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Effect of the He-Ne Laser Radiation on the Reproduction Rate and Protein Synthesis of the Yeast

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The reproduction rate and protein synthesis of the yeast-like fungus *Endomyces magnusii* and yeast *Torulopsis sphaerica* was studied after the He–Ne laser ($\lambda = 632.8$ nm) irradiation. The synthesis of protein in E. magnusii was activated in dose range from 0.42 to 0.84 J/cm^2 with maximum at 0.63 J/cm^2. In *T. sphaerica* the maximal activation took place at 0.42 J/cm^2. The lag-period was not changed, but the exponential period of the growth was reduced by 1.5 hours for irradiated cells.

The low intensity radiation of He–Ne laser (632.8 nm) has been used in clinical practice for wound healing for the past ten years.[1,2] Despite the application of this kind of treatment in many clinics, the mechanism of the stimulating action of red light is still not clear. Most experimental works about the red light action has been performed on the mammalian organisms and in these cases the observed stimulation occurs as a result of many complex reactions. In our previous paper[3] tissue culture was used to illustrate that the "red light effect" at the molecular level is

characterized by changes in nucleic acids synthesis rate and in cell membrane permeability. The cell culture is a complicated model and some simpler ones are needed to disclose the metabolic processes which form the basis for the mechanism of stimulating action of He–Ne laser. The cells of microorganisms, yeast in particular, are a good model for biochemical studies. They have all the features of eucaryotic cells and at the same time are simple and handy objects for laboratory research.

Numerous data on the light sensitivity of microorganisms are available in literature.[4] However, it is mainly concerned with the suppressing action of light of different spectral characteristics, basically in the UV region, on the vital activity of cells. There is much less data on the stimulating and inhibiting action of visible light on yeast cells[6,7] and the information about the stimulating action of red light on the vital activity of yeast is inconsistent. This paper is the first step in mastering the model to continue our work to clear up the mechanism of biostimulating action of red light ($\lambda = 633$ nm).

Thus, the tasks of this work are:

1. To study the action of different irradiation doses of He–Ne laser ($\lambda = 632.8$ nm) on the rate of reproduction and synthesis of protein in the *Endomyces magnusii* and *Torulopsis sphaerica* yeast.
2. To clear up the activating doses of He–Ne laser irradiation and optimal terms of the stimulation of the vital activity of yeast.

METHODS

The yeast-like fungus *Endomyces magnusii* V.K.M. reproducting by division and the yeast organism *Torulopsis sphaerica* V.K.M. reproducting by budding was used by us as models in studying the action of red light.

The culture of *Endomyces magnusii* was grown for 12 to 16 hours in liquid Rieder medium or in wort (7 Bal) in rocker-mounted flasks, then separated from the nutrient medium by centrifuging. *Torulopsis sphaerica* was grown in Petri dishes in agar-wort for 8 hours, then washed off from agar with sterile tap water and centrifuged. The thickness of the yeast suspension used for irradiation, was strictly controlled colorimetrically (1.1×10^7 and $3 \cdot 10^7$ cells in 1 ml for *T. sphaerica* and *E. magnusii* respectively).

The irradiation was performed in a glass bottle (2.5 cm in diameter) by a beam of 5×10^{-3} W. He–Ne laser focused up to the dimensions of the bottle bottom. During irradiation the suspension of *E. magnusii* was stirred with a magnetic mixer all the time. Different radiation doses were realized by varying its duration from 60 to 360 s.

The irradiated yeast was placed into the wort in rocker-mounted flasks at 28°C for further growing. After certain terms of cultivation the yeast was separated from the nutrient medium by centrifugation, washed three times with water, and a suspension of a strictly definite volume was prepared. One milliliter of suspension was used to determine the number of cells and buds by counting them in a Goryaev chamber with a microscope. The rest of the suspension was subjected to hydrolysis in 0.1 M NaOH in a water bath for 10 min and protein was determined by the Lowry method.[5]

In all control samples the cells of yeast subjected to the same procedures as in the test ones, but were kept in darkness while the test samples were irradiated. The amount of accumulated biomass was determined from the quantity of protein synthesized by the cells, the rate of reproduction from the number of cells and buds in the culture.

RESULTS

Table I illustrates the action of radiation at various doses on the synthesis of biomass in two cultures of yeast *T. sphaerica* and *E. magnusii* (8 hours of cultivation).

TABLE I

Effect of different irradiation doses of He–Ne laser light on yeast biomass' accumulation. (The relative amount of protein accumulated by a yeast culture during 8 hours after the irradiation (in % to the control))

Sort of yeast	Dose (J/cm^2)	0.21	0.42	0.63	0.84	1.26	1.89	2.52
Endomyces magnusii		110±5	112±3	123±6	120±7	106±5	99±5	103±9
Torulopsis sphaerica		123±4	145±8	—	—	—	—	27±5

With increasing dose the biomass enhancement reaches maximal values (0.42 J/cm^2 for *T. sphaerica* and 0.63 J/cm^2 for *E. magnusii*) and then decreases to control level. A further increase in radiation dose can even cause the suppression of the vital activity of mecroorganisms as it has been observed for *T. sphaerica* at 2.52 J/cm^2.

Figure 1 shows the dynamics in changes of the number of the cells and buds in 1 ml of exposed and unexposed suspensions of *T. sphaerica.* The number of cells and buds in the exposed yeast culture becomes larger than in the control one after the culture passes from the lag-phase to the exponential phase of growth. The growth curves show that the duration of lag-period for the exposed and control yeast cultures almost does not vary, and the differences in the growth curves are due to the fact that the average generation time for exposed yeast is decreased by 1.5 to 1.8 times.

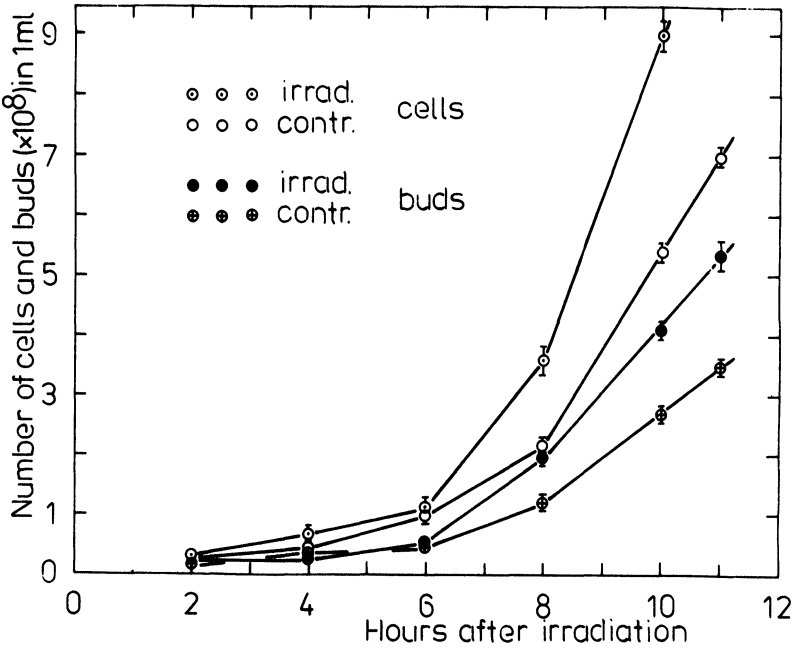

FIGURE 1 The effect of He–Ne laser irradiation (0.42 J/cm^2) on the reproduction of *Torulopsis sphaerica.*

In the next series of experiments we studied the dynamics of biomass' accumulation as a function of the cultivation time of yeast.

The curves given in Figure 2 show that the active accumulation of the biomass begins after the lag-period of the growth is over. The cells of the exposed culture reach the stationary phase of growth about 1.5 hours earlier than the unexposed ones. The maximum excess of the biomass accumulation in the exposed culture over the control one was observed in the second half of the exponential growth phase (Figure 3). Even though the reaction of various yeast organisms to the red-light irradiation is qualitatively similar, it may have essential quantitative differences. For instance, the amount of protein in the irradiated culture of *T. sphaerica* at the end of the exponential growth phase is almost twice higher than in the unirradiated culture, but for *E. magnusii*—only 30%. These differences are well reproduced from

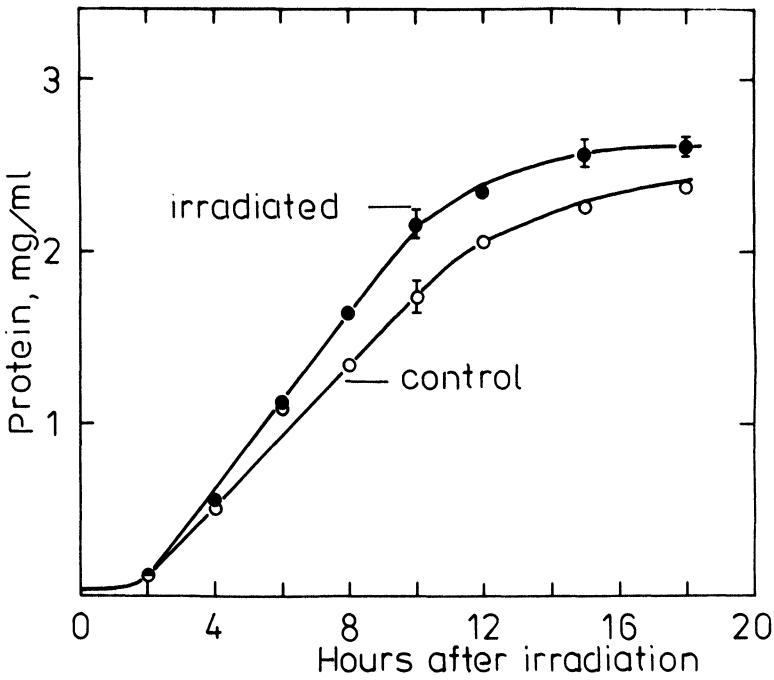

FIGURE 2 The effect of He–Ne laser irradiation (0.63 J/cm^2) on the synthesis of protein in *Endomyces magnusii.*

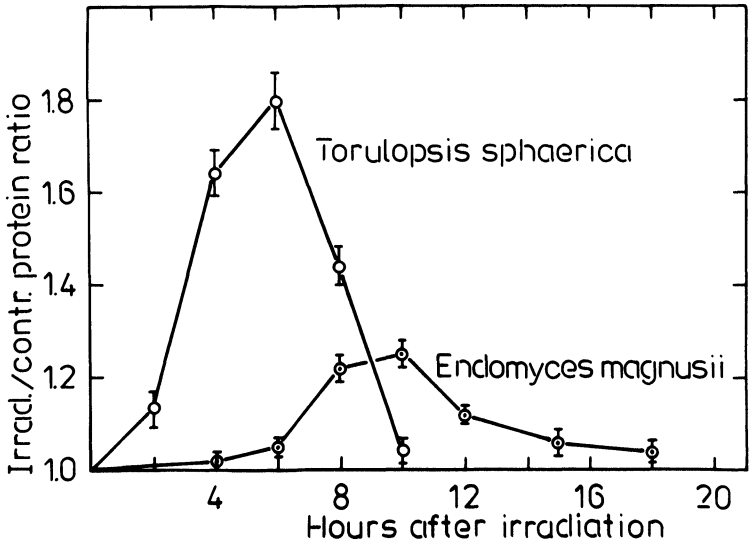

FIGURE 3 The ratio between the rates of reproduction of exposed and control yeasts measured from the quantity of synthesized protein.

the experiment to experiment and trustworthy as the estimations of these differences by the Student *t*-test show.

DISCUSSION OF RESULTS

Both concerned yeast cultures respond to irradiation with He–Ne laser. The reaction of yeast organisms is strongly dependent on radiation dose. The growth curves for the exposed and unexposed cultures have lag-periods of the same duration, and the exponential period of growth for the exposed culture is reduced by 1.5 hours. It can be seen from Figure 3 that the total biomass of yeast, as the exponential phase of growth passes into the stationary one, is almost the same for the exposed and unexposed cultures. On the other hand, the increased accumulation of biomass of the exposed culture was followed by a strictly proportional increase of the number of cells and buds at the exponential growth phase. From this it follows that the size of the cells and the amount of protein in single cell apparently don't differ for the exposed and unexposed cultures. Thus, the irradiation with He-Ne laser in the strictly definite doses leads to intensifying the

synthesis of protein and speeds up the preparation of cells for division and budding. It should be noted that we, unlike Rubin *et al.*,[6] have not observed any reduction in the lag-period, indicating changes in the accomodating reactions of the cell. Since in our work we used yeast organisms of other species than in Ref. 6, it seems to be that various representatives of this class of microorganisms respond in different ways to the stimulating action of visible and particularly red light. The reduction of the lag-period in the yeast after irradiation with red light is related by Rubin to the presence of the phytochrome system regulating the growth of *Candida guilliermondii.* In the yeast organisms *E. magnusii* and *T. sphaerica* studied in our experiments, the light-sensitive system is not clear. The mechanism of activating action of red radiation is still far from being clear. As it seems to us, in solving this problem we should call particular attention to studying the reaction of the enzymes of the red light exposed cell and, first of all, the respiratory enzymes participating in the generation of the energy, essential for protein synthesis and other biochemical processes in the cell.

As it has been noted, we could observe the activation of cell's vital activity over a rather narrow dose range. As the red light radiation dose is exceeded, the stimulating action disappeared, and at even higher doses (Table I) the growth of microorganisms becomes suppressed. Such a behaviour of the cells irradiated with the He–Ne laser, enables the latter to be applied as a subtle instrument for regulating the biochemical processes in cells into one or another direction.

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