creased to $10^{-3}M$ while the calcium was held at $10^{-3}M$, the animals responded for 4 minutes (*9*).

The feeding response elicited by zinc resembled the response activated by glutathione, although occasionally some tentacles assumed a transitory (2 to 3 seconds) hypercontracted appearance. Long exposure (18 hours) to $10^{-3}M$ zinc in the presence of $10^{-3}M$ $CaCl_2$ in the test solution proved toxic to *Hydra*. The zinc-activated response was an effective one in that the *Hydra* ingested small inert objects offered to them. Zinc also activated a response in *Hydra pirardi*.

To determine whether the activation by zinc was a typical response to metals, we tested the effects of copper, nickel, cobalt, cadmium, lead, and uranyl ions. None acted in the same way as zinc. On the contrary, depending upon the experimental conditions, these metals inhibited the feeding response to glutathione, and were toxic to *Hydra littoralis*.

Zinc-calcium interactions are known, for example, both in nutrition, where effects of high calcium diets can be relieved by zinc (*10*), and in muscle physiology, where the twitch potentiation of the frog's sartorius induced by zinc (*2*) can be reversed by calcium-ethylenediaminetetraacetic acid (*11*). In our experiments, calcium might either be competing with zinc for a single site on the receptor-effector system or be preventing zinc from reaching the site of its activity.

Since some of the biological and chemical actions of zinc and other non-tripeptide activators of the feeding reflex (*12*) are known, studies of their effects on *Hydra* might help elucidate the nature of the receptor or of some of the components of the receptor-effector system. For example, Gurd and Goodman (*13*) and Vallee *et al.* (*14*) have shown that zinc can be bound to the histidine, sulfhydryl, or terminal α-amino of proteins. Since studies of the effect of *p*H (*15*) indicate that histidine and an α-amino might be at the glutathione-receptor site, zinc could possibly activate feeding in *Hydra* by binding these groups.

The activation of feeding in nature by free zinc is probably not of ecological significance because a concentration as high as $10^{-3}M$ is toxic and would most likely not be encountered there. It is possible, however, that glutathione acts by making "bound" zinc available to a zinc-requiring apoenzyme. Alternatively, zinc, known to act on muscle (*2*), might cause effector myofibrils to contract. A third possibility is that zinc becomes bound to some of the receptors, causing a slight change in their tertiary structure in a manner analogous to that proposed for glutathione (*2*), thus leading to the activation of the receptor-effector system (*16*).

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9. Experiments such as these stress the necessity for rigorously controlling the medium in which the *Hydra* (and the receptors) are bathed. A list of some factors affecting the glutathione-activated response is given by Lenhoff (*3*, p. 204). This point has especial bearing in evaluating experiments in which non-tripeptide activators are used on *Hydra*, if these experiments are carried out in the conventional filtered pond water, as, for example, were those of H. Forrest [*Biol. Bull.* **122**, 343 (1962)]. Unfortunately, in such experiments we do not know the ionic composition of that solution or even the *p*H. Furthermore, during the filtration procedure significant concentrations of glutathione (only $10^{-8}M$ is needed to elicit feeding) possibly are emitted from the myriad of aquatic plankton found in pond water. Thus, although such experiments might provide insight into mechanisms, it is impossible to repeat the conditions given in the report, much less interpret the experiments.
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Toxohormone Inhibitory Effect on the Growth of an Unstable Strain of Yeast

Abstract. *Toxohormone preparations obtained from yeast mutants with impaired respiration and from cancerous tissues inhibited the growth of an unstable strain of yeast in media containing lactate as the carbon source. Depending on the source of the different toxohormone preparations, amounts ranging from 5 to 9.5 mg/ml inhibited growth by 50 percent. This phenomenon could be utilized in quantitative evaluations of toxohormone activity.*

Unstable strains of yeast produce spontaneously large numbers of respiration-deficient (RD) mutants. In ordinary sugar media, populations of these unstable strains often contain more than 50 percent of RD mutants (*1*). When one such unstable strain was cultured in a medium containing lactate as the carbon source, it grew normally, but the number of living cells decreased rapidly after the 3rd day of inoculation so that the culture was almost sterile after 2 weeks.

Since respiration-deficient mutants

Table 1. Growth of an unstable strain of yeast (R6U2) in a medium containing lactate as the carbon source, to which toxohormone preparations were added at different concentrations. The results are expressed as millions of cells per milliliter after incubation for 24 hours. Each figure represents the mean of five experiments.

Source of toxohormone	Concentration of toxohormone (mg/ml)							
	30	25	20	15	10	5	1	0
T_7 RD yeast mutant	1.3	1.6	2.1	2.8	4.6	6.9	10.0	10.2
Mn6 RD yeast mutant	0.7	1.0	1.5	2.5	4.0	6.2	9.3	10.2
r 6u2 RD yeast mutant	1.1	1.7	2.4	3.4	4.9	7.3	9.5	10.2
S_2 normal yeast strain	9.9	9.5	11.0	11.3	10.8	10.0	10.6	10.2
Mammary carcinoma	1.0	0.8	1.2	1.4	2.5	5.0	8.6	10.2
Parotid gland carcinoma	0.8	0.8	1.0	1.9	3.3	5.7	9.1	10.2
Noncancerous muscular tissue	9.9	10.0	10.0	12.1	10.6	11.4	10.2	10.2

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