Location and Activity of the Respiratory Enzymes of Baker's Yeast and Brewer's Bottom Yeast Grown under Anaerobic and Aerobic Conditions

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SUMMARY

The activity of the electron-transport enzymes of baker's yeast or brewer's bottom yeast, grown under anaerobic conditions, was very low. When anaerobic baker's yeast was cultured aerobically to the mid-exponential phase with limited carbon source, the activity of the electron-transport enzymes increased 3- to 10-fold and, correspondingly, the activity in the stationary phase rose 10- to 50-fold. For brewer's bottom yeast the increase of activity induced by oxygen in the aerobic stationary phase was only about 3- to 4-fold and the activity was clearly lower than that of baker's yeast. The activity of the electron-transport enzymes accumulated in the 10,000 g sediment, which under aerobic conditions contained 60–80% of the total activity; the NADPH₂ oxidase system formed an exception. The activity of the enzymes of the citric acid cycle also increased under aerobic conditions but only 2- to 10-fold in baker's yeast of the aerobic stationary phase; in brewer's bottom yeast the increase during oxygen adaptation was proportionally greater. The bulk of the enzymes of the citric acid cycle were found in the postmitochondrial supernatant, while the 10,000 g sediment contained 20 to 40% of the total activity.

The 10,000 g sediment of anaerobically grown baker's yeast contained mitochondrial precursors, while the 10,000 g sediment from the aerobic exponential phase contained mitochondria with a more developed structure, showing a respiratory control ratio of 1.4–1.7 with several substrates. The internal structure of the mitochondria was not completely developed until the aerobic stationary phase, where the uptake of oxygen with several substrates also increased many fold.

INTRODUCTION

The existence of mitochondria in yeast cells has been clearly proved by electron microscopy (e.g. Vitols, North & Linnane, 1961) and by isolating mitochondria from yeast cells disrupted by mechanical means (e.g. Vitols & Linnane, 1961) or by enzymic methods (Duell, Inoue & Utter, 1964; Ohnishi, Kawaguchi & Hagihara, 1966). It is found that yeast grown anaerobically on glucose loses its capacity for oxidation (Tustanoff & Bartley, 1962). In the presence of oxygen and at a low glucose concentration respiration again takes place. Electron microscopy has revealed that besides the development of respiration, changes appear in the cell structure of the yeast, such as the formation of mitochondrial precursors and their further development to mitochondria (Yotsuyanagi, 1962; Wallace & Linnane, 1964; Polakis, Bartley & Meek, 1964). Alterations in respiratory particles and in various characteristic enzymic activities on release from glucose repression have been studied by Jayaraman, Cotman,

Mahler & Sharp (1966). We have investigated the activity of the electron-transport enzymes and the enzymes of the citric acid cycle in baker's yeast and brewer's bottom yeast grown under anaerobic and aerobic conditions, as well as their location in the yeast cell, using for cell fractionation the spheroplast method (Nurminen, Oura & Suomalainen, 1965; Suomalainen, Nurminen & Oura, 1967), by which it is possible to isolate subcellular particles in a much less damaged condition than when prepared by methods of mechanical disruption.

METHODS

Growth of yeast. Anaerobically cultured baker's yeast, stage R₃, produced at the Rajamäki Factories of the Finnish State Alcohol Monopoly and brewer's bottom yeast obtained from the brewery Sinebrychoff Ltd., Helsinki, were used as seed yeasts (0.3 g./l. of baker's yeast or 0.6 g./l. of brewer's bottom yeast). The growths were made in White medium (1954) at 30°. When the availability of the carbon source limited the growth, baker's yeast reached the mid-exponential phase after 10 hr, the yield being 6 g./l. under anaerobic and 10 g./l. under aerobic conditions. For the experiments on the aerobic stationary phase, baker's yeast was grown for 20 hr, the yield being 29 g./l. Brewer's bottom yeast took 17 hr to the exponential phase with a yield of 5 g./l., and 2 days to the aerobic stationary phase with a yield of 27 g./l. In aerobic growth 1 l. of air per minute and per litre was forced through the medium, and the anaerobic conditions during growth were obtained by passing a stream of oxygen-free nitrogen from which the traces of oxygen had been removed by alkaline pyrogallol through the growth medium. After growth, the yeast was centrifuged and washed several times with cold water, and used immediately for preparation of spheroplasts. From the anaerobic yeasts the spheroplasts were prepared by using the shortest possible time of digestion in a tightly closed vessel in order to avoid adaptation to respiration.

Preparation of spheroplasts. The yeast cells were suspended to a 10% suspension in a digestion medium containing 1.0 M-sorbitol, 0.01 M-MgSO₄, 0.01 M-tris-HCl, pH 7.5. 'Suc digestif d'*Helix pomatia*' (L'Industrie Biologique Française, Gennevilliers, Seine, France) was centrifuged at 3000 g for 15 min. and 5% (v/v) of this enzyme solution was added to the suspension, which was then incubated at 30° for the time reported. For yeast cells in the aerobic stationary phase 2-mercaptoethanol treatment was used before digestion. In this procedure the yeast was suspended to 10% in the digestion medium, 0.01 M-2-mercaptoethanol was added and the suspension incubated at room temperature for 30 min., after which the yeast was washed by centrifugation 3 times with cold digestion medium, and the digestion proceeded as above. The formation of spheroplasts was studied by phase-contrast microscopy and by measuring the extinction (Nurminen *et al.* 1965) as well as by determining, in connexion with fractionation, the amount of protein released into the 1000 g supernatant. Thus, the anaerobic and aerobic log.-phase yeast cells were digested for 60 and 90 minutes respectively and the yeast in the aerobic stationary phase, after pretreatment with 2-mercaptoethanol, for 3 hr. Instead of true protoplasts, spheroplasts, i.e. osmotically sensitive, digested cells with some cell wall remaining, were partly used in order to protect the inside of the cell against the digestion liquid.

Fractionation. The spheroplasts were centrifuged at 1000 g for 10 min. and washed twice by centrifugation with cold digestion medium. The fractionation was essentially

accomplished to the method of Duell *et al.* (1964). The spheroplasts were suspended in a hypotonic medium (medium A), containing 0.25 M-sucrose, 0.02 M-potassium phosphate buffer (pH 6.8) and 0.001 M-EDTA and, thus, lysis of the spheroplasts was caused by the osmotic shock. The suspension was homogenized for 30 sec. in a Potter-Elvehjem tissue homogenizer. The remaining whole cells, the nuclei and cell debris were removed by centrifugation at 1000 *g* for 10 min. It was possible to increase the yield in the 1000 *g* supernatant by repeated suspension of the 1000 *g* sediment in medium A, followed by slight homogenizing and centrifuging at 1000 *g* and, in some cases, this method was used. In order to prepare the mitochondrial fraction or the corresponding anaerobic sediment, the 1000 *g* supernatant was centrifuged at 10,000 *g* for 10 min. The 10,000 *g* sediment obtained was also washed by centrifugation, usually twice, by suspending in a medium, containing 20 % sucrose, 0.02 M-potassium phosphate buffer (pH 6.8) and 0.001 M-EDTA. When necessary on the basis of phase-contrast microscopy, the debris of the 1000 *g* sediment was removed by repeating the 1000 *g* centrifugation. The washings of sediments were combined with the corresponding supernatants.

Analytical methods. The protein was determined by the biuret method (Racusen & Johnstone, 1961) with crystalline serum albumin as reference. The dry matter of the yeast was determined gravimetrically.

Determination of the enzyme activities. The fractions isolated for enzyme determinations were kept overnight in 1 to 2 ml. lots (about 20 mg. protein per ml.) at -20° and only thawed once. However, cytochrome *c* oxidase and succinate cytochrome *c* reductase were measured immediately after fractionation, since their activity was found to decrease during storage. The enzyme activities of the seed yeast were determined in the homogenate obtained by shaking in a Mickle disintegrator with Ballotini beads for 30 min. at 4° .

The activity of cytochrome *c* oxidase was measured by observing the rate of enzymatic oxidation for cytochrome *c* (Cooperstein & Lazarow, 1951; Yonetani & Ray, 1965) reduced by the method of Chantrenne (1955). NADH₂ cytochrome *c* reductase and succinate cytochrome *c* reductase were determined by registering the reduction of cytochrome *c* in a system where cytochrome *c* oxidase was inhibited with cyanide (Green & Ziegler, 1963). The NADH₂ oxidase system was examined by the method of Green & Ziegler (1963) and the NADPH₂ oxidase system correspondingly, with NADPH₂ as substrate. The activities of the primary NADH₂ dehydrogenase and succinate dehydrogenase were determined by using potassium ferricyanide as electron acceptor in the presence of cyanide (Rabinowitz & DeBernard, 1957). NAD-malic dehydrogenase was determined according to Ochoa (1955), NADP- and NAD-iso-citrate dehydrogenase according to Kornberg (1955*a, b*), and fumarase (Racker, 1950; Massey, 1955) and aconitase (Racker, 1950; Anfinsen, 1955) according to Racker. The enzyme activities were determined spectrophotometrically at 25° by following the changes of extinction at 15-second intervals. The references were measured without the sample or without the substrate.

Measurement of oxygen consumption. The uptake of oxygen in some substrates was measured polarographically at 25° , using vibrating platinum electrode (Oxygraph, Gilson Medical Electronics, Middleton, Wisconsin, U.S.A.). The reaction medium contained 250 mM-sucrose, 20 mM-potassium phosphate buffer (pH 6.8), 1 mM-EDTA and 10 mM-KCl. Succinate, α -ketoglutarate, isocitrate, citrate, ethanol or lactate was

used as substrate in a concentration of 10 mM; for pyruvate and malate the concentration was 5 mM, and for NADH₂ 0.5 mM. The mitochondrial concentration in the assay was 0.14 to 1.25 mg. protein per ml. of reaction mixture on a total volume of 2.5 ml.

Electron microscopy. After fixation with osmium tetroxide the samples were dehydrated with successive alcohol concentrations and propylene oxide before being embedded in epoxy resin. Electron micrographs of thin sections were taken with a Siemens Elmiskop I or a Philips EM 200 electron microscope.

RESULTS AND DISCUSSION

Electron-transport enzymes. The specific activities of the electron-transport enzymes in homogenates and subcellular fractions of anaerobically and aerobically grown baker's yeast and brewer's bottom yeast are presented in Table 1. The activity of the electron-transport enzymes is very low under anaerobic conditions in both baker's yeast and brewer's bottom yeast. When baker's yeast had grown aerobically under conditions when the carbon source was limited until the middle exponential phase, the activity of the electron-transport enzymes in the cell homogenates had increased 3- to 10-fold, and reached its maximum, 10- to 50-fold, in the aerobic stationary phase. A similar, although clearly lower, oxygen-induced increase of activity was observed in brewer's bottom yeast, being in the aerobic stationary phase only 3- to 4-fold.

NADH₂ cytochrome *c* reductase was usually more active than succinate cytochrome *c* reductase. The same phenomenon was observed with the corresponding primary dehydrogenases when potassium ferricyanide was used as electron acceptor.

The activity of NADPH₂ oxidase in aerobic log.-phase cells of baker's yeast was only a fifth of that of NADH₂ oxidase, and the activities also differed in other ways. The NADPH₂ oxidase activity was essentially the same in baker's yeast grown to the exponential phase under both anaerobic and aerobic conditions and only 10 to 18 % of the total activity was found in the 10,000 g sediment. According to Schatz & Klima (1964), the NADPH₂ oxidase system in yeast is associated with the microsomal fraction, but NADPH₂ oxidase activity is also found in the mitochondrial fraction (Schuurmans Stekhoven, 1966).

By electron microscopy it has been established that the presence of oxygen is necessary for the synthesis of mitochondria in yeast (Linnane, Vitols & Nowland, 1962; Wallace & Linnane, 1964), at least when glucose is used as carbon source. Studies on the enzyme activities in homogenates and electron micrographs of whole cells have revealed that glucose in high concentration represses the synthesis of respiratory enzymes and the development of mitochondria (Yotsuyanagi, 1962; Polakis, Bartley & Meek, 1964). This is in agreement with the observation that the activity of the electron-transport enzymes increased also in the log.-phase cells during aerobic growth on a medium containing a lowered concentration of glucose (Table 1). The greatest proportional increase occurred in the activity of succinate cytochrome *c* reductase which, according to Schatz (1963), is very sensitive to conditions inhibiting the development of mitochondria and is not to be found in the mitochondrial precursors.

In all the fractionations, the specific activity of the electron-transport enzymes was highest in the 10,000 g sediment (Table 1), and depended on the growth conditions in

Table 1. *Specific activities of some electron-transport enzymes in homogenates and subcellular fractions of baker's yeast and brewer's bottom yeast, grown under varying conditions*

	Cytochrome <i>c</i> oxidase	Succinate cytochrome <i>c</i> reductase	NADH ₂ cytochrome <i>c</i> reductase	NADH ₂ oxidase
Baker's yeast				
Seed yeast homogenate	0.003*	0.004	0.003	0.012
Anaerobic culture				
Homogenate	0.004	0.001	0.012	0.019
10,000 g sediment	0.014	0.005	0.046	0.104
10,000 g supernatant	0.000	0.001	0.006	0.011
Aerobic, middle exponential phase†				
Homogenate	0.042	0.005	0.038	0.066
10,000 g sediment	0.139	0.022	0.140	0.176
10,000 g supernatant	0.010	0.002	0.010	0.024
Aerobic, exponential phase‡				
Homogenate	0.042	0.025	0.054	0.054
10,000 g sediment	0.118	0.123	0.660	0.400
10,000 g supernatant	0.026	0.004	0.051	0.055
Aerobic, stationary phase				
Homogenate	0.205	0.028	0.226	0.174
10,000 g sediment	1.095	0.245	1.905	1.029
10,000 g supernatant	0.111	0.011	0.095	0.097
Brewer's bottom yeast				
Seed yeast homogenate	0.008	0.000	0.005	0.005
Anaerobic culture				
Homogenate	0.023	0.005	0.007	—
10,000 g sediment	0.067	0.017	0.011	—
10,000 g supernatant	0.002	0.000	0.004	—
Aerobic, exponential phase				
Homogenate	0.044	0.008	0.004	—
10,000 g sediment	0.229	0.085	0.008	—
10,000 g supernatant	0.028	0.000	0.006	—
Aerobic, stationary phase				
Homogenate	0.078	0.016	0.022	0.037
10,000 g sediment	0.100	0.018	0.051	0.051
10,000 g supernatant	0.058	0.014	0.021	0.024

* Specific activities expressed as μ moles of transformed substrate or electron acceptor per minute per mg. protein. 5 % (initial conc.) sucrose used as carbon source.

† Actual carbon source concentration about 2 %.

‡ Actual carbon source concentration about 0.6 %.

the same way as in the homogenates. Table 2 shows the distribution of the total activity in aerobically grown baker's yeast cells. The 10,000 g sediment contained 70–80 % of the total activity of cytochrome *c* oxidase, succinate cytochrome *c* reductase and NADH₂ cytochrome *c* reductase and about 60 % of the total activity of the NADH₂ oxidase. The latter could be inhibited by addition of antimycin A to 90 % in the homogenate and completely in the 10,000 g sediment of log-phase cells. Hence 70 % of the NADH₂ oxidase activity, which was inhibited by antimycin A, was located in the 10,000 g sediment. The electron-transport enzymes investigated are found to be typical of electron-transport particles isolated from yeast by mechanical disintegration (Biggs & Linnane, 1963; Mackler *et al.* 1962; Mahler, Mackler, Grandchamp &

Slonimski, 1964) and thus also of mitochondria. By centrifugation of homogenate of mechanically disrupted baker's yeast cells at 26,300 *g* for 15 min., Schatz, Tuppy & Klima (1963) obtained a sediment from which they have isolated a mitochondrial fraction and which contained 89 % of the total activity of NADH₂ oxidase and 88 % of that of cytochrome *c* oxidase. Duell *et al.* (1964) have reported that the mitochondrial fraction separated from yeast by means of the spheroplast method (at a noticeably lower *g* value, 5000 *g* for 20 min.) contains almost all the cytochrome *c* oxidase activity. The conclusion can be drawn that the danger of damaging the mitochondria is markedly smaller when the spheroplast method is used than with methods of mechanical disintegration. Further, the recovery of the electron-transport enzymes, found in the fractions of the log.-phase cells, corresponded to 90 to 110 % of the total activity of the homogenate before fractionation. Thus, the activity of the electron transport enzymes found in the post-mitochondrial supernatant apparently does not originate from mitochondria but from other parts of the cell, perhaps from mitochondrial precursors or other membraneous parts.

Table 2. *Distribution of total activities of some electron-transport enzymes and enzymes of the citric acid cycle in aerobically grown baker's yeast*

	Aerobic baker's yeast							
	Middle exponential phase 10,000 <i>g</i>				Stationary phase 10,000 <i>g</i>			
	Supernatant		Sediment		Supernatant		Sediment	
	Total activity*	%†	Total activity*	%†	Total activity*	%†	Total activity*	%†
Cytochrome <i>c</i> oxidase	5.5	24	17.4	76	12.3	27	32.4	73
Succinate cytochrome <i>c</i> reductase	0.7	18	3.1	82	1.6	30	3.7	70
NADH ₂ cytochrome <i>c</i> reductase	5.2	27	13.8	73	12	26	35	74
NADH ₂ oxidase	12.8	38	20.7	62	12	40	18	60
NAD-malic dehydrogenase	54	70	23	30	233	74	80	26
NADP-isocitrate dehydrogenase	2.6	53	2.3	47	7.0	79	1.9	21
Aconitase	5.8	62	3.6	38	26	76	8	24
Fumarase	9.6	60	6.3	40	—	—	—	—

* Specific activity multiplied with the amount of protein in the fraction.

† Distribution of total activity between the 10,000 *g* supernatant and 10,000 *g* sediment as a percentage of the sum.

Enzymes of the citric acid cycle. The specific activities of the enzymes of the citric acid cycle found in homogenates and fractions of anaerobically and aerobically grown baker's yeast and brewer's bottom yeast are shown in Table 3. The activities of these enzymes were also observed to increase on transfer from anaerobic growth conditions to the aerobic stationary phase, but here the differences relating to the growth conditions were smaller than those occurring in the electron-transport enzymes. Thus, NAD-malic dehydrogenase, NADP-isocitrate dehydrogenase, aconitase and fumarase were of the same order of magnitude in anaerobically as well as in aerobically grown log.-phase cells of baker's yeast. The reduction of the glucose concentration only partly increased the activities in the aerobic exponential phase. The specific activity of NAD-

malic dehydrogenase increased to 10-fold, while NADP-isocitrate dehydrogenase and aconitase rose only to 2-fold in aerobically grown baker's yeast in the stationary phase. In brewer's bottom yeast the corresponding increase of activity was proportionally larger because of the very low activity of the enzymes of the citric acid cycle in anaerobically grown brewer's bottom yeast.

Table 3. *Specific activities of some enzymes of the citric acid cycle in homogenates and subcellular fractions of baker's yeast and brewer's bottom yeast, grown under varying conditions*

	NAD-malic dehydro- genase	NADP- isocitrate dehydro- genase	Aconitase	Fumarase
Baker's yeast				
Seed yeast homogenate	0.167*	0.010	0.035	—
Anaerobic culture				
Homogenate	0.126	0.018	0.134	0.227
10,000 g sediment	0.852	0.096	0.140	0.454
10,000 g supernatant	0.063	0.013	0.072	0.089
Aerobic, middle exponential phase†				
Homogenate	0.141	0.018	0.078	0.197
10,000 g sediment	0.350	0.094	0.234	0.836
10,000 g supernatant	0.099	0.007	0.048	0.118
Aerobic, exponential phase‡				
Homogenate	0.994	0.036	0.085	—
10,000 g sediment	1.666	0.033	0.200	—
10,000 g supernatant	0.853	0.037	0.061	—
Aerobic, stationary phase				
Homogenate	1.424	0.039	0.327	—
10,000 g sediment	5.114	0.145	4.728	—
10,000 g supernatant	1.126	0.031	0.200	—
Brewer's bottom yeast				
Seed yeast homogenate	0.135	0.006	0.043	—
Anaerobic culture				
Homogenate	0.028	0.008	0.008	—
10,000 g sediment	0.033	0.013	0.045	—
Aerobic, exponential phase				
Homogenate	0.027	0.006	0.026	—
10,000 g sediment	0.015	0.015	0.050	—
Aerobic, stationary phase				
Homogenate	1.451	0.023	0.066	—
10,000 g sediment	2.560	0.042	0.125	—
10,000 g supernatant	0.468	0.007	0.014	—

* Specific activities expressed as μ moles of transformed substrate per minute per mg. protein. 5 % (initial conc.) sucrose used as carbon source.

† Actual carbon source concentration about 2 %.

‡ Actual carbon source concentration about 0.6 %.

It is interesting to note the considerable activity of the enzymes of the citric acid cycle in baker's yeast, occurring even under anaerobic growth conditions with low activity of electron-transport enzymes and no developed mitochondria. Similar conclusions have been reached by Jayaraman *et al.* (1966) from studies on the glucose repression. They found that the NADH₂ oxidase is subject to strong repression while

mitochondrial malic dehydrogenase appears less sensitive. Polakis *et al.* (1965) suggest as a possible explanation that enzymes of the citric acid cycle are necessary for amino acid and protein synthesis even under conditions where mitochondria and electron-transport enzymes are not needed for energy production because of the abundant energy supply from glycolysis.

NAD-malic dehydrogenase was observed to be the most active of the enzymes of the citric acid cycle, which apparently means participation in the regulatory mechanisms. NAD-isocitrate dehydrogenase was present in baker's yeast in smaller amounts than the NADP-linked enzyme; under anaerobic conditions the specific activity was 0.002 in the homogenate and 0.008 in the 10,000 g sediment. The specific activity of the enzymes of the citric acid cycle reached a maximum in the 10,000 g sediment and the activity depended on the growth conditions in the same way as in the homogenate. Contrary to the electron-transport enzymes, the bulk of the total activity of the enzymes of the citric acid cycle was found in the post-mitochondrial supernatant, while the 10,000 g sediment contained 20–40 % (Table 2). Schatz *et al.* (1963) have reported that the sediment obtained by centrifugation of baker's yeast homogenate at 26,300 g for 15 min. contained 14 % of the total fumarase activity, 13 % of the aconitase and 8 % of the NAD-malic dehydrogenase activities. A similar result was obtained by us as regards the distribution of the above enzymes in the mitochondrial and cytoplasmic fractions, although the portion of the 10,000 g sediment was clearly larger. Duell *et al.* (1964) have isolated the mitochondrial fraction of yeast by means of the spheroplast method. They found that the fraction contained about 26 % of the NAD-malic dehydrogenase activity, while the bulk appeared in the post-mitochondrial supernatant, an observation that agrees well with our results. The location of isocitrate dehydrogenase in the yeast cell has not previously been investigated. In assays on animal cells it has been found that the soluble fraction contained 80 % of the NADP-linked enzyme, while the mitochondrial fraction contains only 12 % (Hogeboom & Schneider, 1950). In yeast, NADP-isocitrate dehydrogenase was likewise found mainly in the cytoplasmic fraction (Table 2). The enzymes of the citric acid cycle and the electron-transport enzymes had a different intracellular distribution and their activities depended on the growth conditions in different ways. The enzymes of the citric acid cycle have a synthetic function beside their role in energy production and are for the most part soluble. The synthesis of these enzymes is not as sensitive to glucose repression and oxygen induction as is the synthesis of the enzymes bound in the lipoprotein structures of mitochondria.

Oxidative properties and structure of the particles of the 10,000 g sediment. Particularly interesting are the properties of the particles of the 10,000 g sediment obtained from log.-phase yeast cells grown aerobically under glucose repression. The oxidative properties of the 10,000 g sediments prepared from middle log.-phase cells and from cells harvested during the stationary phase were compared (Table 4). As was previously observed in enzyme determinations, the uptake of oxygen when NADH₂ was used as substrate was markedly higher than when succinate was used. With NADH₂ the uptake of oxygen in the 10,000 g sediment obtained from the stationary phase was about 10-fold as compared with the uptake in the exponential phase. With succinate as substrate the corresponding increase was about 8-fold. The exogenous NADH₂ cannot permeate into the animal mitochondria if these are intact, while the yeast mitochondria are able to rapidly oxidize NADH₂, with a clear respiratory control

(Ohnishi *et al.* 1966). Even on ethanol or lactate as substrate, the uptake of oxygen in the stationary phase rose about 3-fold. However, it is to be noted that in several substrates the particles obtained at the exponential phase already had a respiratory control ratio of 1.4 to 1.7.

Table 4. *Oxidative properties of the 10,000 g sediment isolated from baker's yeast at different aerobic growth phases*

Substrate	Aerobic baker's yeast		
	Middle exponential phase 10,000 g sediment		Stationary phase 10,000 g sediment
	Oxidative rate*	RCR†	Oxidative rate
NADH ₂	0.041	1.4	0.400
Succinate	0.008	1.4	0.062
Pyruvate + malate	0.034	1.4	0.040
Citrate	0.039	1.5	0.050
Isocitrate	0.013	1.7	0.014
α -Ketoglutarate	0.016	1.7	0.034
Lactate	0.064‡	1.4	0.208
Ethanol	0.033	1.0	0.101

* μ atoms oxygen/min./mg. protein.

† Respiratory control ratio, rate with ADP/rate without ADP.

‡ When 5 μ g./ml. antimycin A was added, the oxidative rate was 0.047. On the other hand, antimycin A completely inhibited oxygen uptake with NADH₂, succinate, pyruvate + malate or citrate as substrate, whether the sample was taken from the exponential or stationary phase.

As far as the mitochondria synthesis of *Saccharomyces cerevisiae* is known on the basis of electron micrographs Yotsuyanagi, 1962 and Wallace & Linnane, 1964 showed that the 10,000 g sediment isolated from baker's yeast cells by the spheroplast method contained mitochondrial precursors of different stages when the yeast had been grown under anaerobic conditions. At the aerobic exponential phase the cells contained, in addition to the precursors, further developed mitochondria (Pl. I, fig. 1). The mitochondria were not completely developed until the cells had reached the aerobic stationary phase. So the structure of the isolated particles correlates with what has been observed as regards the activities of the respiratory enzymes during oxygen adaptation under carbon source limited growth.

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EXPLANATION OF PLATE

Fig. 1. 10,000 g sediment isolated from baker's yeast cells grown aerobically to the mid-exponential phase. $\times 10,000$.