisiae.

By 1×10^{-2} M of sodium fluoride the respiration on exogenously upplied glucose suffered only slight inhibition, whereas it was perfectly inhibited after 30 minutes of the addition of 2×10^{-2} M of sodium fluoride (Figure 33).

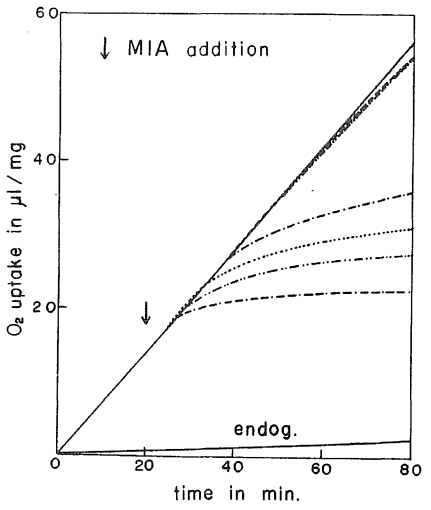

Fig. 20. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Schizosaccharomyces pombe*. The reaction conditions were the same as those for Fig. 16.

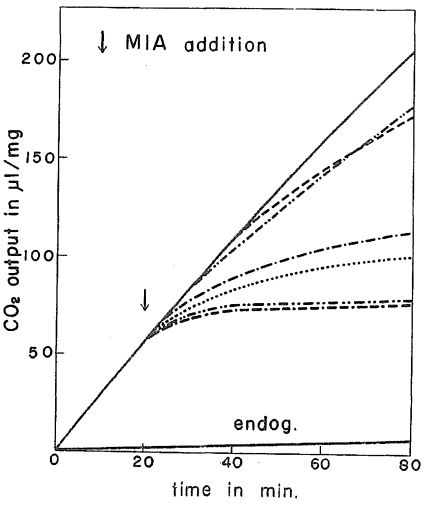

Fig. 21. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Schizosaccharomyces pombe*. The reaction conditions were the same as those for Fig. 17.

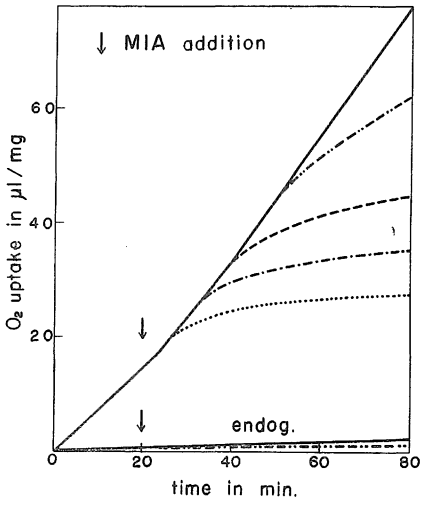

Fig. 22. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Saccharomyces cerevisiae*. The reaction conditions were the same as those for Fig. 16.

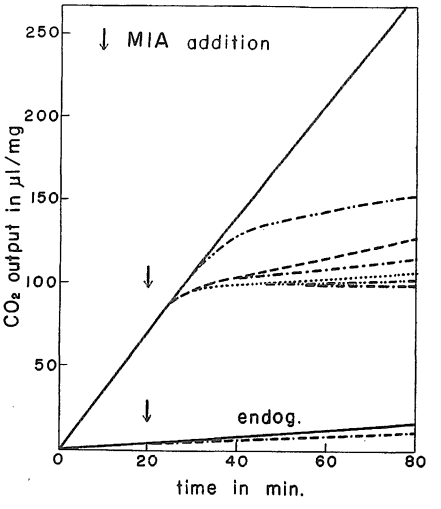

Fig. 23. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Saccharomyces cerevisiae*. The reaction conditions were the same as those for Fig. 17.

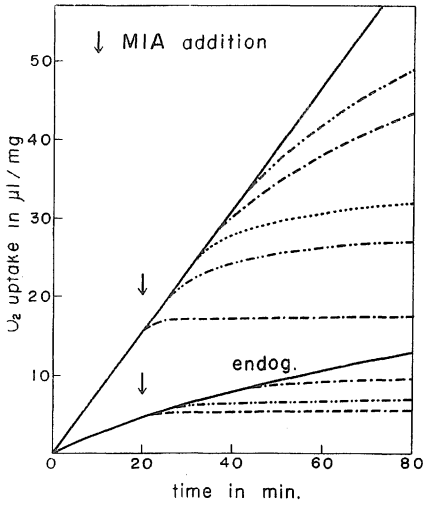

Fig. 24. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Saccharomyces sake*. The reaction conditions were the same as those for Fig. 16.

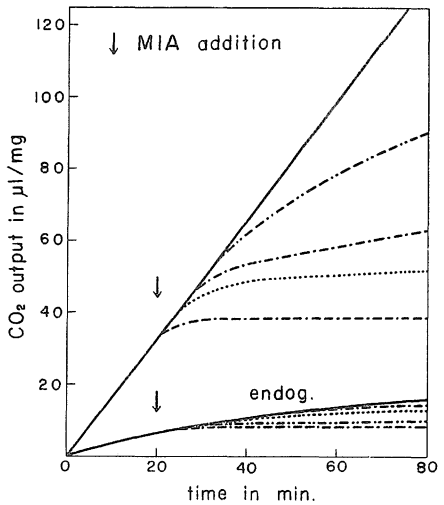

Fig. 25. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Saccharomyces sake*. The reaction conditions were the same as those for Fig. 17.

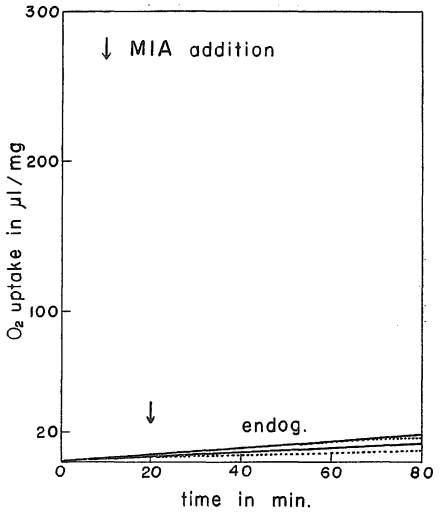

Fig. 26. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Saccharomyces carlsbergensis*. The reaction conditions were the same as those for Fig. 16.

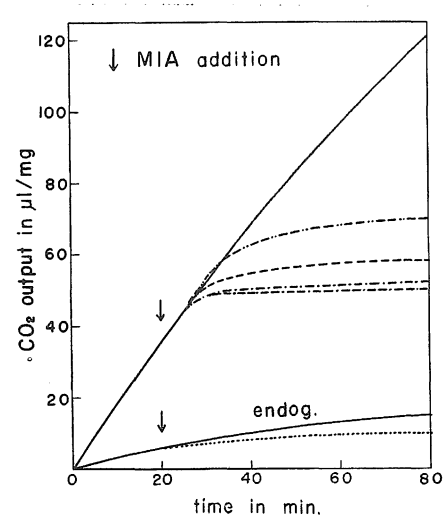

Fig. 27. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Saccharomyces carlsbergensis*. The reaction conditions were the same as those for Fig. 17.

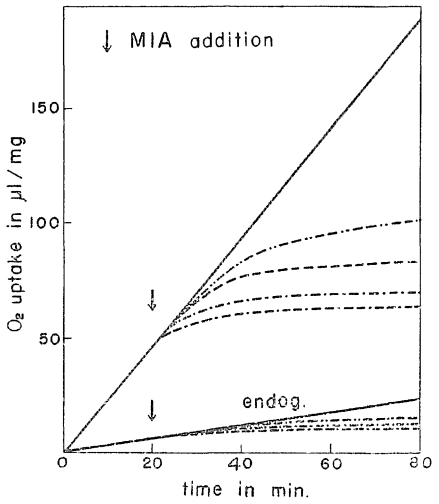

Fig. 28. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Mycoderma cerevisiae*. The reaction conditions were the same as those for Fig. 16.

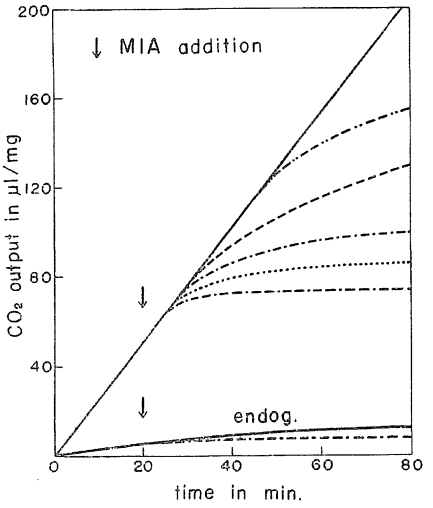

Fig. 29. The effect of monoiodoacetic acid on CO_2 output by washed resting cells of *Mycoderma cerevisiae*. The reaction conditions were the same as those for Fig. 17.

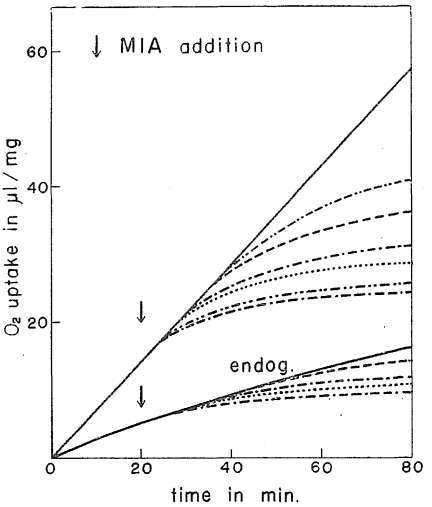

Fig. 30. The effect of monoiodoacetic acid on O_2 uptake by washed resting cells of *Torula candida*. The reaction conditions were the same as those for Fig. 16.

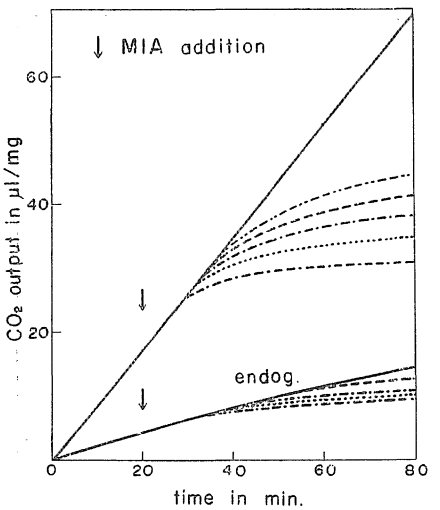

Fig. 31. The effect of monoiodoacetic scid on CO_2 output by washed resting cells of *Torula candida*. The reaction conditions were the same as those for Fig. 17.

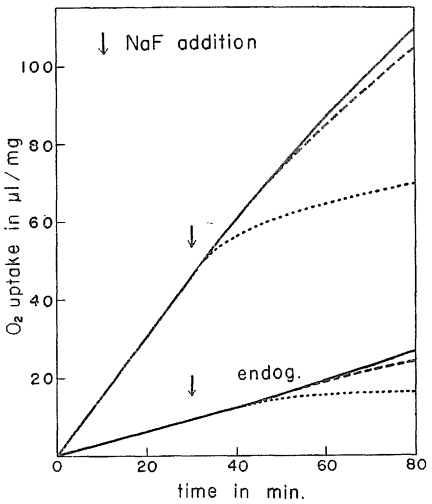

Fig. 32. The effect of NaF on glucose oxidizing activity of washed resting cells of *Rhodotorula glutinis*. The reaction conditions were the same as those for Fig. 16, except that NaF was used instead of monoiodoacetic acid.

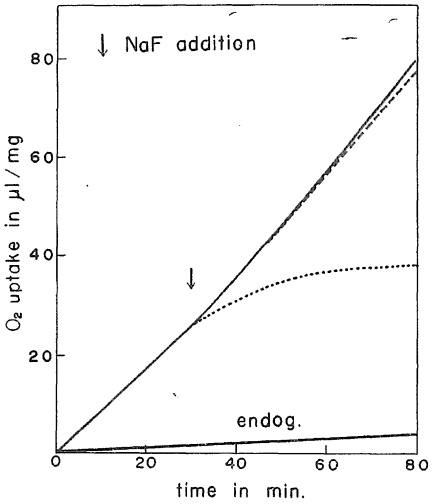

Fig. 33. The effect of NaF on glucose oxidizing activity of washed resting cells of *Saccharomyces cerevisiae*. The reaction conditions were the same as those for Fig. 32.

Concentration of added NaF (from Fig. 32 to Fig. 33): ——— non
- - - - - - - - - - 1×14^{-2}
..... 2×10^{-2}

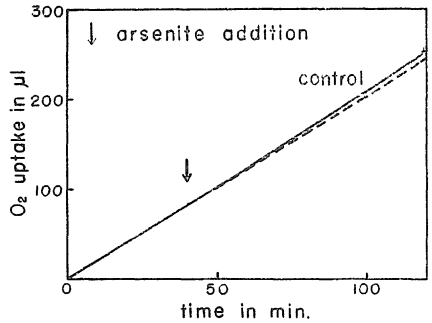

Fig. 34. The effect of arsenite on glucose oxidizing activity of washed resting cells of *Rhodotorula glutinis*. The reaction conditions were the same as those for Fig. 32, except that 5×10^{-4} M of arsenite was used instead of NaF.

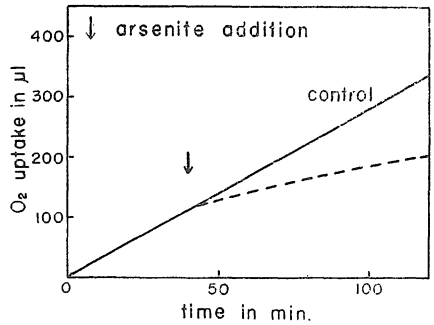

Fig. 35. The effect of arsenite on glucose oxidizing activity of washed resting cells of *Saccharomyces sake*. The reaction conditions were the same as those for Fig. 32, except that 5×10^{-4} M of arsenite was used instead of NaF.

(3) Inhibition by Arsenite.

In the test of respiratory inhibition by arsenite, *Rhodotorula glutinis* and *Saccharomyces sake* were used.

Rhodotorula glutinis.

Arsenite of 5×10^{-4} M inhibited the oxidation of glucose only slightly (Figure 34).

Saccharomyces sake.

On the contrary, this organism received severe inhibition by 5×10^{-4} M of arsenite (Figure 35).

IV. COMPARISON OF CYTOCHROME COMPONENTS IN YEASTS

(1) Microspectroscopic Study.

Components of cytochrome in eight kinds of yeasts were observed (Figure 36). Four kinds of yeasts which have comparatively low RQ values, viz., *Candida utilis*, *Mycoderma cerevisiae*, *Pichia membranaefaciens* and *Rhodotorula glutinis* showed the cytochrome a, b and c bands. On the contrary, only two kinds of yeasts showed the cytochrome a, b and c bands among the other four kinds of yeasts which have comparatively high RQ values, viz., *Schizosaccharomyces pombe*, *Saccharomyces sake*, *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. The band of cytochrome c appeared clearly in all tested yeasts. The band of cytochrome b appeared clearly in *Candida utilis*, *Mycoderma cerevisiae*, *Saccharomyces sake* and *Saccharomyces cerevisiae*. This band was shown relatively clearly on *Rhodotorula glutinis* and *Schizosaccharomyces pombe*. In *Pichia membranaefaciens* and *Saccharomyces carlsbergensis* this band was faint. Dense bands of cytochrome a were observed in *Candida utilis*, *Mycoderma cerevisiae* and *Saccharomyces cerevisiae*. This band was comparatively clear in *Rhodotorula glutinis* and *Saccharomyces sake*, but was quite dim in *Pichia membranaefaciens*. It could not be observed in *Schizosaccharomyces pombe* and *Saccharomyces carlsbergensis*. The β−bands of cytochromes were observed only on *Mycoderma cerevisiae* and *Saccharomyces cerevisiae*.

(2) Spectrophotometric Study.

Figure 37−44 show the absorption spectra of cytochromes in intact cells of eight kinds of yeasts. Among these yeasts there were some variations in types of spectra. However, all cytochromes a, b and c were found in each yeast.

In the case of *Candida utilis* the peaks at 604, 563 and 551 mμ are considered to be due to the cytochromes a, b and c respectively (Figure 37). It is suspected that the peak at 522 mμ is caused by the β−band of the cytochrome c. No peak caused by the β−band of the cytochrome b was found.

In *Mycoderma cerevisiae* cytochromes a, b and c were observed at 603, 562 and 548 mμ. Peaks at 525 and 520 mμ are considered to belong to the β−bands of the cytochromes c_1 and c respectively (Figure 38). In *Pichia membranaefaciens* the peaks caused by cytochromes a, b and c appeared at 603, 563 and 557 mμ respectively. Peaks at 525 and 521 mμ seem to be due to the β−bands of the cytochromes c_1 and c (Figure 39).

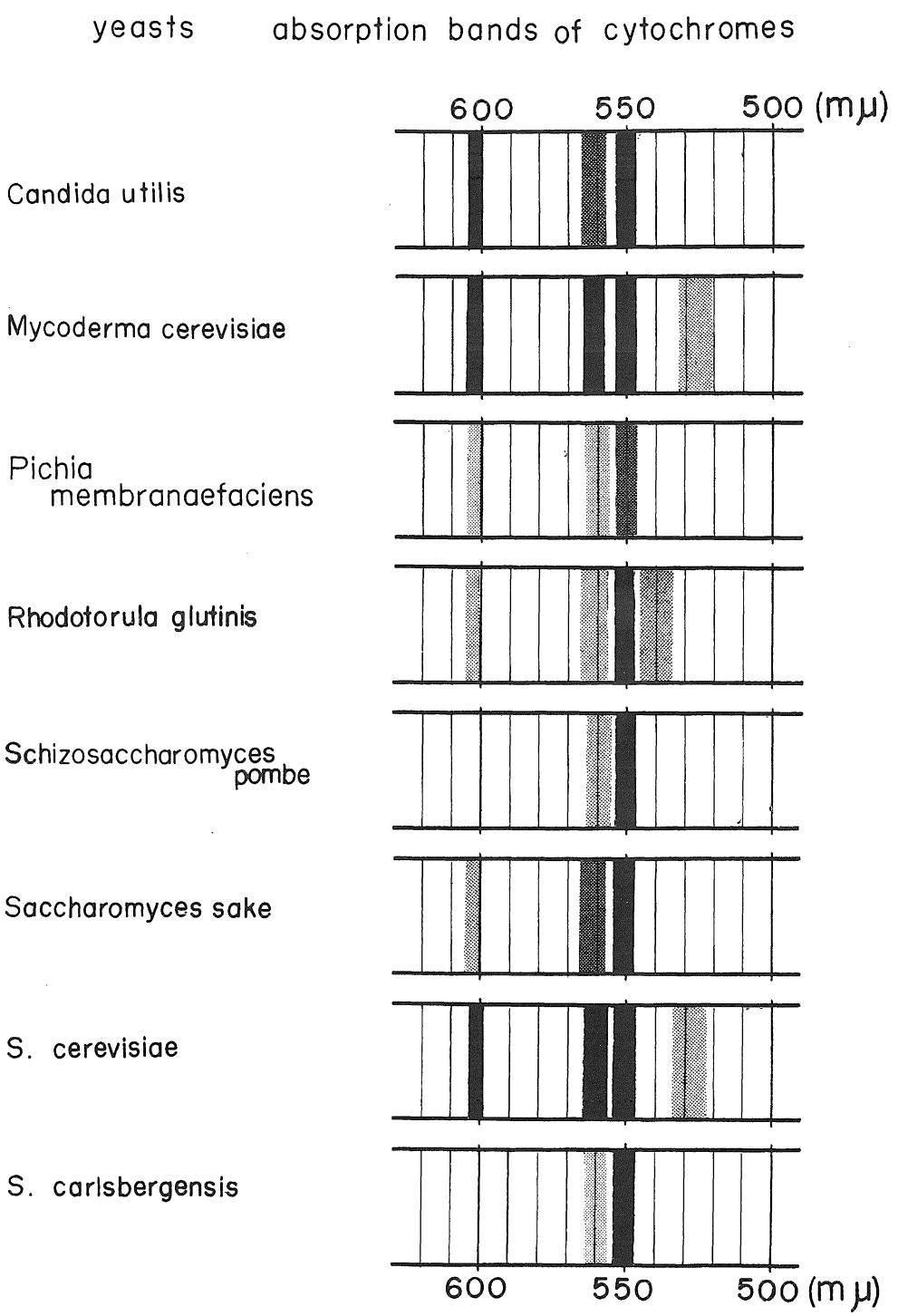

Fig. 36. Comparison of absorption bands of cytochromes. All observations were performed in anaerobic state on resting cell suspensions.

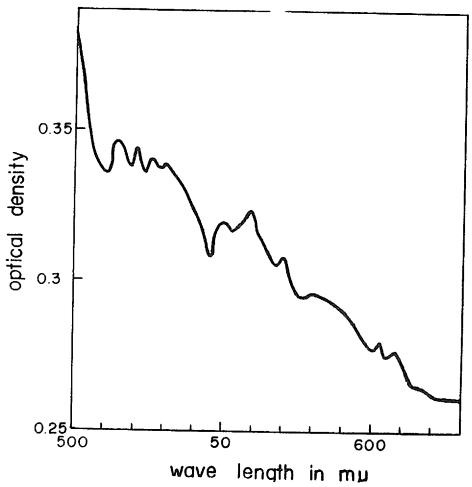

Fig. 37. Absorption spectra of resting cell suspension of *Candida utilis*. The suspension contains 34μg of nitrogen per ml. Sodium dithionite was used as a reductant.

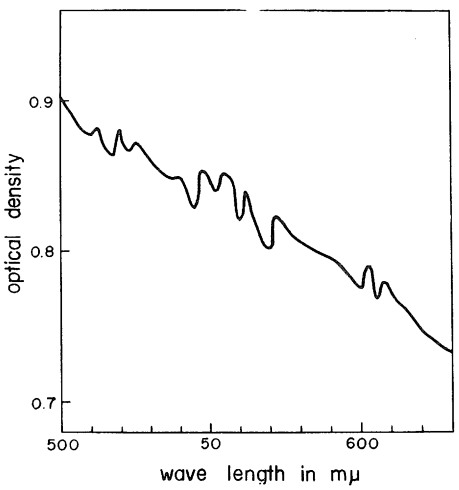

Fig. 38. Absorption spectra of resting cell suspension of *Mycoderma cerevisiae*. The suspension contains 9.5 μg of nitrogen per ml. Sodium dithionite was used as a reductant.

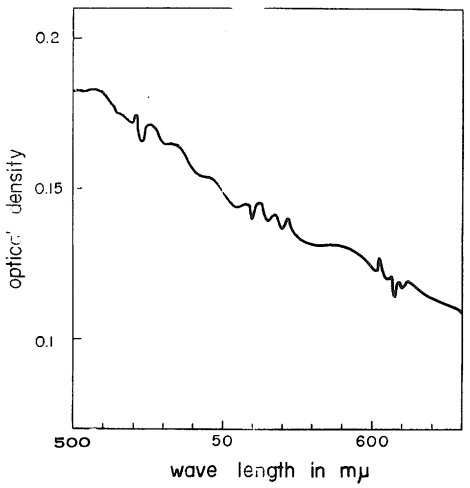

Fig. 39. Absorption spectra of resting cell suspension of *Pichia membranaefaciens*. The suspension contains 6.0 μg of nitrogen per ml. Sodium dithionite was used as a reductant.

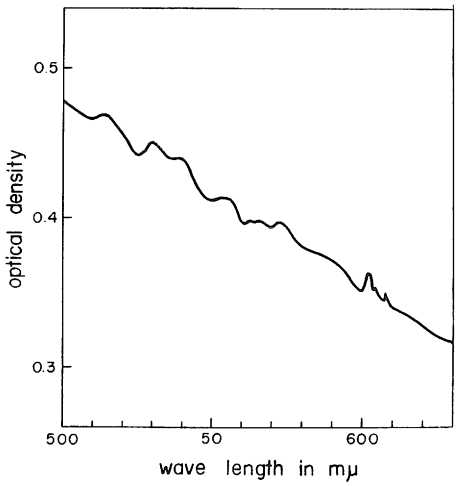

Fig. 40. Absorption spectra of resting cell suspension of *Rhodotorula glutinis*. The suspension contains 3.0μg of nitrogen per ml. Sodium dithionite was used as a reductant.

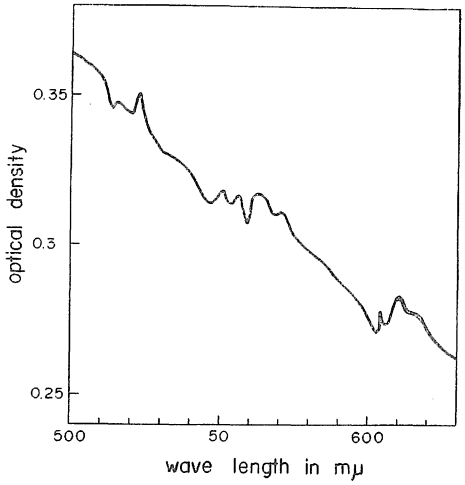

Fig. 41. Absorption spectra of resting cell suspension of *Schizosaccharomyces pombe*. The suspension contains 1.9 μg of nitrogen per ml. Sodium dithionite was used as a reductant.

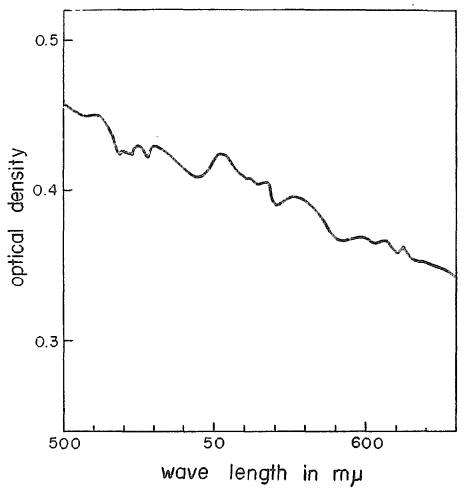

Fig. 42. Absorption spectra of resting cell suspension of *Saccharomyces sake*. The suspension contains 32 μg of nitrogen per ml. Sodium dithionite was used as a reductant.

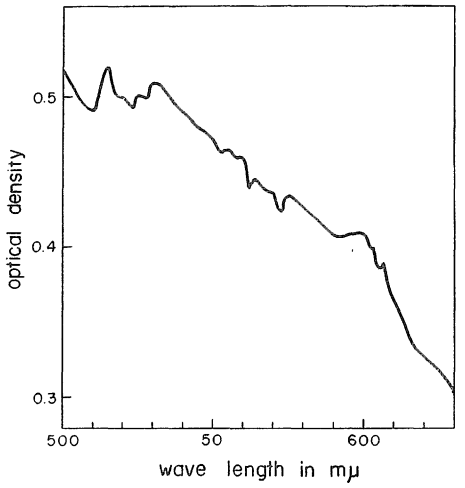

Fig. 43. Absorption spectra of resting cell suspension of *Saccharomyces cerevisiae*. The suspension contains 128μg of nitrogen per ml. Sodium dithionite was used as a reductant.

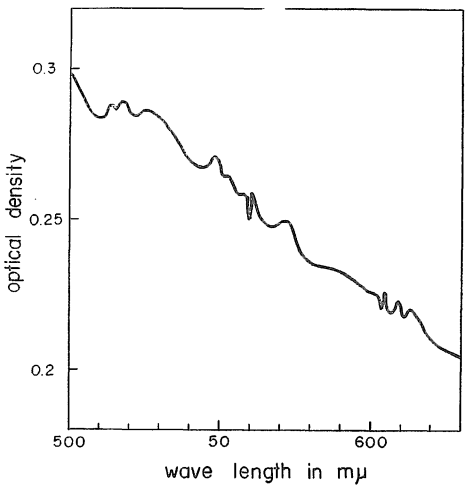

Fig. 44. Absorption spectra of resting cell suspension of *Saccharomyces carlsbergensis*. The suspension contains 8.0 μg of nitrogen per ml. Sodium dithionite was used as a reductant.

In *Rhodotorula glutinis* the peaks of the cytochromes a, b and c appeared at 603, 563 and 553 mμ respectively (Figure 40). A peak of β−band of the cytochrome b was observed clearly at 530 mμ. However, the peak of the β−band of cytochrome c was not detected. It is considered that the shoulder at 540 mμ is caused by rosy pigments included in this organism. This peak at 540 mμ was not observed in any other tested yeasts.

In *Schizosaccharomyces cerevisiae* the peaks of cytochromes a, b and c were found at 603, 559 and 550 mμ respectively (Figure 41). The β−bands of cytochromes b, c_1 and c were detected at 530, 525 and 520 mμ respectively.

In *Saccharomyces sake* the peaks at 600, 562 and 552 mμ are considered to have been induced by cytochromes a, b and c respectively (Figure 42). The peaks at 530, 525 and 520 mμ represent the β−bands of the cytochromes b, c_1 and c respectively.

In *Saccharomyces cerevisiae* the peaks at 600 and 564 mμ and the shoulder at 550 mμ are considered to be caused by cytochromes a, b and c respectiyely (Figure 43). The peaks and shoulder of the β−bands of cytochromes b, c_1 and c were observed at 530, 525 and 520 mμ respectively.

In *Saccharomyces carlsbergensis* the peaks at 605, 561 and 549 mμ and the shoulder at 552 mμ are considered to be due to the cytochromes a, b, c and c_1 (Figure 44). The peak at 525 mμ is considered to represent the β−band of the cytochrome c_1.

DISCCUSSION

A. OXIDATION OF GLUCOSE, ARABINOSE, ETHANOL, PYRUVATE, GLUCONATE AND THE TRICARBOXYLIC ACID CYCLE INTERNEDIATES.

In the past few years it was demonstrated by numerous investigators that various organisms utilize the hexose monophosphate (HMP) cycle in carbohydrate metabolism[23-33]. Holzer and Witt[27], Karasevich[28] and Imsenetskii[29] demonstrated the utilization of the HMP cycle by baker's yeast *Candida tropicalis* and genus *Torulopsis* respectively. Hence, it is evident that the yeasts possess the system of the HMP cycle for the catabolism of carbohydrate.

Among numerous strains of yeasts, however, some differences seem to exist in respect to the degree of utilization of the HMP cycle. The investigation reported here was performed with intact cells being employed for the most part, therefore, it will be necessary to study cell extract also in order to make the matter more certain. As for the results obtained here, it was observed that there are some differences among the yeasts in oxidative activity on various substrates as carbon source. Especially some interesting relationships were found between the oxidations of exogenously supplied glucose, gluconate, ethanol and the TCA cycle intermediates and the RQ values of yeast. More specifically, the intact cells of *Rhodotorula glutinis*, *Candida utilis* and *Schizosaccharomyces pombe* oxidized

glucose more actively than ethanol. On the contrary *Saccharomyces cerevisiae* and *Torula candida* oxidized ethanol more actively than glucose. Gluconate was utilized actively by the former, viz. *Rhodotorula glutinis* and *Candida utilis* and by *Mycoderma cerevisiae* which oxidized both glucose and ethanol at similar rate.

On the other hand, concerning oxidations of the TCA cycle intermediates, different activities were seen according to the strain of yeast. The oxidation of the TCA cycle intermediates, without citrate, was more active in *Candida utilis, Mycoderma cerevisiae, Pichia membranaefaciens* and *Rhodotorula glutinis.* However, in case of other yeasts, *Schizosaccharomyces pombe, Saccharomyces sake, Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis,* oxygen uptake increased only slightly as a result of the addition of the TCA cycle intermediates. The former four kinds of yeasts have comparatively low RQ values. Contrarily, the latter four kinds of yeasts have comparatively high RQ values. Citrate was oxidized actively only by *Candida utilis.* Consequently, the yeast which oxidized gluconate actively also utilized the HMP cycle intermediates. On the contrary the added TCA cycle intermediates were oxidized fairly well by the cell-free extract of *Saccharomyces cerevisiae* compared to endogenous oxidation. Concerning this problem, Barnett and Kornberg have expressed their opinion in a recent paper[6]. Probably the differences in oxidation between these yeasts may not be due only to the differences of enzyme activities in the yeast cells. Cell permeability may be concerned in this problem.

The oxidative activity on the TCA cycle intermediates in various concentrations was tested by washed resting cells of *Candida utilis* which utilized citrate most markedly among the eight strains of yeasts tested. The optimum concentration of oxidation of glucose by *Candida utilis* was comparatively low. This observation coincides with the result obtained by use of baker's yeast by Aldous[48]. However, on oxidation of four kinds of substrates of the TCA cycle intermediates, higher concentrations had to be used necessary to obtain active oxidation. These phenomena also support the opinion mentioned above that oxidative activity of the intact cells on the TCA cycle intermediates is restricted by the permeability of the cell surface.

B. PREINCUBATION EFFECTS

Non-treated *Rhodotorula glutinis* could scarcely oxidize ethanol, acetaldehyde and acetate. However, this ability was recovered by preincubation for about four hours. Ethanol, acetaldehyde and acetate were all effective as inducers. According to Haboucha and Masschelein[49] the causes of the lack of ability have been explained by the inactivity of aceto-CoA-kinase, cytochrome oxidase and succinic dehydrogenase. They have explaned this problem by mutant "petite colonie" of *Saccharomyces carlsbergensis. Rhodotorula glutinis* employed here is able to oxidize succinate. For this reason, the lack of ability of *Rhodotorula glutinis* in the oxidations of ethanol, acetaldehyde and acetate is considered to be caused by the decreased activity of aceto-CoA-kinase but not by lowering of activities of cytochrome oxidase and succinic dehydrogenase. For the restoration of the activity, the use of ethanol, acetaldehyde and acetate were all effective. Even in the presence of

chloramphenicol, at 200 γ per ml., together with the inducer, ethanol, the induced synthesis of the oxidation system of ethanol was not inhibited. This concentration of chloramphenicol is considerably higher than in the case of bacteria[50]. It has already been observed that in case of other inhibitors higher concentration was required for inhibiting the activity of enzymatic systems in yeasts[51].

C. EFFECTS OF RESPIRATORY INHIBITORS

It is certain that the mechanism of enzyme inhibition by monoiodoacetic acid has relationship with radical SH of apo-enzyme[52]. 3-Phosphoglyceraldehyde dehydrogenase, alcohol dehydrogenase, pyruvic carboxylase and succinic dehydrogenase are known to be inhibited by monoiodoacetic acid [39,53]. It is also widely accepted that sodium fluoride inhibits specifically the activity of enolase. Further, the inhibition of the EMP pathway by arsenite has been reported[23].

In general, yeast follows two courses in catabolizing glucose. These are the EMP pathway—the alcoholic fermentation or—the TCA cycle system and the HMP cycle system. The degree of utilization of these systems varies according to the strain of yeast. In the studies here reported, examination was made of the rate of decrease of oxidizing activity by monoiodoacetic acid, sodium fluoride and arsenite. Results of inhibition experiments are summarized in Table IV. It can be tentatively concluded that the yeast, the glucose respiration of which is severely inhibited by these substances, is utilizing the EMP pathway considerable extents during glucose breakdown.

The tested eight kinds of yeasts can be classified into two groups according to the type of inhibition by monoiodoacetic acid : a) *Rhodotorula glutinis*, *Candida utilis* and *Schizosaccharomyces pombe*, the respiration of which are comparatively insensitive to monoiodoacetic acid, and b) *Saccharomyces sake*, *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Mycoderma cerevisiae* and *Torula candida*, the respiration of which are sensitive monoiodoacetic acid inhibition.

Table IV *Rate of inhibition of oxidation of glucose by various inhibitors*

Yeasts	Inhibition rate (%) (Inhibitors : concentration in M)					
	MIA			NaF		Arsenite
	5×10^{-4}	2×10^{-3}	5×10^{-3}	10^{-2}	2×10^{-2}	5×10^{-4}
Rhodotorula glutinis	2	67	70	3	66	5
Candida utilis	19	49	81			
Schizosaccharomyces pombe	4	77	100			
Mycoderma cerevisiae	84	100	100			
Saccharomyces sake	46	57	100			71
Saccharomyces cerevisiae	54	92	97	5	96	
Saccharomyces carlsbergensis	60	100	100			
Torula candida	79	93	95			

In general, inhibition was intense in the cases of yeast with comparatively high RQ values. However, the latter two genera have comparatively low RQ values and suffered intense inhibition.

The test of inhibition by sodium fluoride was done with *Rhodotorula glutinis* which has comparatively low RQ values, and *Saccharomyces cerevisiae* which has comparatively high RQ values. The inhibition appeared more severely on *Saccharomyces cerevisiae.* The test of inhibition by arsenite was carried out with *Rhodotorula glutinis* which has comparatively low RQ values, and *Saccharomyces sake* which has comparatively high RQ values. The effect was heavier on *Saccharomyces sake.* Therefore, it seems that the inhibition by these three enzyme inhibitors is more effective in the case of yeasts which have comparatively high RQ values. It could be considered that probably in the cells of these yeasts which have comparatively high RQ values the EMP pathway — TCA cycle system is predominantly functioning.

D. CYTOCHROME COMPONENTS

Clear absorption bands of cytochrome c were observed in the case of all tested yeasts. From these results it is considered that all these yeasts have essentially an ability of respiration. This opinion was supported by the spectrophotometric study reported here. The strengths of absorption spectra caused by cytochromes are reported to be remarkably varied depending upon the stage and condition of growth[54]. Nevertheless, under the conditions reported, there were no essential differences in the type of absorption spectra of yeast.

Experimental results obtained may be summarized as follows : the tested nine kinds of yeasts are divided into at least two main groups. The first group includes *Rhodotorula glutinis, Mycoderma cerevisiae, Candida utilis* and *Pichia membranaefaciens.* The second group iucludes genus *Saccharomyces,* viz., *Saccharomyces sake, Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* and other two species, *Schizosaccharomyces pombe* and *Torula candida.*

The first group has the following characteristics : (1) comparatively low RQ values; (2) stronger activity of oxidation of glucose than that of ethanol; (3) the activity of oxidation of the TCA cycle members and gluconate ; (4) relatively low inhibition rate in glucose oxidation by monoiodoacetic acid, sodium fluoride and arsenite compared with the case of the second group. The second group has the following natures : (1) comparatively high RQ values cxcept Torula candida ; (2) stronger activity in respect to the oxidation of ethanol than that of glucose ; (3) negligibly small activity of oxidation of the TCA cycle members and unavailability of gluconate, except *Torula candida* ; (4) relatively high inhibition rate in glucose oxidation by monoiodoacetic acid, sodium fluoride and arsenite compared with the case of the first group.

Therefore, these findings seem to indicate that the first group of yeasts utilizes the members of the HMP and TCA cycles and acquires the energy by aerobic system. On the contrary, as to the second group of yeasts, the acquirement of energy is rather per-

formed by the fermentative system.

SUMMARY

Nine strains of yeasts in intact cells were investigated with respect to the oxidation of exogenously supplied substrates : glucose, arabinose, ethanol, pyruvate, gluconate and the TCA cycle intermediates and the effects of monoiodoacetic acid, sodium fluoride and arsenite on oxygen uptake and carbon dioxide output.

1. In general, yeasts which have comparatively low RQ values oxidized these above named exogenously supplied substrates more actively than the yeasts with comparatively high RQ.

2. The sensitivity to monoiodoacetic acid varies with the kind of yeast. The inhibition was small on *Rhodotorula glutinis* and *Candida utilis* which have comparatively low RQ values and scarcely ferment. Severe inhibition was observed on genera *Saccharomyces* which actively ferment alcohol. The highest inhibition, however, appeared on *Mycoderma cerevisiae* which has comparatively high RQ values and on *Torula candida* which has comparatively low RQ values.

3. Relatively high concentrations of sodium fluoride were needed to inhibit the respiration of yeast. The inhibition was less on *Rhodotorula glutinis* which has comparatively low RQ values. contrary to this, Saccharomyces cerevispae which has comparatively high RQ values is remarkably inhibited.

4. Similar effect was observed as a result of the addition of arsenite to yeasts. *Rhodotorula glutinis* was insignificantly inhibited. *Saccharomyces sake* which has comparatively high RQ values was greatly inhibited.

5. The results obtained by means of microspectroscopic study showed some variations of absorption bands of cytochromes. However, by the study using a spectrophotometer, it is found that there are no great differences among cytochrome components of yeast tested.

6. The variations in permeability of the intact cells and the decrease of enzyme activities may cause the differences in oxidation rate of exogenously supplied substrates and in sensibility against inhibitors.

7. The ability in respect to oxidation of ethanol in *Rhodotorula glutinis* was adaptively acquired by preincubation in either ethanol, acetaldehyde or acetate.

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