its combination of high solubility and blue color in solution. It was important here to achieve smooth density variation in the fluid, for sharp boundaries could lead to separate layers of the liquid spinning down independently, as mentioned above. To effect a smooth density distribution, cupric nitrate solution of specific gravity 1.20 was placed in the crystallizing dish; water was poured on cautiously, initially forming a sharp interface with the dense blue solution. Careful stirring yielded a smooth color gradient, after which four specifically prepared floats of densities 1.05, 1.10, 1.15, and 1.20 could be placed in the solution to give quantitative information on the density distribution. Typically, the distribution had a linear z-dependence, ranging from 1.20 g/cm^3 at $z = 0$, to 1.00 g/cm^3 at $z = 5$ cm.

Figure 1 shows angular displacements relative to the container plotted against the time for surface floats on two types of liquids: (i) density stratified $Cu(NO_3)_2$ solution; (ii) distilled water. In both cases the liquid was allowed half an hour to assume the container's rotational speed. At $t = 0$, a change of 0.21 rev/min was made in rotational speed (for the particular observation chosen to represent type i, the speed change was positive, while that for type ii was negative).

Fourteen observations on density-stratified liquids were made; seven each for increased and decreased container rotational speeds. Angular displacements per minute, $\Delta\theta$, as described earlier, were plotted against time on semilog coordinates. It was found that after the liquid had been rotated for about 2 minutes, the expected purely exponential decrease in surface velocities did set in. Exponential decay was seen for approximately 15 minutes, after which surface velocities were too low to be measured accurately. The e-folding times for the linear portions of the semilog curves in the cases of decreased rotational speed of the container averaged 342 seconds, whereas those for increased rotation averaged 394 seconds. The average of these is 368 seconds, which is to be compared with the 266-second decay time found in Eq. 5.

The difference in the first two decay times can be understood qualitatively. As noted above, for stratified fluids the rotational speed and density distributions are interconnected. Thus, after initial transient circulatory currents have ceased, the distribution of rotation will be dependent upon both the initial density distribution and the change in angular velocity. The rotational distribution will generally not be that of a normal decay mode and will be different in the two cases of spin-up and spin-down.

Three liquids of constant density [including a constant-density $Cu(NO_3)_2$ solution] were observed, all giving results of the type presented in Fig. 1 (lower curve). Surface velocities for these liquids depart grossly from decay mode (*3*). Damping was much stronger than for the stratified fluids investigated: final angular displacements of surface floats following equal changes in container rotation were about ten times greater for stratified fluids than for constant-density fluids. One is led to conclude from this great difference in damping rates that the occurrence of Ekman pumping and the ensuing "spin-down" in a fluid can indeed be inhibited or eliminated by a sufficient density gradient in the fluid. When these observations are related to our initial discussion of hydrodynamic processes within the sun, our experiments suggest that the density stratification in the solar interior (*7*) should preclude "spin-down" damping of differential rotation.

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25 September 1967

Biochemical Genetics of Oxidative Phosphorylation

Abstract. *The usefulness of mutants in the unraveling of complex, highly organized, membrane-bound processes such as oxidative phosphorylation is illustrated by a study of a single recessive gene mutation in yeast, designated* op_1, *which has abolished the efficiency in vivo and in vitro of oxidative phosphorylation without impairing the electron transfer.*

During the last two decades oxidative phosphorylation has been studied by a variety of methods. These include the kinetic, functional, and chemical analyses of mitochondrial components in both the isolated and *in situ* states, the investigation of the sites of action of specific inhibitors, and the fractionation of individual components followed by their reconstitution into more complex functional units. Despite extensive efforts of many laboratories, the fundamental mechanism underlying oxidative phosphorylation is still far from being completely understood (see *1*).

The usefulness of mutants as a means for the analysis of metabolic pathways no longer needs to be stressed. It is obvious that if this approach could be systematically applied to the study of complex, highly organized, membrane-bound processes such as oxidative phosphorylation new insights into its mechanisms might be gained.

In yeast a total absence of respiration leads only to a conditional lethality, since fermentative energy supply is able to palliate the deficiency. The same should be true for a block in oxidative phosphorylation in this organism. After introduction of the genetic methodology in the study of electron transfer (*2, 3*) it seemed logical to extend this approach to the oxidative phosphorylation research. The purpose of this report is to present first examples of the use of mutants in this field.

Numerous respiratory-deficient mutants in yeast have been isolated which lack one or several components of the respiratory chain. Genetically they result either from a chromosomal gene mutation (abbreviated p) or from an extrachromosomal, mitochondrial DNA mutation (abbreviated p^-) (*2–4*). The phenotype used to screen such "petite" mutants is based on the fact that they are unable to grow on nonfermentable substrates (for example, glycerol, lac-

tate, ethanol, and so forth) but grow on the fermentable ones (for example, glucose, galactose, and so forth). It is to be expected that the same growth phenotype may be caused by a lesion in oxidative phosphorylation. However, it should be possible to distinguish p or ρ^- (petite) mutants from oxidative phosphorylation–deficient mutants by the fact that the former do not respire while the latter should have a normal if not higher oxygen uptake.

A search of such mutants was made after HNO_2 mutagenesis of a *Saccharomyces cerevisiae* haploid strain 18-27. This strain is wild type in respect to the chromosomal genes and to the cytoplasmic factor controlling respiration (that is, $P\ \rho^+$). It grows on lactate as carbon source and contains genetic markers α, ad_{3-6} and me_1. The conditions for the mutagenic treatment with HNO_2 are described elsewhere (see 5).

Master plates using glucose as the carbon source were replicated on plates using DL-lactate as carbon source. From the lactate negative strains, we selected those colonies that stained bright red with tetrazolium when grown on glucose, indicating a capacity for oxidative metabolism (6). This step eliminates p or ρ^- mutants which do not stain or stain very weakly with tetrazolium. A single cell isolate called 18-27-T12 was retained for further analysis.

This strain did not grow aerobically on glycerol, D- or L-lactates, ethanol, or acetate. It could not utilize either the carbon or the energy sources present in yeast extract and peptone. At first glance its growth behaviour seemed identical to that of a cytoplasmic ρ^- petite mutant (Fig. 1). A close inspection reveals differences: 18-27-T12 does grow, although extremely slowly, on nonfermentable carbon and energy sources; its mean doubling time is of the order of 30 to 40 hours as compared to 2 or 3 hours for the mother strain.

This extremely slow growth is not due to reversions, as every cell of 18-27-T12 gives rise, after 3 weeks, to a minute colony on glycerol plates. Under these conditions there is absolutely no growth of ρ^- cells. The mutation that has occurred in the strain 18-27-T12 will be henceforth designated as op_1 because it will be shown that (i) it impairs the oxidative phosphorylation and (ii) it results from a single chromosomal gene mutation. The corresponding wild-type allele will be designated *OP*.

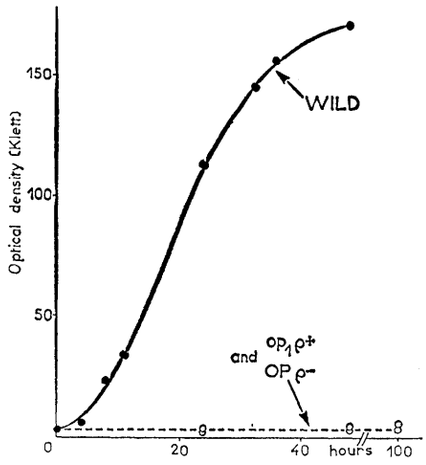

Fig. 1. Growth on glycerol of a phosphorylation-deficient mutant 18-27-T12 ($op_1\ \rho^+$), a cytoplasmic respiratory-deficient mutant ($OP\ \rho^-$), and a wild-type strain ($OP\ \rho^+$). The medium contains 1 percent of Difco yeast extract, 1 percent of bactopeptone, and 2 percent glycerol. Culture was at 28°C with full aeration.

In spite of salient differences in the growth pattern between the op_1 mutant and wild type strains, there are no major differences in the rate of respiration. Substrates such as lactate, glycerol, ethanol, and pyruvate, that do not support growth, are readily oxidized by the 18-27-T12 strain. The rate of oxygen uptake ($Q_{O_2}^{air}$ per milligram of protein) reaches values between 100 and 200, quite comparable to those of normal yeast. The balance between ethanol utilized, CO_2 produced, and the RQ values indicate that oxidation of ethanol is complete under resting conditions and that practically no carbon is assimilated (in contrast to wild type). Furthermore, the impairment of growth cannot be due to a deficiency in the Krebs-cycle enzymes.

Analysis of overall glucose metabolism gives results consistent with those mentioned above. The Pasteur effect is abolished in the mutant strain. The rate of glucose fermentation of the op_1 mutant in air is similar to that measured under anaerobic conditions, whereas the wild-type strain shows a typical decrease in the CO_2 output (Fig. 2). It should be stressed that both strains oxidize glucose at the same rate (Fig. 2) and have a normal cytochrome spectrum with typical bands of cytochrome $a + a_3$, b, c_1, and c. Aerobic respiration by the mutant has no effect on its growth yield per mole of glucose consumed (Table 1). Wild-type yeast has

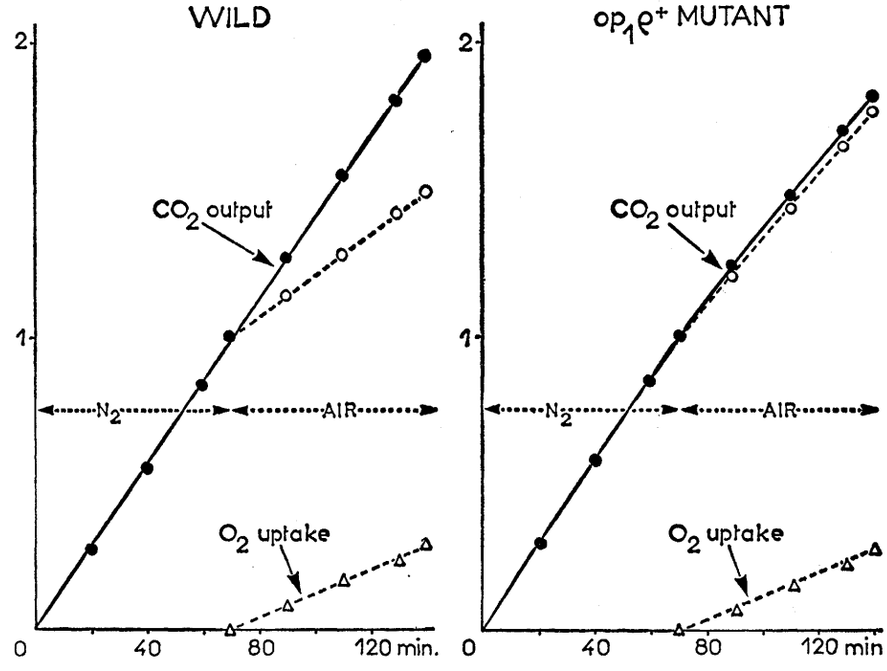

Fig. 2. Fermentation and respiration of $op_1\ \rho^+$ mutant and a wild-type strain. Standard direct Warburg method was used. During the first period the conditions are anaerobic in all flasks (N_2); after 70 minutes one control flask is continued under the same conditions (●———●) while in other flasks air replaces N_2. The rate of oxygen uptake ($Q_{O_2}^{air}$, △———△) and the rate of fermentive CO_2 output ($Q_{CO_2}^{air} - Q_{O_2}^{air}$, ○———○) are normalized with respect to $Q_{CO_2}^{N_2}$ measured during the first period, which is taken as 1.

Table 1. Molar growth yields ($Y_{glucose}$); grams, dry weight, of cells formed per mole of glucose consumed (see 7) of a wild-type yeast, phosphorylation-deficient mutant, and a cytoplasmic respiratory-deficient mutant.

Genotype	Conditions of growth	$Y_{glucose}$
$OP/OP\ \rho^+$ and $OP/op_1\ \rho^+$	Anaerobic	25
	Aerobic	325
$op_1/op_1\ \rho^+$	Anaerobic	25
	Aerobic	25
$OP/OP\ \rho^-$	Anaerobic	25
	Aerobic	25

Table 2. Oxidative phosphorylation efficiencies in wild type and op_1 mitochondria. Mitochondria were isolated by a modified procedure of Duell *et al.* (9). Oxidative phosphorylation was measured in a Warburg apparatus for 20 minutes at 30°C. The main compartment of the Warburg flasks contained, in 2.0 ml: 0.48*M* mannitol, 1.1 m*M* ethylenediaminetetraacetate (EDTA), 10 m*M* tris maleate, 10 m*M* potassium phosphate, 0.5 m*M* adenosine triphosphate (ATP), 7.5 m*M* $MgCl_2$, 0.15 percent bovine serum albumin, 25 m*M* glucose, 1 mg hexokinase (Sigma, Type III), substrate as indicated above, and 1.4 mg of mitochondrial protein; final *p*H, 6.4.

Substrate	Mitochondria	
	Wild type (P/O)	op_1 (P/O)
Citrate	1.5	0.22
Succinate	1.4	.09
α-Ketoglutarate	1.5	.12
D-Lactate	1.0	.17
L-Lactate	1.0	.12

the growth yield per mole of glucose utilized about 13 times greater aerobically than under anaerobic conditions. This value is quite close to the theoretical one expected with tight coupling between oxidation and phosphorylation. In contrast, the op_1 mutant as well as the ρ^- petite strain give the same low growth yields in air as are obtained during anaerobic growth (Table 1). In the case of ρ^- (petite) mutant the explanation is obvious, since there can be no oxygen uptake. In the op_1 mutant oxidative respiration is of normal intensity, but is ineffectual and does not produce chemical energy which can be used for cellular synthesis. These results strongly suggest that the mutation designated as op_1 has abolished or severely diminished the efficiency in vivo of oxidative phosphorylation.

Genetic analysis of crosses and backcrosses between 18-27-T12 and the wild type shows a single-gene segregation in tetrads: two spores are unable to grow on glycerol and two spores are able to do so. The mutation is therefore chromosomal. Furthermore, op_1 mutant complements cytoplasmic respiratory deficient mutants (ρ^-) and chromosomal respiratory deficient mutants belonging to the following classes: p_1, p_2, p_3, p_4, p_5, p_6, p_7 (see 3). The op_1 mutation is therefore functionally nonallelic to the above-mentioned genes. Data on recombination further support this idea.

No indication of genetic linkage has been found between the op_1 gene and the chromosomal genes that impair the formation of cytochromes $a + a_3$ or cytochrome *b* or both. Diploid heterozygous strains op_1/OP have a wild-type phenotype as judged by the growth pattern on nonfermentable energy sources and by the molar growth yield on glucose (Table 1). It can be concluded that the gene op_1 is recessive and not linked to the known genes controlling the synthesis of the electron transfer chain.

Although the physiology of op_1 mutants clearly suggests a deficiency in oxidative phosphorylation, final proof can be obtained only by studies of isolated mitochondria. As shown in Table 2, phosphorylation efficiency of mitochondria isolated from the op_1 strain is greatly decreased as compared with mitochondria from the wild type. The deficiency is general for all substrates tested. The results may be interpreted as indicating a lesion in the energy-coupling mechanism. The fact that addition of adenosine diphosphate to respiring op_1 mitochondria does not increase the rate of oxygen uptake (Fig. 3) may be used as an argument in favor of this idea. However (Fig. 3), the addition of 2,4-dinitrophenol in concentrations which uncouple phosphorylation in wild strain also increases the O_2 uptake of the op_1 mutant mitochondria. It may therefore be concluded that the first step of the energy-conservation mechanism, the coupling process, is preserved and that the subsequent reactions are affected by the mutation. This conclusion is supported by a systematic study of adenosine triphosphatase activity in mitochondria.

The adenosine triphosphatase activity of wild-type yeast mitochondria displays two *p*H optima, 6.2 and 9.5, and two different patterns of inhibition corresponding possibly to two different enzymes. As shown in Table 3, the "6.2" component is not modified by the op_1 mutation as judged by its specific activity and the inhibition pattern with respect to oligomycin, azide, and fluoride. On the other hand, the "9.5" component of the adenosine triphosphatase is considerably diminished in the op_1 mutant.

In spite of the diminution of the specific activity, the mutant's enzyme is still oligomycin- and azide-sensitive, and

Table 3. Adenosine triphosphatase activity of mitochondria from wild-type yeast, phosphorylation-deficient mutant, and cytoplasmic-deficient mutant. Specific activity is expressed in micromoles of P_i liberated per hour per milligram of mitochondrial protein. The reaction mixture contained, in 1.0 ml: 4 m*M* ATP, 80 m*M* KCl, 0.5 m*M* EDTA, 20 m*M* tris maleate and 5 m*M* $MgCl_2$ (at *p*H 6.2) or 20 m*M* tris chloride and 2 m*M* $MgCl_2$ (at *p*H 9.5), 0.014 to 0.18 mg of mitochondrial protein, and inhibitors as indicated. Incubation lasted 10 minutes at 30°C. The values are means from two to five experiments. Specific activity is expressed in moles of P_i liberated per hour per milligram of protein. Since the extent of oligomycin inhibition depended from oligomycin/protein ratios, which varied in different experiments, only approximative values are presented.

*p*H	Characteristics	Genotype		
		$OP/OP\ \rho^+$	$op_1/op_1\ \rho^+$	$OP/OP\ \rho^-$
6.2	Specific activity	33	39	36
6.2	Inhibition by 10 m*M* NaF (%)	82	73	85
6.2	Inhibition by 4 m*M* NaN_3 (%)	5	8	6
6.2	Approximative inhibition by oligomycin at 50 μg oligomycin/mg protein (%)	8	5	6
9.5	Specific activity	183	68	72
9.5	Inhibition by 10 m*M* NaF (%)	10	30	25
9.5	Inhibition by 4 m*M* NaN_3 (%)	85	60	71
9.5	Approximative inhibition by oligomycin at 50 μg oligomycin/mg protein (%)	70	60	10
9.5	Km with ATP, m*M*	2.2	2.5	3.0

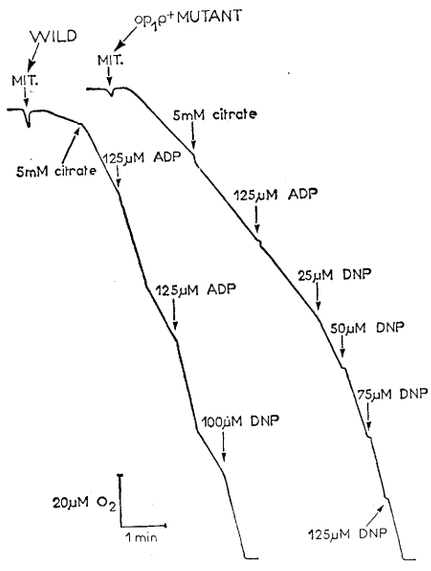

Fig. 3. Effect of adenosine diphosphate (ADP) and 2,4-dinitrophenol (DNP) on oxidation rates of mitochondria from a wild strain and $op_1\ \rho^+$ mutant. Respiration was measured polarographically at 30°C. The reaction mixture contained in 2.0 ml: 0.6*M* mannitol, 20 m*M* KCl, 1.5 m*M* EDTA, 10 m*M* tris maleate, and 10 m*M* potassium phosphate; final *p*H, 6.4. To start the experiments, 1.6 and 2.3 mg of wild-type and mutant mitochondrial protein respectively were added. The other additions are indicated in final concentrations.

fluoride-resistant like the wild-type adenosine triphosphatase.

Two important conclusions may be drawn from the study of the op_1 mutants. First, a single recessive gene mutation can lead to a deficiency that is common to all the phosphorylation steps in the respiratory chain. It can be inferred from it that either the same enzyme is involved in the energy transfer at all the phosphorylation sites or that the same structural component of mitochondria is required for the normal functioning of all sites. Second, this common component or enzyme is correlated with the high activity of the *p*H 9.5 oligomycin-sensitive adenosine triphosphatase.

It is conceivable that the diminution of adenosine triphosphatase activity is not a primary effect of the op_1 mutation but is secondary to other modifications in the organization of proteins or structures in mutant mitochondria. Another possibility would be a lesion in the last step of the mitochondrial phosphorylation system, that is, in the adenine nucleotide translocation through the membrane. All these possibilities will be considered in further studies of the mutant.

It is clear that an analysis of a single mutant is unable to solve the problem of the mechanism of oxidative phosphorylation. It seems to us, however, that by systematically applying the approach presented in this report one should be able to dissect in a stepwise manner the mechanisms of this highly integrated process. It may be hoped that the use of nonallelic complementing mutants could permit a reconstitution of functional machinery from its mutationally deficient parts. It may be of interest, in this respect, to mention that the mutants that have lost cytochromes $a + a_3$ and b (ρ^- cytoplasmic mutants) still maintain at least part of the oxidative phosphorylation reactions. This is indicated by the fact that their assimilation processes are still sensitive to dinitrophenol and azide (*8*). As shown in Table 3, ρ^- mutant has a normal activity of the *p*H 6.2 adenosine triphosphatase while the *p*H 9.5 component is very similar in its specific activity to that present in the op_1 mutant but is no longer sensitive to oligomycin.

Although yeast presents considerable advantages in the mutant approach to oxidative phosphorylation the same methodology could be tried with other eucaryotic microorganisms that possess mitochondria, or with bacteria.

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19 September 1967

Lambda Transition in Liquid Sulfur

Abstract. *Density measurements on liquid sulfur near the transition at 160°C show a logarithmic singularity and discontinuous density. The transition shows the kinetic behavior of a zero-order reaction. The transition is expected to have a latent heat characteristic of a first-order phase transition but is of the cooperative nature associated with second-order transitions such as the lambda point in liquid helium.*

First-order phase transitions are characterized by a discontinuous first derivative of the Gibbs free energy, so that volumes and entropies are discontinuous. Second-order transitions have discontinuous second derivatives, so that heat capacities and expansion coefficients are discontinuous. Many second-order lambda-type transitions are known in magnetic substances, explained by the alignment of spins of electrons or nuclei. The classical example of a second-order transition occurs in liquid helium at 2.17°K. Spin ordering is not, however, an explanation in this case. The transition in liquid sulfur at 160°C is therefore of great interest, since it is the only recognized example (other than helium) among elementary liquids (*1*). The low-temperature μ form is structurally an eight-member ring, whereas the high-temperature λ form is a folded linear polymer of high molecular weight. Heat capacity measurements have shown the transition to have the form of a lambda point, unusual in the sense that the discontinuity falls on the low-temperature side of the transition (*2*). The transition is comprehensive. The viscosity changes by two orders of magnitude within a few degrees. The color changes from a yellow liquid to the dark-brown polymer. Surface-tension measurements show a