Effect of Choline on Yeast Bioassay of Inositol.* (19284)

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During the application of the inositol microbiological assay of Atkin, Williams, Schultz, and Frey(1) to the determination of lipid inositol in blood plasma, it was noted that inhibition of the yeast growth occurred in the presence of plasma hydrolysates. This inhibition occurred with completely hydrolyzed lipid samples, insofar as all the lipid P was recovered as inorganic phosphate and was partially overcome with increasing amounts of the hydrolysate. It was found further that the free choline present was solely responsible for this effect. In this paper are summarized the effects of different amounts of choline and several related substances on yeast growth with standard amounts of inositol.

Methods and results. The basal medium was prepared according to the authors' method as reported by György(1), except that a commercial casein hydrolysate was used (Nutritional Biochemicals Corp.) and the pH of the medium was adjusted to 4.38. The assay was carried out in 25 ml Erlenmeyer flasks in a 30°C water bath for 16 hours. Growth was read turbidimetrically in a Coleman Universal Spectrophotometer at 600 μ. Identification of the inhibiting agent in plasma hydrolysates was ascertained after a check assay of a synthetic "hydrolysate," containing 21 $\mu\gamma$ M of inorganic phosphorus, 33 glycerol, 20.9 choline, 1.16 ethanolamine, 2.32 dl-serine, 60 μg inositol. With this mixture, typical inhibition occurred. It was found that choline alone was responsible for this inhibition. The other substances did not inhibit at the above levels or at levels equivalent to 31 times the inositol present on a molar basis.

Other compounds related chemically or metabolically to choline were also tested with inositol at molar ratios of 30 or 60 to 1. It was found that dimethyl ethanolamine inhibited growth of the yeast to the same extent as choline, whereas methionine, betaine, and choline phosphatides from liver had no effect.

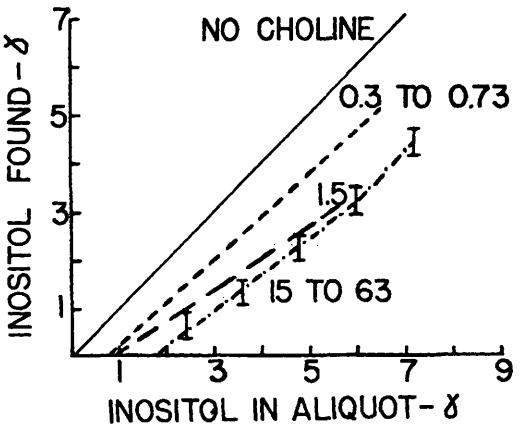

FIG. 1. Effect of choline on inositol assay using *S. carlsbergensis.* The figures accompanying the above curves represent the choline to inositol molar ratios of the particular samples assayed.

Variations in the choline content of hydrolysates of lipid extracts made it necessary to determine the effect of choline at various levels. Solutions were assayed containing choline to inositol molar ratios of 0.3 to 63. These results are given in Fig. 1. It is apparent that molar ratios as low as 0.3 cause measurable inhibition whereas maximum inhibition is reached at a ratio of 15:1 and possibly lower. Therefore, it is possible to establish a standard curve with choline present in excess of a 15:1 molar ratio and obtain values for plasma hydrolysates directly from this curve. A comparison of values obtained from such a curve with those read from the regular, "non-choline" curve has been made and is recorded in Table I. It appears that results calculated from the "choline" curve in this manner are more consistent and essentially valid.

Discussion. There is little information available on the effect of tissue preparations or biochemical substances on the growth of *Saccharomyces carlsbergensis* using this assay. Williams *et al.*(2) have reported that extracts of many biological materials contain sufficient amounts of stimulatory or inhibiting sub-

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TABLE I. Comparison of Plasma Lipid Inositol Values Based on Standards With and Without Added Choline.

Plasma hydrolysate dog sample	Inositol found—calculated from normal standard curve		Inositol found—calculated from standard curve with added choline	
	Range γ moles	Avg γ moles	Range γ moles	Avg γ moles
A, B	4.49–6.64	5.47	10.7 –12.17	11.46
C	1.01–2.41	1.97	3.70– 3.99	3.89

stances to interfere with the inositol assay using *S. cerevisae*. These substances were not identified and their effects were blanked out by addition of an autolysate of the material to be tested. In the basal medium for *S. cerevisae* devised by Woolley(3,4) the choline to inositol molar ratio at the assay level is approximately 5:1 on the basis of added choline alone. Hence if *S. cerevisae* is inhibited by choline similarly to *S. carlsbergensis* this effect would largely be obscured by the choline content of the basal medium. On the other hand, the choline effect may be specific for the *S. carlsbergensis*.

The low molar ratios of choline to inositol capable of inhibiting the growth of *S. carlsbergensis* limit the use of this assay under these conditions. Many tissues or tissue extracts would be expected to contain choline equivalent to a choline to inositol molar ratio of at least .3. However, this effect can be readily corrected for by the addition of sufficient quantities of choline to the basal medium.

It should be emphasized that the capacity of the organism to overcome the inhibition is enhanced with higher levels of inositol for the amount of inhibitor is also proportionately increased. Inositol then appears to be necessary for the removal of choline. The failure of betaine, ethanolamine, and choline phosphatides to inhibit suggests metabolic pathways by which the inositol fed yeast might reduce the choline content of the medium. It is possible that one or more enzymes concerned with the conversion of choline to these substances may require inositol for its synthesis or action.

Summary. Growth inhibition of *S. carlsbergensis*, caused by choline during routine inositol assays has been described. The effect was noted at a choline to inositol molar concentration of 0.3 to 1 and maximum inhibition was reached at a ratio of 15:1. Similar inhibition occurred with dimethylamino ethanol but was not observed with ethanolamine, serine, betaine, methionine or choline phospholipides. For assay purposes, this inhibition was corrected for by the addition of comparable amounts of choline to the standard inositol tubes.

1. Atkin, L., Williams, W. L., Schultz, A. S. and Frey, C. N., Preprint of unpublished method (1944) (As reported by Gyorgy, Paul, *Vitamin Methods,* 1950).

2. Williams, Roger J., Stout, Anne King, Mitchell, Herschel, K., and McMahan, J. Raymond, Univ Tex. Pub. No. 4137, 1941, 27. (As reported by P. Gyorgy, *Vitamin Methods,* 1950).

3. Woolley, D. W., *J. Biol. Chem.,* 1941, v140, 453.

4. ———, *Biol. Symposia,* 1947, v12, 279.

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