

Factors Not Synthesized by Yeast in the Presence of Cyanide.

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This study was undertaken as a result of difficulties encountered in growing yeast in a synthetic medium in the presence of potassium cyanide.¹ Other investigators^{2,3} have reported no difficulty in culturing *Saccharomyces cerevisiae* in a medium containing the same concentration of cyanide, but their media contained malt extract or yeast extract. Presumably the extracts supply one or more substances which the yeast is unable to synthesize with sufficient rapidity in the presence of cyanide. Direct experiments supported this view.

A method for quantitative study of the active principle or principles present in yeast extract was devised, the procedure being patterned after that used in the assay method for biotin.⁴ Initially, the only modification in the culture medium was the addition of a sufficient amount of potassium cyanide to give a concentration of 0.001 *M*. Later, pantothenic acid (2 mg per liter) was substituted for β -alanine, and cysteine (20 mg per liter) was added as a supplement. The standard of activity was Difco yeast extract which was added over a range of 0 to 10 mg. The test organism was *Saccharomyces cerevisiae*, F.B., and the extent of growth was measured turbidimetrically after an incubation period of 15 to 16 hours at 30°C. Table I shows the growth response of F.B. yeast to various levels of yeast extract under test conditions.

A large number of known compounds including the B vitamins, most of the familiar

amino acids, various saturated, unsaturated, and hydroxy acids, certain purines and pyrimidines, adenosine, adenylic acid, glutathione, sodium thioglycolate, and ascorbic acid were found to be inactive. Only cysteine and methionine showed any activity and neither of these produced an effect comparable with that of yeast extract.

None of the various source materials tested was as active as yeast extract. A commercial hydrolysate of casein (casamino acids) was found to resemble methionine in effect, but by measuring the growth stimulation of mixtures of this preparation and yeast extract and of methionine and yeast extract it was established that the activity of the hydrolysate did not depend solely on its content of methionine.

Substitution of pantothenic acid for β -alanine in the test medium resulted in an enhanced growth stimulation by casamino acids. A concentration of 10 γ per tube of the vitamin was as effective as 100 γ and was considerably more effective than 100 γ of β -alanine. If only 2 factors, pantothenic acid (present in yeast extract in nonlimiting concentration) and some other substance (contained in both yeast extract and casamino acids), were involved in the reversal of cyanide inhibition of growth, the growth stimulation produced by adding yeast extract to casamino acids in a pantothenic acid medium should represent an additive effect.

TABLE I.
Growth Response of F.B. Yeast to Yeast Extract in Cyanide Medium.

Yeast extract added per tube, mg	Galvanometer reading
0	5
0.2	11
0.5	22.5
1.0	32
5.0	62
10.0	81

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¹ Eppright, M. A., and Williams, R. J., *J. Gen. Physiol.*, in press.

² Pett, L. B., *Biochem. J.*, 1936, **30**, 1438.

³ Stier, J. B., and Castor, J. G. B., *J. Gen. Physiol.*, 1941, **25**, 229.

⁴ Williams, R. J., *Univ. Texas Pub.*, 1942, **4237**, 7.

TABLE II.
Anti-Cyanide Activity of Yeast Extract and Casamino Acids, Separately and Combined, in Media Containing β -Alanine or Pantothenic Acid.

Amt of yeast extract added, mg	Extent of growth in various media					
	β -alanine medium		β -alanine medium + casamino acids*		Pantothenic acid medium	
	Galvanometer readings	Apparent mg units†	Galvanometer readings	Apparent mg units	Galvanometer readings	Apparent mg units
0	6	0	23	0.65	6	0
0.5	21	0.5	49	3.0	22	0.6
1.0	28	1.0	56	4.4	28	1.0
5.0	59	5.0	69	>5	59	5.0
					73	>5

* The concentration of casamino acids was 10 mg per tube.

† The response to yeast extract alone in the β -alanine medium was used as standard.

The results in Table II show this not to be the case. It appeared, therefore, that at least 2 factors other than pantothenic acid must be involved. For all subsequent tests pantothenic acid was substituted for β -alanine in the culture medium.

The growth-promoting effect of yeast extract was improved by including in the medium any one of several reducing agents (ascorbic acid, sodium thioglycolate, glutathione, sodium hydrosulfite or cysteine). The optimum growth stimulation occurred in the medium containing 20 mg per liter of cysteine. For all of the experiments which followed this observation, the cyanide medium was supplemented with cysteine.

Further investigation provided evidence confirming the supposition that at least 2 substances other than pantothenic acid were responsible for the growth-promoting activity of yeast extract in a cyanide medium. These factors could be separated by adsorption with Darco G-60. The active material in the unadsorbed fraction was designated as the filtrate principle while that in the adsorbed portion which was eluted with an aqueous mixture of alcohol (40%) and ammonia (10%) was termed the eluate principle.

Although attempts to identify the filtrate and eluate principles with known compounds were unsuccessful, some information concerning their properties was obtained. Both principles are soluble in 70% ethyl alcohol and are unaffected by autoclaving in the presence of normal solutions of acid and base or by exposure to light. The filtrate principle is partially inactivated by treatment with nitrous acid and completely inactivated by hydrogen peroxide while the eluate principle is virtually unaffected by both of these reagents.

The results in Table III show other differences between the filtrate and eluate principles. The similar behavior of the filtrate principle and casamino acids made it seem likely that the filtrate principle was an amino acid. However, of a number of amino acids tested, all were inactive except norleucine which was inhibitory. The possibilities remain that the filtrate principle is an amino

TABLE III.
Growth Promoting Effect of Various Materials in Cyanide Medium.

Material tested	Apparent potency
Yeast extract (standard of reference)	1.0
Filtrate preparation	0.18
Eluate preparation	0.07
Casamino acids	0.20
Yeast extract (1 mg) + filtrate (1 mg equivalent)	2.1
Yeast extract (1 mg) + eluate (1 mg equivalent)	1.0
Yeast extract (1 mg) + casamino acids (1 mg)	2.0
Filtrate + eluate (equivalent amts of each)	1.0
Filtrate (1 mg equivalent) + casamino acids (1 mg)	0.40
Eluate (1 mg equivalent) + casamino acids (1 mg)	1.1
Filtrate + nicotinic acid (10 γ per tube)	0.27
Filtrate + folic acid (20 γ per tube)	0.22
Filtrate + nicotinic acid (10 γ) + folic acid (20 γ)	0.36

acid other than the ones tested or that its effect is a composite one due to several amino acids.

Even though nicotinic acid (or its amide) and folic acid were found to be somewhat stimulatory, neither individually nor variously combined did they behave in the same manner as did the eluate principle.

By repeated adsorption and elution from charcoal, a preparation was obtained which was approximately 8 times as active as that resulting from the initial treatment of yeast extract. A 6-fold concentration of the eluate principle was achieved by refluxing yeast extract with 95% ethyl alcohol and treating the soluble fraction once with Darco G-60.

Evidence indicating that the activity of eluate preparations does not depend on a single substance was derived by subjecting the principle to electrolysis at high voltage in the electrical transport apparatus described

by Williams and Truesdail.⁵ The active principle was not concentrated in a single cell but was distributed throughout the 4-cell system employed.

Summary. Yeast extract has been shown to reverse the inhibition of yeast growth caused by cyanide. No known compound was found which duplicated the action of yeast extract, although methionine and cysteine had some "anti-cyanide" activity. Excess pantothenic acid had a beneficial effect on growth under certain conditions.

It has been demonstrated that the action of yeast extract depends on at least 2 substances which can be separated by charcoal adsorption. Indirect evidence indicates that the activity of both filtrate and eluate principles is due to more than a single substance.

⁵ Williams, R. J., and Truesdail, J. H., *J. Am. Chem. Soc.*, 1931, **53**, 4171.

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Effects of Various Anesthetic Agents on the Blood Pressure of the White Rat.*

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In an investigation involving the role of the autonomic nerves in cardiovascular disorders it was found desirable to measure the

blood pressure repeatedly in rats without injury to the animals and to determine the effect of anesthetic agents on the blood pressure. A survey of the literature showed that the apparatus used was varied and that de-

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