Table 2. Genotype and phenotype of adenine-requiring yeasts. AD and ad signify genes controlling adenine synthesis. AER and aer signify respiratory phenotype.

Genotype	Respiratory phenotype	Synthetic adenineless media	
		Agar 2 days	Broth 2 days
AD	AER	+	+
AD	aer	− or +/−	+
ad	AER	−	−
ad	aer	−	−

trolling the rates of carbohydrate fermentation in yeasts should be clarified by tests of the competence of the respiratory mechanisms of the segregants involved.

Similar complications are involved in the diagnosis of phenotype and genotype in adenine-requiring (ad) yeasts. The pinkish-red pigmentation of these yeasts is controlled by (i) genes, (ii) extrachromosomal factors, and (iii) environment (*11*). Both pink and brownish cultures give rise to adenine-requiring white colonies spontaneously, with relatively high frequencies. The pink color is restored on outcross to normal, and genetic tests revealed that gene mutation was not involved in the spontaneous change from red to white. In the absence of contact with air, such cultures form little or no pigment. Certain adenine-requiring, respiration-deficient pigmented strains form white colonies on 0.1 percent glucose agar (*12*), brownish colonies on 2 percent glucose agar, and deep red colonies on 8 percent glucose agar. Other nutritional factors (the concentration of yeast extract) influence color. In several strains, adenine-requiring, respiration-deficient clones can be distinguished from adenine-requiring, respiration-sufficient clones on 2 percent agar, on which the former are brown, while the latter are pink (*13*).

For some unknown reason, the phenotype of adenine-independent (AD), respiration-deficient yeasts is different in synthetic broth and on synthetic agar (Table 2) when scored over the usual period of from 1 to 2 days. Such cultures do not develop the positive phenotype (confluent growth) on adenineless synthetic agar plates, although they are phenotypically adenine-independent (show rapid growth) in broth. If an adenine-independent, respiration-deficient culture is diagnosed erroneously as adenine-dependent (because of the phenotype on agar), the vigorous growth which the hybrid with an adenine-dependent AER culture (AD aer × ad AER) displays on agar might be interpreted incorrectly to indicate the presence of two nonallelic genes controlling adenine-synthesis.

In certain matings (*8*) between (i) adenine-dependent mutants of strain 8256 which had been altered from pink to brown by ultraviolet irradiation and (ii) adenine-dependent mutants of strain 8282 which had changed spontaneously from pink to brown, the hybrid was pink—that is, brown × brown produced pink—suggesting the presence of nonallelic, synergistically acting genes. Subsequent crosses showed that the brown parents were both respiration-deficient and that the hybrid was respiration-sufficient. In 14 tetrads from three such hybrids, segregation was 2 ad AER pink: 2 ad aer brown. Tetrad analysis showed that respiratory competence was under genic control in one parent and cytoplasmic control in the other, and that the "nonallelism" was attributable to the restoration of respiratory competence in the hybrid by the complementary action of gene and cytoplasm.

A few respiration-deficient progeny may be expected from any AER × AER hybrid. For example, 31 tetrads from such a mating yielded the following:

AER	AER	AER	AER	22
AER	AER	AER	aer	2
AER	aer	aer	aer	2
aer	aer	aer	aer	5

It may be inferred that the respiratory granules are often lost during the process of cutting out the spore plasm from the epiplasm.

In view of these results, a fundamental ambiguity prevails in certain genetical analyses of the inheritance of slow fermentation, adenine-synthesis, and pigmentation. This ambiguity arises from the absence of information concerning the respiratory competence of hybrids and segregants. Routine tests of segregants for their respiratory phenotypes are an essential complement to any genetical analysis, regardless of the respiratory competence of the parents.

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References and Notes

1. A number of experimental criteria have been employed in the diagnosis of respiration deficiency (*2*). In our laboratory a respiration-sufficient (AER) strain is defined operationally in terms of (i) $Q_{O_2}(N)$ about 1000 with glucose as substrate; (ii) growth on lactate agar (*3*); (iii) intensely colored colonies after overlay with 2,3,5-triphenyl tetrazolium chloride (TTC) (*4*); and (iv) alkali production in an acetate medium (*5*). A respiration-deficient (aer) strain is defined operationally in terms of (i) $Q_{O_2}(N)$ 0–50 with glucose as substrate; (ii) inability to grow on lactate agar (*3*); (iii) uncolored or faintly colored colonies after overlay with TTC (*4*); and (iv) no alkali production in an acetate medium (*5*).
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"Lethal" Variant of Candida albicans, a "Petite Colonie" Mutant

Two spontaneous and irreversible hereditary variants may be easily distinguished in the pathogenic yeast *Candida albicans* (*1*). The colonies of the normal (wild) form, strain 582, are smooth and are built up by typical yeast cells ("Y" form). One variant, strain 806, gives wrinkled, hard, rough colonies, and, microscopically, it is filamentous or mycelial ("M" form or variant). The second variant was called "lethal," and it is characterized by smaller sized colonies that perish rather rapidly.

Nickerson carried out studies with strains 582 ("Y" form) and 806 ("M" variant), and, in a recent paper, Nickerson and Falcone (*2*) state that the "M" variant is deficient in a protein-disulfide reductase that is apparently essential for cell division but not for growth.

We have studied the biochemical behavior of the "lethal" variant (strain 807), and our results show that the respiration of the mutant is nearly abolished. In manometric studies in a Warburg apparatus (yeast, 4 mg; buffer, 0.05M KH_2PO_4; substrate, 100μM glucose; temperature, 30°C; atmosphere, air or N_2) the normal form gave: $Q_{CO_2}{}^{N_2} = 13.2$ (μl/mg of yeast per 10 minutes); $Q_{CO_2}{}^{air} = 8.9$; $Q_{O_2}{}^{air} = 6.8$; respiratory quotient = 1.3; and Pasteur effect = 33 percent (inhibition). On the other hand, the following results were obtained with the "lethal" variant: $Q_{CO_2}{}^{N_2} = 12.4$; $Q_{CO_2}{}^{air} = 12.5$; $Q_{O_2}{}^{air} = 0.24$; respiratory quotient = 53; and Pasteur effect = 1.6 percent (inhibition).

Cytochrome-*a* and -*b* bands were absent from the absorption spectrum of the "lethal" mutant. These bands were present in the spectrum of the normal form. The α and β bands of cytochrome *c* were

present in the spectra of both the normal and the "lethal" forms.

Our results demonstrate that the "lethal" variant of *Candida albicans* and the "petite colonie" mutant of *Saccharomyces cerevisiae* described by Ephrussi (*3*) have the same physiological impairment. This fact suggests that the hereditary loss of the respiratory function may be a frequent phenomenon among microorganisms endowed with both aerobic and anaerobic metabolism.

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O-Methylation of Epinephrine and Other Catechols in vitro and in vivo

For the past 20 years the catechol amines, epinephrine and norepinephrine, have been considered to be transformed in the body, mainly by monoamine oxidase. However, recent investigations have shown that these amines may be inactivated by enzymes other than monoamine oxidase (*1*). The important observation of Armstrong and McMillan (*2*), that a major metabolic product of epinephrine and norepinephrine is 3-methoxy-4-hydroxy mandelic acid, suggested that methoxy derivatives of the catechol amines may be the precursors of the deaminated compound. I wish to report the isolation of an enzyme from rat liver which catalyzes the O-methylation of epinephrine and other catechols. In addition, the presence of methoxy epinephrine and methoxy norepinephrine in the urine was demonstrated.

Table 1. Enzymatic O-methylation of epinephrine. The soluble supernatant fraction from 10 mg of rat liver was incubated at 37°C with 0.1 μmole of l-epinephrine bitartrate, 50 μmole of phosphate buffer (*p*H 7.4), 20 μmole of $MgCl_2$, and water to make a final volume of 0.5 ml. After 30 minutes of incubation, the reaction mixture was assayed for epinephrine (*13*).

Additions	l-Epinephrine metabolized (μmole)
None	0.000
S-Adenosylmethionine, 0.05 μmole (*8*)	0.041
S-Adenosylmethionine, 0.1 μmole	0.065

Enzymes have been found which transfer the methyl group of S-adenosylmethionine to the nitrogen of nicotinamide (*3*) and guanidoacetic acid (*4*). These findings suggested the possibility that phenols also could accept methyl groups by a similar mechanism. Incubation of the soluble supernatant fraction of rat liver with epinephrine, adenosine triphosphate, and methionine resulted in the disappearance of the cathechol amine. In the absence of either cofactor, no metabolism took place. Cantoni has shown that an enzyme in the liver can form S-adenosylmethionine from adenosine triphosphate and methionine (*5*). When S-adenosylmethionine was substituted for adenosine triphosphate and methionine, a more rapid disappearance of epinephrine was observed (Table 1). The metabolic product was extracted into an ether-isoamyl alcohol mixture at *p*H 9.0 and was then returned to dilute hydrochloric acid. The acid extract contained a phenolic compound which did not give a reaction for catechols (*6*). These observations were taken as evidence that an enzyme present in rat liver can transfer the methyl group of S-adenosylmethionine to one of the phenolic groups of epinephrine.

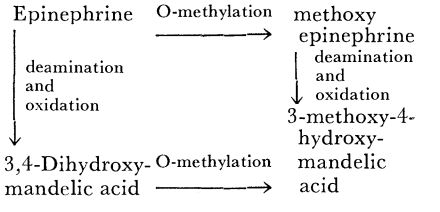
Several other catechols (norepinephrine, 3,4-dihydroxyphenylethylamine, and 3,4-dihydroxybenzoic acid) also served as substrates for the enzyme. The enzyme has been purified 20-fold and is stimulated considerably by magnesium ions. Supernatant fractions of brain, kidney, and spleen can also O-methylate epinephrine, but to a lesser extent than the liver.

Evidence for the identity of methoxy epinephrine formed by the enzyme was obtained by deaminating and oxidizing the metabolite with a monoamine oxidase preparation to yield 3-methoxy-4 hydroxymandelic acid as follows. Methoxy epinephrine was incubated with rat-liver mitochondria, and the metabolic product was extracted with ethyl acetate at *p*H 1, returned to a dilute sodium bicarbonate solution, and reextracted into ethyl acetate at *p*H 1. The resulting compound, when subjected to paper chromatography with several solvent systems (*7*), had the same R_f values and color reaction as an authentic sample of 3-methoxy-4-hydroxymandelic acid (*8*). A marked depression in the formation of the deaminated derivative was observed when the mitochondrial preparation was obtained from a rat that had been pretreated with iproniazid, an inhibitor of monoamine oxidase.

Urine of rats was examined for methoxy epinephrine after incubation with β-glucuronidase. Phenolic amines were extracted from a sodium chloride-saturated urine at *p*H 9.0 into an ether-isoamyl alcohol mixture and returned to dilute hydrochloric acid. The acid extract was saturated with sodium chloride, adjusted to *p*H 9.0, and shaken with *n*-butanol. After the butanol extract was evaporated to dryness, the residue was dissolved in a small volume of an ether-methanol mixture and subjected to two-dimensional chromatography (solvent systems I and III). A compound was found that had the same R_f values (0.70 and 0.52) and color reaction as the enzymatically formed methoxy epinephrine. The amount of methoxy epinephrine excreted in the urine of rats was markedly elevated after the intraperitoneal administration of iproniazid and l-epinephrine bitartrate (*9*).

Rat urine was also examined for methoxy norepinephrine after the intraperitoneal administration of l-norepinephrine bitartrate. Urine was incubated with β-glucuronidase and extracted as described in the preceding paragraph. After the extract had been subjected to two-dimensional chromatography (solvent systems I and III), a compound was found that had the same R_f values (0.54 and 0.43) and color reaction as enzymatically formed methoxy norepinephrine (*10*).

On the basis of the experiment described, the following scheme for the metabolism of epinephrine and its congeners is suggested:



Barger and Dale (*11*), as well as other investigators, have shown that numerous derivatives of epinephrine have sympathomimetic action. It is conceivable that some of the activity of the catechol amines could be mediated through their methoxy transformation products (*12*).

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