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A STUDY OF THE EFFECTS OF 17-ß-ESTRADIOL ON THE GROWTH OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

[1]*Saccharomyces cerevisiae* possesses a high-affinity estrogen binding protein and endogenous ligand that effectively displaces [^{3}H]17-ß-estradiol from the yeast binding protein and mammalian estrogen receptors. [2]Semi-purified preparations of this ligand have been shown to exhibit estrogenic activity in mammalian systems. [3]This study investigated the possibility that estradiol functioned in yeast to increase cell number and/or cell size. [4]Yeast cells were introduced to culture media containing concentrations of estradiol ranging from 0.66pM to 25uM. [5]These cultures were monitored spectrophotometrically and microscopically for changes in the cells. [6]In cultures with estradiol concentrations ranging from 0.66pM to 12.5uM there were no changes observed in cell size or number. [7]However, when the yeast was exposed to estradiol in a concentration of 25uM, growth was completely inhibited. [8]Therefore, estradiol, in physiologically tolerable concentrations, had no effect on *Saccharomyces cerevisiae* cell growth.

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INTRODUCTION

The origin of hormones during evolutionary development is unclear. That unicellular organisms communicate by message molecules is well documented (Barksdale et al., 1969; Gooday et al., 1974). Roth et al. (1982) provided evidence that vertebrate peptide hormones are present in unicellular organisms, however, much less is known about steroid hormones. The existence of binding proteins with high affinity for vertebrate steroid hormones has been demonstrated for several unicellular fungi including *Candida albicans* (Loose et al., 1981; Loose et al., 1982; Loose et al., 1983a), *Paracoccidioides brasiliensis* (Loose et al., 1983b), *Coccidioides immitis* (Powell et al., 1983) and *Saccharomyces cerevisiae* (Feldman et al., 1982; Feldman et al., 1984b; Burshell et al. 1984).

In recent years workers have discovered that *S. cerevisiae* does indeed contain receptors for estradiol and that they have shown that estradiol is actually a yeast product. They extracted ~800ng/1.5kg yeast, however, the amount of estradiol being produced and a physiological function associated with it has not been determined (Feldman et al., 1984a).

According to Feldman et al. (1984b) the two most likely possibilities for the presence of estradiol in *S. cerevisiae* are that the yeast uses this hormone system to communicate about food or sex. An effect on either of these should reflect on the overall amount of growth and the rate at which such growth would take place. This study investigated the possibility that this relationship did indeed exist, hypothesizing that under the influence of an appropriate concentration of estradiol, the yeast will respond with an increase in cell number and/or cell size.

The finding of estradiol (and other hormones) in this unicellular organism has significance in a variety of areas. Firstly, the presence of estradiol in this simple yeast indicates that the enzymatic system necessary for steroid synthesis must exist very early in evolution (Loumaye et al., 1982).

Second, the presence of hormonal levels of the fungal product, in addition to the estrogen binding protein, adds support to the hypothesis that *S. cerevisiae* posesses a hormone-receptor system analagous to those present in vertebrates (Burshell et al., 1984; Feldman et al., 1982). However, "although the hormone appears the same, we do not have data indicating the nature of the functional response mediated by this system in yeast" (Feldman et al., 1982).

A third area of significance involves the possible use of yeast as a model system to gain a better understanding of the mechanism of steroid hormone action. "Although we have not proven that estradiol acts as a fungal 'hormone,' the presence of both a receptor-like protein and a hormonal endogenous ligand makes this more feasible" (Feldman et al., 1984a).

The last area of significance is that of bidirectional hormonal interactions between fungi and higher organisms (Loose et al., 1981; Loose et al., 1982; Loose et al., 1983a; Loose et al., 1983b; Feldman et al., 1982; Burshell et al., 1984). This has been proven in the yeast *Paracoccidioides brasiliensis* by the inhibition of maturation in the presence of estradiol and in *Coccidioides immitis* by an increase in dissemination in females as opposed to males.

MATERIALS AND METHODS

A commercially dried culture of *S. cerevisiae*, type YSC-1, containing a mixture of both a and mating types, was purchased from the Sigma Chemical Co., St. Louis. A stock culture was maintained in 150ml. Difco Yeast Nitrogen Base medium (YNB) (6.7g/1) (Difco, 1984), with a glucose solution added (10g/1), and incubated in a Chicago Surgical and Electrical incubator at 27° C (Byrd et al., 1982; Feldman et al., 1982). Cultures were harvested at 3-4 days growth and centrifuged at 2000 RPM for 10 minutes in a Beckman J2-21 refrigerated centrifuge. The pellet was then washed three times with a saline solution as described by Loose et al. (1983b). The pellet was then resuspended in 150ml. of fresh YNB media and incubated once again.

The estradiol and all other chemicals used were reagent grade, purchased from Sigma unless otherwise stipulated. All experimentation was done using aseptic technique unless indicated otherwise.

Preliminary experimentation was conducted to determine: a mean growth curve, an appropriate concentration of yeast to be used in the actual experimentation, and the proper incubation time and temperature. These tests were also performed to determine the best instrumentation.

In the actual experimentation 20ml. of eight (8) hour log phase yeast cells were harvested from the stock culture and transferred to the appropriate flask containing 130ml. of YNB and varying amounts of estradiol for a total of 150ml. The amounts of estradiol used were: 0.66pM, 1.2pM, 6.6pM and 660pM. These concentrations were based on information that the estradiol was extracted in a concentration of ~800ng/1.5 kg (~0.66pM) of yeast (Feldman et al. 1984a). Respectively, these concentrations were: the same, 2X, 10X and 100X strength. Other concentrations picked randomly were the following: 25nM, 2.5uM, 12.5uM and 25uM.

These concentrations of estradiol were first dissolved in ethanol and placed into a water bath at 50°C so as to overcome the insolubility of estradiol in water. Upon complete dissolution in the ethanol, serial dilutions were carried out, using YNB media, to obtain the milli, micro and nanogram amounts of estradiol to be used (Pinto et al., 1984a).

The cultures were subsequently incubated at 27°C and their absorbances were measured on a Bausch and Lomb Spectronic 21 at 600nm (Dawes, 1983).

Microscope slides were also prepared of the various concentrations at randomly selected intervals during their incubation. These slides were stained with methylene blue and observed under light microscopy using a Bausch and Lomb One-Fifty microscope and under light, dark and phase contrast microscopy using a Nikon LABOPHOT microscope. Upon careful observation appropriate pictures were taken of the important morphological characteristics of certain cells using a Nikon FX 35 and Nikon Land cameras.

RESULTS

The results obtained, compared with those of the controls, showed no effect on the cells' growth in any of the cultures with an estradiol concentration ranging up to but not including 25uM, where complete inhibition was observed (Fig. 1).

Three points can be made from these data: (1) the steroid tested does not cause a change in the time to the onset of exponential growth, (2) there was no change

in the rate of exponential growth, implying no change in the length of the cell cycle, and (3) there was no change in the cell concentration at the plateau phase after treatment.

In regard to the 25uM concentration's inhibition of growth, its cause can only be speculated at this time; the most probable being that in such a high concentration the molecule exhibited a toxic effect on the yeast.

Upon observation of the slides and photographs it was concluded that there was no effect on the morphology of any of the cultures except the 25uM culture. All other cultures appeared normal for their particular life stage.

The lag phase cells appear less dense and smaller than those of the log phase. There was also an obvious decrease in the number of budding cells in the lag phase as compared to the log phase. This was the case for all of the cultures except that of the 25uM. In the early stage of the 25uM culture (0-12h) cellular budding was virtually absent. This was also the case in the late stage of the 25uM culture (12-48h) lending support to the spectrophotometric evidence that there was no growth.

It was also determined, through microscopic examination of the cultures, that there was no bacterial or other contamination which may have interferred with the results obtained.

DISCUSSION

As the results imply, there is no growth response associated with 17-ß-estradiol in *S. cerevisiae*. None of the experimentation showed any change in the growth except that of the 25uM culture, leading to the conclusion that the original hypothesis was incorrect.

Another hypothesis correlating estradiol with *S. cerevisiae* would be to repeat the experimental protocol but with separate haplotype populations (a and). Then mix the two haplotypes and determine if the mixed culture resulted in an increase in the absorbance compared to the separated culture. If an increase was observed in the mixed culture it would indicate that estradiol functions to increase the fusion of the haplotypes (Feldman, 1985).

The most recent hypothesis being proposed (Feldman, 1985) is that the yeast are using an enzyme system of their own and producing estradiol (and other hormones) from the nutrients in the media. However, this hypothesis is still under investigation so no valid conclusions may be drawn at this time.

One other hypothesis that is in need of further investigation is that it is not estradiol that functions in the yeast but a precursor or product of it. A molecule such as testosterone may be the active steroid that affects growth or sex and after it is used it is converted to estradiol. This is why there is such a high specific-binding of estradiol in the yeast. The other possibility is that estriol or estrone is being used as the primary steriod for growth or sex and that before it is produced the estradiol is being detected in the binding assays.

Both of these possibilities show some degree of validity since many different steroids have been detected in yeast (Loose, 1983b). Other support comes from the fact that humans can produce estradiol from testosterone as well as produce estriol and estrone from estradiol (Hadley, 1984). Therefore, if it is correct to assume that these hormones were conserved evolutionarily, then the precursors, products and enzymes necessary for conversion may have been conserved as well.

SUMMARY

The mammalian hormone 17-β-estradiol has recently been discovered in the yeast *Saccharomyces cerevisiae* (Feldman, 1984b). However, no one has as yet associated its presence with any physiological function. This study investigated the hypothesis that estradiol functioned to increase cell number and/or cell size in *Saccharomyces cerevisiae*. The methods used to examine this hypothesis were to culture *S. cerevisiae* in the presence of estradiol in various concentrations ranging from 0.66pM to 25uM. These cultures were monitored spectrophotometrically and microscopically for any deviation from the controls. The results obtained showed that, in cultures whose estradiol concentrations ranged from 0.66pM to 12.5uM, there was no deviation from that of the controls. However, the 25uM culture showed complete inhibition of growth which was most likely due to a toxic effect of the estradiol on the yeast. These results led to the conclusion that estradiol had no effect on *S. cerevisiae* when introduced in physiologically tolerable concentrations. Therefore, the estradiol must serve another function in the yeast but to date there is no evidence of any particular function.

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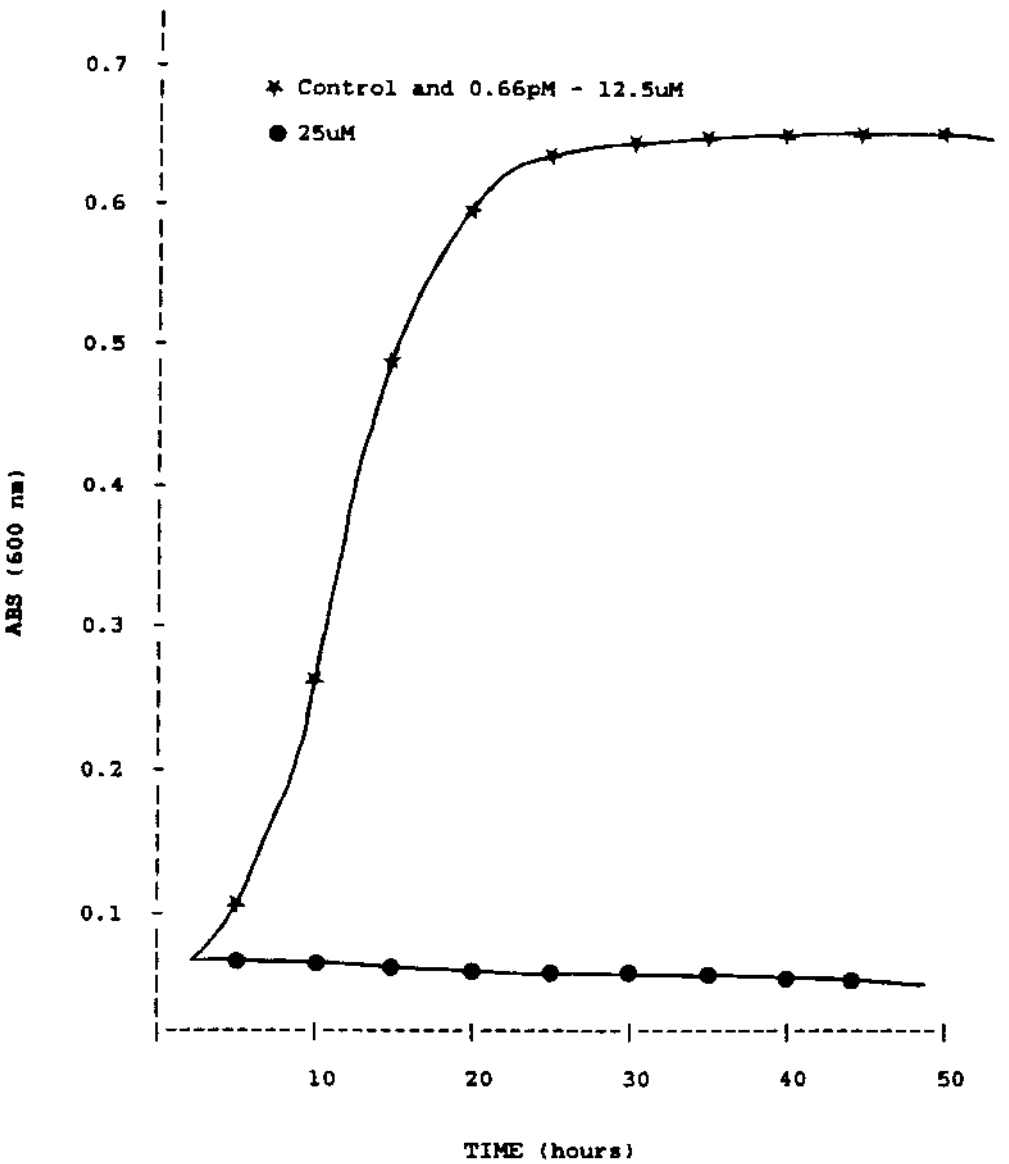

Fig. 1 Growth curves of estradiol concentrations ranging from 0.66pM to 25uM compared with the control.