dinitrophenylhydrazine could convert XMal, but not YMal, into a new, chromatographically separable yellow product.

In searching farther afield, at this point, for an intermediate in reaction (3) capable of reacting with NEM to yield an α-ketoacid, it may be necessary to question some of the restrictions imposed by the effects of NEM on reactions (1) to (4). In one possibility, suggested by conversations with Dr. J. S. Fruton, the mobile electrons of compound IV (Fig. 3) could be considered to constitute a negative charge on the γ-carbon which, rather than acquiring a solvent proton, could react with the maleimide double bond. Hydrolysis of the resultant Schiff's base would yield a substituted aminocrotonate (see IV → V → IX) which would decompose to compound XII.

There is no intermediate comparable to compound IV in the scheme for β-elimination. However, it would be necessary to explain why maleimides react with compound IV in reaction (3), but not in reaction (1), if compound IV is an intermediate in both reactions.

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Biochemical Correlates of Respiratory Deficiency. III. The Level of Some Unsaponifiable Lipids in Different Strains of Baker's Yeast*

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Two wild-type (respiratory-sufficient, "grande") and one cytoplasmic-mutant (respiratory-deficient, "cytoplasmic petite") yeasts have been analyzed for their content of unsaponifiable lipids, with special emphasis on their content of ubiquinone (coenzyme Q). The methods used involve saponification with methanolic pyrogallol, alumina chromatography, and spectrophotometric identification and analysis. Under controlled physiological conditions the differences in content of ubiquinone, tocopherol, vitamin A, ergosterol, total steroid, and total unsaponifiable lipids between the wild type and its "petite" mutant are not considered significant. Only one homolog, ubiquinone-30 (coenzyme Q_6), could be identified in any of the strains examined; it is present to the extent of ~80 μg/g dry wt in the parent strain 59 R harvested in the exponential phase and attains a level with "cytoplasmic petite" 59 RA of ~68 μg/g under comparable conditions.

The ability of *Saccharomyces cerevisiae* to grow aerobically on nonfermentable substrates is under stringent genetic control. As shown by Ephrussi and collaborators either loss (or modification) of a cytoplasmic factor ($\rho^+ \rightarrow \rho^-$) or single mutations of any of a number of unlinked chromosomal loci ($P \rightarrow p_x$) gives rise to phenotypically identical cell populations incapable of aerobic growth on, e.g., glycerol or lactate, and yielding characteristic small colonies when grown aerobically on glucose-agar (Ephrussi *et al.*, 1949; Tavlitzki, 1949; Chen *et al.*, 1950; Ephrussi and Hottinguer, 1951; Ephrussi, 1956). Biochemically this class of "respiratory-deficient" or "petite colonie" mutants has been characterized by an almost total absence of cytochromes (a plus a_3) and cytochrome b, a normal (or elevated) content of cytochrome c and probably cytochrome b_2, and an inability of intact cells or cell-free extracts to catalyze the aerobic oxidation of a number of characteristic substrates such as glucose, D- or L-lactate, succinate, and NaDH (Tavlitzki, 1949; Slonimski, 1949; Slonimski and Hirsch, 1952; Slonimski, 1953; Ephrussi *et al.*, 1956; Gregolin and Ghiretti-Magaldi, 1961). The enzymological corollary of these findings is the virtual or complete absence of cytochrome c oxidase and of antimycin A–sensitive NADH- and succinic-cytochrome c reductase activities in these mutants (Tavlitzki, 1949; Slonimski, 1949, 1953; Slonimski and Hirsch, 1952; Schatz *et al.*, 1963; Mahler *et al.*, 1964; Kovachevich, 1964), while ample evidence is accumu-

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TABLE I
LIPID ANALYSIS FOR DIFFERENT YEAST SAMPLES[a]

Component	Springer		59 R		59 RA			
	1	2	1	2	1a	2a	1b	2b
Total unsaponifiable (mg)	11.2	12.5	10.8	11.1	11.0	10.7	6.3	6.1
Total sterol (mg)	8.2	10	8.21	8.38	7.12	6.5	4.3	4.2
Ergosterol (μg)	591	—	715	857	690	718	515	950
Vitamin A (IU)	3.1	2.4	5.5	6.1	3.2	3.1	3.9	4.2
Ubiquinone (μg)	122	166	79	82	66	69	42	43
Identified as	UQ-30	UQ-30	UQ-30	UQ-30	UQ-30	UQ-30	UQ-30	UQ-30
Tocopherol (μg)	13.3	—	13.6	13.1	3.1	4.3	29.4[b]	30.6[b]

[a] All per mg dry wt. Analyses were performed on 20-g (dry wt) samples by the procedure described below; all analyses in duplicate, both sets of values are shown. A dash (—) means not determined. The different strains of yeast used were: "Springer," a commercial baker's yeast; 59 R, a wild-type strain harvested in the exponential phase; 59 RA, a cytoplasmic "petite colonie" mutant isolated from strain 59 R by acriflavine treatment (Slonimski and Ephrussi, 1949); for samples 1a and 2a the culture was harvested toward the end of the exponential phase in a peptone-containing medium; for samples 1b and 2b the culture was allowed to approach the stationary phase, but at a cell density corresponding to that observed with 59 R. All identifications were made by both reversed-phase and thin-layer chromatography. Hydrolysis conditions: dried cells (20–25 g) were added to methanolic pyrogallol (5 g pyrogallol/liter; 1.4 cc/g of cells) plus 0.9 cc of aqueous KOH (600 g/liter) per g of cells. When all liquid had been absorbed, an additional 1.4 cc/g of MeOH was added and refluxed for 50 minutes. Four cc of H_2O/g of cells was then added and work-up was continued essentially as described by Pennock *et al.* (1962). Chromatography on alumina (Pennock *et al.*, 1962): elution with light petroleum ether and 2% ether in petroleum ether yielded colorless residue plus small amounts of chromophore with λ_{max} = [238], 252, 273, 285–87, [302] mμ tentatively identified as sterol (ergosterol?) ester; UQ eluted with 4% and 6% ether–petroleum ether, λ_{max} = 272 mμ; tocopherol (λ_{max} = 295 mμ) in 6% and 10% ether–petroleum ether; sterol (ergosterol) with λ_{max} = 262, 272, 282, and 292 mμ and vitamin A (λ_{max} = 325 and 342 mμ) found in 30% ether–petroleum ether and pure ether fractions. [b] These values are the spectrophotometrically determined (Emmerie-Engel) amounts; actual isolation by preparative thin-laye chromatography, followed by elution and spectrophotometric identification as tocopherol, are 24.0 and 12.5, respectively.

lating that the level of the various primary dehydrogenases involved is not significantly different from that found in the parent wild-type strain (Linnane and Still, 1956; Gregolin and Ghiretti-Magaldi, 1961; Schatz *et al.*, 1963; Mahler *et al.*, 1964).

The most attractive hypothesis advanced to account for these diverse observations ascribes the primary biochemical lesion to a functional and/or structural alteration of the particulate entity concerned with electron transport, i.e., the mitochondria of the intact cell (Ephrussi and Slonimski, 1955). One approach to this problem has been direct electron-microscopic examination of various types of mutant cells which has disclosed that although "vegetative petites" ($P\rho^-$) do appear to possess structurally altered particles (but in approximately normal numbers!) "segregational petites" ($p_z\rho^+$) contain mitochondria which are quantitatively and qualitatively indistinguishable from those seen in the wild type (Yotsuayanagi, 1963).

Another approach has been the isolation from wild-type and mutant strains of mitochondria (Schatz *et al.*, 1963) and of the subunits of mitochondria responsible for electron transport, and a critical appraisal, characterization, and comparison of various component parts (Mahler *et al.*, 1964). These parallel studies by two groups of investigators have indicated that there exists an almost complete homology between respiratory particles isolated from the two types of strains.

In this publication we have set ourselves a more modest aim: Since coenzyme Q (ubiquinone) is now generally regarded to be a key component of mitochondrial electron-transport systems[1] (albeit of as yet uncertain function; see, for instance, Blair *et al.*, 1963; Wolstenholme and O'Connor, 1961; Massey and Veeger, 1963; Szarkowska and Klingenberg, 1963) we have compared various yeast strains including a "cytoplasmic petite" and its parent wild type with regard to their content of ubiquinone and other nonsaponifiable lipids. The relevant data are shown in Table I. In all strains examined we found exclusively coenzyme Q_6 (ubiquinone 30); under carefully controlled conditions of culture the mutant can approach the wild-type cells in content of this material (>80%). The pattern with regard to the other nonsaponifiable lipids was similar also.

It is quite evident that qualitatively the different samples show a remarkable uniformity of composition with regard to these components: Whatever quantitative differences do appear are certainly referrable to alterations in physiological conditions rather than to the change in genetic makeup responsible for the transition from respiratory sufficiency to deficiency [compare the differences between the two "grandes," Springer and 59R, or between the two sets of conditions for the "petite" 59 RA].

In their communication on the same subject Sugimura and Okabe (1962) report a content of 85 μg of UQ 30/100 mg N in their mutant as compared to 500 μg of the same quinone/100 mg N in their parent yeast.* Assuming that the same proportion relating nitrogen content and dry weight obtained for their yeast as for ours, this corresponds to 70 μg/g dry wt in the mutant and 420 μg/g for the wild type. We see then that their values for their mutant are quite comparable to ours; it is their wild-type yeast which appears to be unusually high in ubiquinone content. To what extent their findings are referrable to the particular physiological conditions and analytical techniques employed is not known.

The ubiquinone has been unambiguously identified as the UQ-30 (coenzyme Q_6) homolog in all the strains examined, both by reversed-phase-paper and thin-layer chromatography. This is the homolog reported previously (Gloor *et al.*, 1958; Lester and Crane, 1959; Sugimura and Rudney, 1960) to be characteristic for baker's yeast. No evidence for the presence of any other homolog nor of any ubichromenol could be adduced in the course of these studies. There is some indication of the appearance of a compound similar to cholesta-3.5-dien-7-one on thin-layer chromatograms.

[1] Coenzyme Q content of yeast appears to vary widely as a function of physiological environment and appears to be maximal under conditions of maximal "respiratory adaptation" (Lester and Crane, 1959; Sugimura and Rudney, 1960).

The ubiquinone found in a respiratory-deficient mutant is therefore identical in structure and almost equal in amount to that of the corresponding wild-type yeast. Whatever the nature of the primary phenotypic difference between the two it is probably also not referrable to any gross differences in the synthesis or utilization of unsaponifiable lipids. These statements hold true so far only for the intact cell; whether or not they are applicable to respiratory particles and whether there are differences in the intracellular localization of these components is presently under study.

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ADDED IN PROOF

In a more recent publication T. Sugimura, K. Okabe and H. Rudney (1964, *Biochim. Biophys. Acta 82*, 350) report values of 315 and 411 μg Q/100 mg N for wild type and 24-85 μg Q/100 mg N for their mutants.

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Generation of Hydrogen Peroxide in Erythrocytes by Hemolytic Agents*

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The generation of H_2O_2 in intact erythrocytes was detected by demonstration of catalase-H_2O_2 complexes I and II. 3-Amino-1,2,4-triazole reacted with complex I to yield irreversibly inhibited catalase; inhibition was prevented but not reversed by employing ethanol to decompose complex I. Complex II was detected by its lack of catalatic activity which could be recovered by treatment with ethanol. The presence of H_2O_2 in intact cells was detected after the addition of several 8-aminoquinolines (e.g., primaquine) but not after the addition of the nonhemolytic 4-aminoquinoline, chloroquine; after the addition of the hemolytic agents phenylhydrazine and menadione; after the addition of the hydroquinone–*p*-quinone redox system, but not after the addition of the nonautoxidizable resorcinol; and, last, after the addition of exogenous H_2O_2. The generation of H_2O_2 from the 8-aminoquinolines required the presence of oxyhemoglobin and could be blocked by preliminary conversion of oxyhemoglobin to methemoglobin. The generation of H_2O_2 from phenylhydrazine was derived in part from a reaction with oxyhemoglobin and in part from autoxidation. The detection of H_2O_2 generated from hemolytic agents supports the concept that H_2O_2 toxicity plays a major role in drug-induced hemolysis of glucose-6-phosphate dehydrogenase–deficient erythrocytes.

Hemolysis in individuals with deficient erythrocyte levels of glucose-6-P dehydrogenase is known to occur upon exposure to various drugs (Beutler, 1960; Tarlov *et al.*, 1962). It has been suggested that the oxidative changes observed during hemolysis, e.g., loss of GSH and oxidation of hemoglobin, are manifestations of the presence of H_2O_2 (Mills and Randall, 1958; Cohen and Hochstein, 1961, 1963). Glucose-6-P dehydrogenase–deficient erythrocytes are sensitive to H_2O_2 by virtue of diminished generation of NADPH.

In this paper we present evidence that H_2O_2 is in fact generated in intact erythrocytes when hemolytic agents are added. Since H_2O_2 does not accumulate

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