are produced continuously from unstable strains of yeast, and since it is known (*2*) that these mutants produce a toxohormone-like substance, we thought this substance might be responsible for the death of the unstable yeasts in lactate media. In the same way, toxohormones obtained from cancerous tissues might have a similar effect. To test this hypothesis, we studied the effects of various toxohormone preparations (TH) on the growth of an unstable yeast strain, R6U2 (*3*), in media containing lactate as the carbon source.

Three toxohormone preparations from respiration-deficient yeast mutants and two from cancerous tissues were tested. The yeast TH preparations were obtained from the following RD mutants: T_7, obtained by treating *Saccharomyces cerevisiae*, strain S_2, with tripaflavine; Mn_6, obtained by treating strain S_2 with manganese salts; and r6u2, isolated from the RD mutants produced spontaneously from the unstable R6U2 strain. Carcinomas of the mammary and parotid glands of humans were used as sources for the cancerous toxohormones. Similar preparations were obtained from the parent yeast strain, S_2, and from noncancerous human tissue, for use in control experiments.

The toxohormone preparations, obtained according to the method of Yunoki and Griffin (*4*), depressed significantly the activity of liver catalase in mice injected with 25 mg. Preparations obtained in the same way from the parent yeast strain and from noncancerous human muscular tissue had no effect on liver catalase, even when mice were injected with 50 mg of the preparation.

The toxohormone preparations were dissolved in the semisynthetic medium of Lindegren *et al.* (*5*), containing sodium lactate instead of glucose, and the solutions were diluted to obtain final TH concentrations of 1 to 30 mg/ml. Samples (2 ml) of each dilution were placed in test tubes and sterilized at 110°C; each was inoculated with 0.1 ml of a suspension of R6U2, containing 2 million cells per milliliter, and incubated at 30°C for 24 hours with continuous shaking. Toxohormone-free controls were included, and similar experiments with a normal strain of *S. cerevisiae*, S_2, were performed.

Table 1 shows the results obtained in the experiments with strain R6U2. Toxohormone preparations from yeasts, as well as from cancerous tissues, inhibited the growth of strain R6U2. When the concentration of toxohormone was from 5 to 9.5 mg/ml, growth was inhibited by 50 percent. Similar preparations from normal yeasts and noncancerous tissues had no effect on the growth of strain R6U2. None of the preparations tested inhibited the growth of the normal yeast strain, so we conclude that the toxohormones produced by respiration-deficient yeast mutants and by cancerous tissues have a toxic effect on strain R6U2, inhibiting its growth when it is cultured on media containing lactate as the carbon source.

These results appear to support the hypothesis that the toxohormones obtained from respiration-deficient yeast mutants and from cancerous tissues have identical effects and could be the basis of a new method for the quantitative evaluation of toxohormone activity.

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30 September 1963

Zinc Stimulation of RNA and Protein Synthesis in Rhizopus nigricans

Abstract. *Addition of 1.7 × 10^{-5}M zinc sulfate to cultures of* Rhizopus nigricans *increases growth and substrate utilization. Analysis of cells during the course of growth, after addition of the metal, showed that there was an immediate increase in RNA, followed by a corresponding increase in protein and cell mass. The DNA content was affected to a lesser extent. It is postulated that Zn^{++} stimulates growth through a primary effect on RNA synthesis.*

It has been recognized for a long time that zinc has a profound effect on the growth and physiological behavior of many fungi. Because fungi synthesize such large amounts of mycelium under favorable conditions, metal deficiencies can be accentuated; thus a response to zinc can be demonstrated easily by adding a small amount of this metal to media prepared from reagent grade chemicals. Foster and Waksman (*1*) showed that Zn^{++} caused a marked increase in the growth and efficiency of glucose utilization, and a corresponding decrease in acid accumulation in a fumarate-producing strain of *Rhizopus nigricans*. The relative amounts of enzyme in fungi have been shown to be modified by Zn^{++}; Nason *et al.* (*2*) found that zinc deficiency in *Neurospora crassa* caused a diminution in the amounts of alcohol dehydrogenase and tryptophane synthetase while increasing the amount of nicotinamide adenine diphophatase. Protein content was reduced during the period of zinc deficiency. Zinc has been implicated in nucleic acid synthesis in other organisms. Webley *et al.* (*3*) reported that zinc-deficient *Nocardia opaca* had a lowered content of both RNA and DNA. Wacker (*4*) found that there was an increase in the amount of DNA and a marked reduction in RNA when *Euglena gracilis* was grown under conditions producing zinc deficiency.

Table 1. Nucleic acid and protein content of *Rhizopus nigricans* at various times after the addition of zinc to the growth medium.

Time after addition of Zn^{++} (hr)	Component in % of dry weight							
	RNA		Protein		DNA		Nucleotides	
	$-Zn^{++}$	$+Zn^{++}$	$-Zn^{++}$	$+Zn^{++}$	$-Zn^{++}$	$+Zn^{++}$	$-Zn^{++}$	$+Zn^{++}$
0	8.32	8.32	31.2	31.2	0.622	0.622	0.107	0.107
3.5	7.36	12.16	30.4	36.8	.612	.738	.091	.327
9	6.40	8.83	29.6	38.5	.593	.680	.082	.186
15.5	6.13	7.57	28.8	34.4	.582	.612	.075	.110

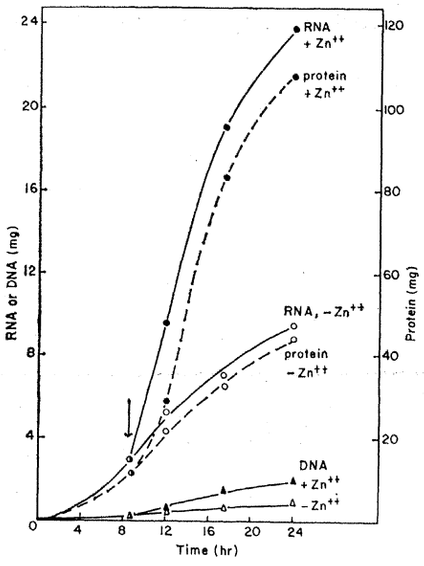

Fig. 1. Effect of zinc on rates of formation of RNA, protein, and DNA; $1.7 \times 10^{-5} M$ Zn^{++} added at time indicated by arrow.

We have determined the effect of adding 5 parts per million of $ZnSO_4$ ($1.7 \times 10^{-5} M$) to a growing culture by comparing the rates of formation of RNA, DNA, and protein in cultures with and without the addition of zinc. Cells were grown in a medium containing 9 g of glucose, 5 g of acid-hydrolyzed, salt-free, vitamin-free casein hydrolysate, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $Fe_2(SO_4)_3$, and 0.01 g of $CaCl_2$ per liter of de-ionized distilled water. Cultures (50 ml in 250-ml flasks) were incubated on a rotary shaker (250 rev/min) at 32°C. Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was added after an initial incubation period of 8.5 hours and samples were taken at suitable intervals thereafter for analysis. Cell weight was determined by filtering on tared filter assemblies and then drying to constant weight at 70°C. Protein content was determined (*5*) after extraction of dried cells with 2*N* NaOH at 100°C for 15 minutes. Free nucleotides, RNA, and DNA were measured by the method of Ogur and Rosen (*6*). Residual glucose in culture filtrates was determined by the method of Folin and Malmros (*7*).

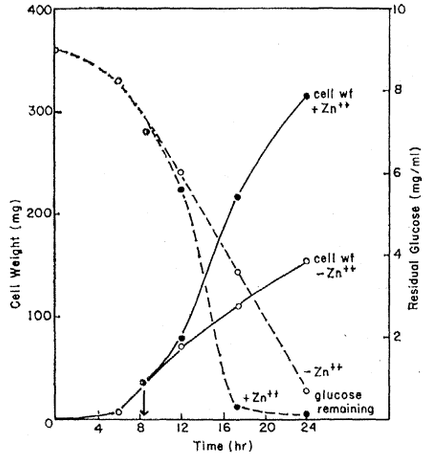

Fig. 2. Effect of zinc on growth and glucose utilization; $1.7 \times 10^{-5} M$ Zn^{++} added at time indicated by arrow.

Addition of zinc immediately stimulated RNA synthesis (Fig. 1). Protein synthesis was also greatly stimulated, but there was a lag before the rate of synthesis was maximum. The increase in RNA during the first 3.5 hours after Zn^{++} addition was 2.7 times as great in the presence of the metal as it was in its absence; in contrast, during the same period, the increase in protein was only 1.6 times as great in the presence of Zn^{++} as in its absence. In the subsequent 5.5-hour period, both RNA and protein increased at the same relative rate. The DNA levels were not increased to the same extent as were RNA and protein.

The pattern of growth and glucose utilization after stimulation by zinc was similar to that found for protein synthesis. Rates did not reach a maximum until 4 hours after the zinc was added (Fig. 2).

The early disproportionate increase in RNA occasioned by Zn^{++} is also shown in Table 1. The RNA content was greatest immediately after the Zn^{++} was added, and it decreased as other cell constituents were synthesized. Protein content was not at a maximum until 9 hours after the addition of Zn^{++}.

The mechanism by which Zn^{++} stimulates RNA synthesis is not known. The increase in free nucleotides, after addition of the metal (Table 1) may indicate a role in the synthesis of purines and pyrimidines or nucleotides, but other explanations are possible. Wacker and Vallee (*8*) found zinc associated with RNA from diverse sources and postulated that the metal may play a role in maintaining the configurational stability of the molecule. Also, it is not yet known whether zinc stimulates synthesis of all RNA's, or whether there is a specific effect on soluble, messenger, or ribosomal RNA (*9*).

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Bromine Analysis in 5-Bromouracil-Labeled DNA by X-ray Fluorescence

Abstract. *The degree of replacement of thymine by 5-bromouracil in the deoxyribonucleic acid (DNA) of the thymine-deficient bacterium* Escherichia coli B *15 T⁻ has been determined by a nondispersive x-ray fluorescence method. A measure of the fraction by weight of bromine in the purified DNA is required for determining the degree of thymine replacement. This fraction is experimentally determined on 35-μg samples of DNA by x-ray spectroanalysis.*

A number of investigators have observed the changes in radiosensitivity of deoxyribonucleic acid (DNA) resulting from incorporating halogenated pyrimidines and purines into nucleic acids. The change in radiosensitivity of bacteria (*1*), viruses (*2*), and cell cultures (*3*) as a result of replacing the thymine of DNA by halogenated pyrimidines has been studied.

In this type of study it is essential to establish the degree of replacement of thymidine by its analog. From a review of the literature the extent of replacement has usually been determined by paper chromatography, density gradient studies, or radioactivity measurements of incorporated Br^{82}-bromouracil. Recent work in our laboratory has required a knowledge of the degree to which thymine is replaced by 5-bromouracil in the DNA of the thymine-deficient mutant *Escherichia coli B* 15 T⁻. In order to measure the degree of replacement we have developed a relatively simple technique that depends upon the determination of the content (percent by weight) of bromine in the isolated and purified DNA by nondispersive x-ray emission analysis.