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THE TOLERANCE OF HAPLOID YEAST CELLS *SACCHAROMYCES CEREVISIAE* TO THE PRESENCE OF CADMIUM IONS

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Introduction

The toxicity of heavy metals within the scope of total pollution of human environment has become a significant problem in present day society. Since the production, processing and consumption of articles containing heavy metals are growing daily, their concentration rises in all parts of the biosphere. Cadmium plays a considerable and significant part in this pollution problem because of its high toxicity to all living organisms. Extensive and multiform scientific research has been carried out in the world with the main purpose of investigating the effect of cadmium ions on living systems in order to safeguard the health of human beings (Piscator 1972, Friberg 1974, Webb 1976). However, this is not the only purpose of research work. The problem of the effect of cadmium has been studied on lower biological systems to discover how the cell mechanism works and how cadmium affects the living cell.

Cadmium has no natural function in the cell although it is sometimes found in traces only. After entering the organism of a mammal it is mainly retained in the liver and in the kidneys. There it has been found bound to specific proteins of small molecular weight, the so called metaloproteins (Cd.-binding proteins). It is considered that there are inducible enzymes which bind cadmium and thus protect the cell from its toxic effect. In lower biological systems, in bacteria *Escherichia coli* (Mitra 1975) and in fungi (Macara 1978) analogous proteins have not been found so far and it is therefore considered that some other mechanism of protection against cadmium exists in these systems. Bacterial systems tend to adapt to the presence of cadmium ions, which may be considered as a protective mechanism.

Fungi represent a higher biological organization than bacteria and belong to the eucariotic type of cell. Therefore an intermediate type of protection against cadmium may be expected. Besides, fungi are disseminated in nature in different ecologic environments where possibilities exist for their direct contact with various external agents and thus with cadmium ions too. A representative of the fungus *Sacchanromyces cerevisiae* has been used in this work because it is a doubly suitable experimental material : as a potential biologic carrier and vector of cadmium ions from outside into the human organism and as a lower eucariot representing a biological model for fundamental research.

Results of our research of the biological activity of yeast cells under the conditions of growth and in the presence of cadmium ions are presented in this work and possibilities of the adaptation of cells to toxic metals are reviewed.

Material and Methods

Organism: haploid strain of yeast *Saccharomyces cerevisiae,* N 123 (O g u r, 1954) was used as experimental material. The cells were grown in a complete nutrient medium of the composition: Yeast Extract "Difco", 5 g, Pepton "Difco", 10 g, Glucose "Kemika", 30 g, NaCl, 9 g to 1 litre of redistilled water. To achieve a solid medium, 20 g Bacto Agar "Difco", was added.

Methods: the growth of the yeast culture was watched by a cell count in the haemocytometer after Bürker. The cells from the stationary phase of growth were inoculated in the complete medium to which $CdCl_2$ ($1{,}0 \times 10^{-5}$ M; $4{,}0 \times 10^{-5}$ M; $8{,}0 \times 10^{-5}$ M) had been added. The cells were incubated in a thermostatic both at 30° C. The samples for the cell count were taken at fixed time intervals. The ability to form colonies was investigated by means of the plating method. After 2 to 3 days of incubation visible colonies were grown up.

Results

A comparison of the curves of growth (Fig. 1) between the control and the treated cells showed that the culture growth was slowed down if the cells were growing in the presence of cadmium that this was dependent on the concentration in the nutrient medium. Data presented in Table 1 showed that the retarded growth of the culture was induced by a prolongation of the time of cell division in the culture. Time expression of the biological effect of cadmium was also dependent on its concentration in the growth medium: lower concentrations had a weaker effect which was marked in the later phases of growth of the culture, while higher concentrations had a more intensive effect which was marked in the course of the early exponential phase of growth. The highest concentration of cadmium tended to stop growth as early as a few hours after incubation. The impression was gained that the period of activity of cadmium ions was the exponential phase of growth, that is during the period of intensive cell division. This conclusion is supported by the results shown in Fig. 2. By adding the same concentration ($8.0 \cdot 10^{-5}$ M) of cadmium to the culture at different time of exponential growth the cell division ceased during the first hour of growth after Cd^{2+} was added. However, when the same concentration of cadmium was added to the medium at the beginning of incubation, the slowing down of cell division occured later, i. e. after a few hours only.

Table 1. Doubling time of cells divided in the nutrient medium containing different concentrations of Cd^{2+}

Tabela 1. Vrijeme udvostručenja stanica koje se dijele u hranljivoj podlozi s različitim koncentracijama Cd^{2+}

CONC. OF Cd^{2+}	Control	1.0×10^{-6}M	1.0×10^{-5}M	$2.0 + 10^{-5}$M
Duplication time (min.)	90	120	120	150

4.0×10^{-5}M	6.0×10^{-5}M	8.0×10^{-5}M
180	240	540

Table 2. Effect of Cd^{2+} on the colonyforming ability of the cells

Tabela 2. Utjecaj Cd^{2+} na sposobnost formiranja kolonija stanica

Experimental groups Eksperimentalne grupe	Number of colonies (%) Broj kolonija %	
CONTROL GROUP KONTROLNA GRUPA	80	
	Cd^{2+} in liquid medium Cd^{2+} u tečnoj podlozi	Cd^{2+} in solid medium Cd^{2+} u čvrstoj podlozi
1.0—8.0 10^{-6} M Cd^{2+}	80	80
$1.0.10^{-5}$ M Cd^{2+}	70	0
$2.0.10^{-5}$ M Cd^{2+}	60	0
$4.0.10^{-5}$ M Cd^{2+}	20	0
$6.0.10^{-5}$ M Cd^{2+}	10	0
$8.0.10^{-5}$ M Cd^{2+}	1	0

% denote the colonies in relation to the number of plated cells

% označava broj kolonija u odnosu na broj zasijanih stanica

Colony-forming ability of cadmium treated cells

The yeast cells showed a reduced ability to form colonies (Table 2) after a 24-hour growth in the presence of cadmium ions. If the cells which had never been exposed to the effect of cadmium were spread on nutrient agar containing different concentrations of cadmium ions, the ability to form colonies was preserved only within the concentrations of $1.0—8.0 \cdot 10^{-6}$ M Cd^{2+}. High concentrations of cadmium completely inhibited the formation of colonies.

Reversibility of cadmium effect

After a 24-hour period of growth in the presence of cadmium ions the cells were rinsed in the physiological saline solution and inoculated in a nutrient medium without cadmium. The cultures were placed in optimal conditions of growth to continue the growth of the culture in the course of the next 24 hours, that is they reached the stationary phase. The growth of the culture was watched by counting cells at fixed time intervals. The kinetics of cell division presented in Fig. 3 showed that the cells did not divide in the course of the first 6 to 10 hours although they were in optimal conditions for growth. The duration of the *lag* phase depended on the concentration of cadmium ions in the medium in which the cells had been growing previously. After this period the cells began to divide in an approximately similar rhythm regardless of the concentration of cadmium in inoculum. In the stationary phase of growth all cultures treated with cadmium and the untreated controls showed an identical number of cells per millilitre.

Adaptation of yeast cells to cadmium

Yeast cells showed the ability of gradual adaptation to the presence of cadmium (Fig. 4). The cells which at first were grown at lower concentrations of cadmium ($1.0 \cdot 10^{-5}$ M) until they reached the stationary phase of growth (24 hours) were subsequently transplanted for further growth to a complete nutrient medium with a higher cadmium concentration ($4, 6, 8{,}0 \cdot 10^{-5}$ M).

The growth curves for each of these concentrations were watched separately. The results showed that yeasts pre-treated with a lower cadmium concentration grew equally fast as the control culture irrespective of the higher concentration to which they were transferred. In the stationary phase of growth, however, the treated cells showed a somewhat lower number of cells than the controls.

Discusion

Reproduction is one of the fundamental characteristics of the living world. A disturbance of this basic feature is a sign of significant jeopardy of biological existence. It was for these reasons that we used this biological parameter for the assessment of the effect of cadmium

ions on yeast cells. The results of our work show that cadmium ions affect the cells, causing retardation and stoppage of division. Since the cells of the control cultures continued to grow, this may be a sign that in the growth medium sufficient nutrient substances necessary for cell division were present. These data have prompted us to assume that cadmium ions limit the ability of yeast cells to make use of exogenous sources of energy. In favour of this assumption are some earlier data published in the literature (Lindegren 1973) indicating that cadmium ions induced microscopic changes in the structure of the mitochondria of yeast cells, which resulted in the appearance of respiratory mutant. The same authors succeeded in isolating mutant yeast which grew in the medium with cadmium indefinitely. The authors considered this to be an adaptation to the presence of cadmium ions. However, this mutant could grow only on a base with a certain concentration of metal. In our experiment the yeast showed adaptation to different concentrations, even to relatively high cadmium concentrations. One ought to bear in mind that the tolerance to cadmium depends on many factors in the experimental procedures due to which it is possible to find differences in the results.

The effect of cadmium ions in yeast is reversible. Yeast, in a medium without cadmium, undergoes normal cell division only after a cetrain period of time. It is very likely that within this period reparative processes take place on the molecular level and cause cell division. These processes are the more intensive if deoxyribonucleic acid (DNA) has been damaged by cadmium ions (Eger 1977, Mitra 1978).

The results of this work* show that the reaction of the cell tends to be of a defensive character and that the cell, in the presence of cadmium ions, suitably arranges its physiological activities for survival in the toxic environment.

Conclusion

Cadmium ions affect the yeast cells in the course of cell division, causing retardation and stoppage of division. If the treated cells are placed in conditions of growth without cadmium, the previous effect becomes reversible and cell division follows a normal rhythm. Previous treatment of cells with a lower concentration of cadmium ions makes normal growth possible even at very high concentrations.

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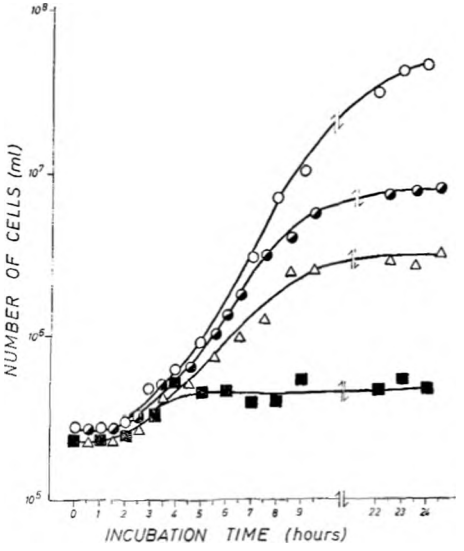

Fig. 1. Growth curves of the cells in the medium with different concentrations of cadmium.
Ordinate: number of cells/ml. Abscissa: incubation time (hours).
Symbols: control (○), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (◑), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8{,}0 \cdot 10^{5}$ MCd^{2+} (■).

Sl. 1. Krivulje rasta stanica u medijima s različitim koncentracijama kadmija.
Na ordinati označen je broj stanica/ml a na apscisi vrijeme inkubacije (sati).
Simboli: kontrola (○), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (◑), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8.0 \cdot 10^{5}$ MCd^{2+} (■).

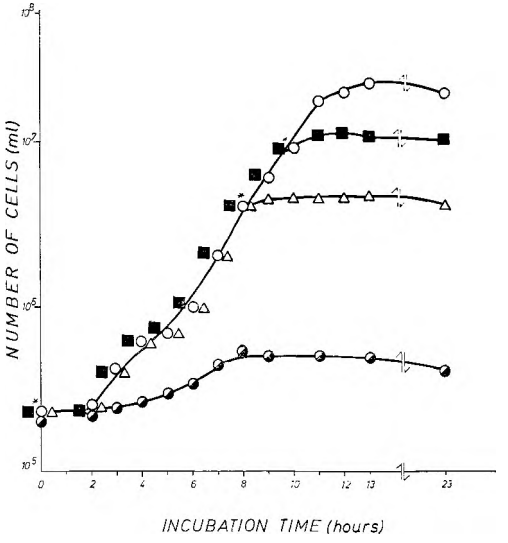

Fig. 2. The effect of cadmium ($8.0 \cdot 10^{-5}$ M) on the cells at different phases of growth.
* denotes the time when Cd^{2+} was added.
Ordinate: number of cells/ml, Abscissa: incubation time (hours).

Sl. 2. Utjecaj kadmija na stanice u različitim fazama rasta.
* označava vrijeme dodavanja kadmija.
Na ordinati označen je broj stanica/ml a na apscisi vrijeme inkubacije (sati).

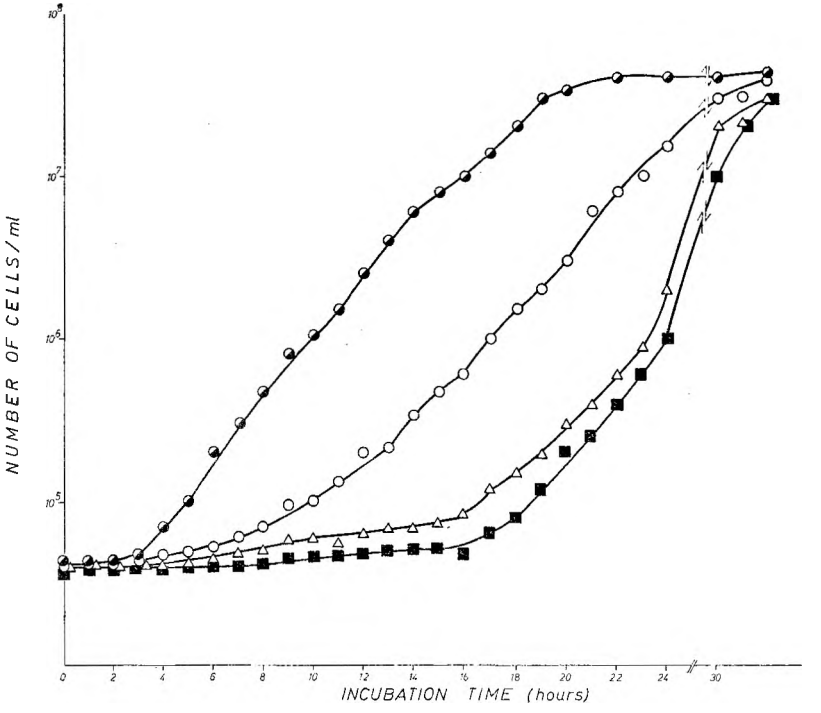

Fig. 3. Growth curves of the cadmium treated cells in nutrient medium without cadmium
Ordinate: number of cells/ml. Abscissa: incubation time (hours).
Symbols: control (◑), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (○), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8{,}0 \cdot 10^{5}$ MCd^{2+} (■).

Sl. 3. Krivulje rasta stanica tretiranih s kadmijem u podlozi bez kadmija.
Na ordinati označen je broj stranica/ml a na apscisi vrijeme inkubacije (sati).
Simboli: kontrola (◑), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (○), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8{,}0 \cdot 10^{-5}$ MCd^{2+} (■).

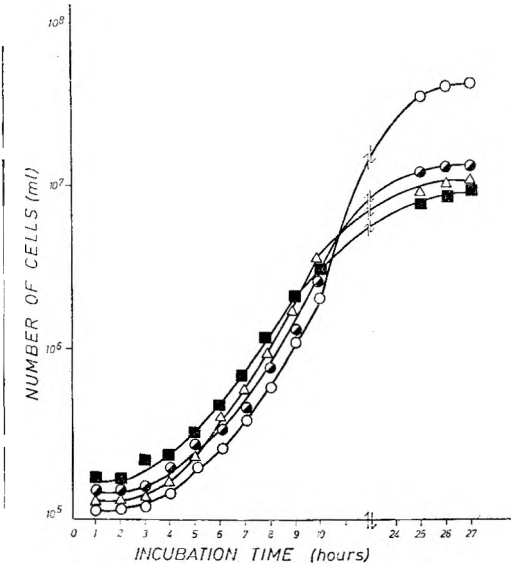

Fig. 4. Growth curves of the cells pre-treated with a lower concentration of cadmium and transferred to higher concentrations.
Ordinate: number of cellls/ml. Abscissa: incubation time (hours).
Symbols: control (○), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (◑), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8{,}0 \cdot 10^{5}$ MCd^{2+} (■).

Sl. 4. Krivulje rasta stanica prethodno obrađenih s nižom koncentracijom kadmija i presađenih u više koncentracija kadmija.
Na ordinati označen je broj stranica/ml a na apscisi vrijeme inkubacije (sati).
Simboli: kontrola (○), $1{,}0 \cdot 10^{-5}$ MCd^{2+} (◑), $4{,}0 \cdot 10^{-5}$ MCd^{2+} (△), $8{,}0 \cdot 0.10^{-5}$ MCd^{2+} (■).

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SAŽETAK

TOLERANCIJA STANICA HAPLOIDNOG KVASCA *SACCHAROMYCES CEREVISIAE* NA PRISUSTVO IONA KADMIJA

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Ioni kadmija usporavaju rast kulture kvasca i to progresivno s povišenošću koncentracije u hranjivoj podlozi. Djelovanje kadmija vezano je za diobeni ciklus stanice. Pri višim koncentracijama diobe stanica zaustavljaju se, iako u podlozi još uvijek ima dovoljno hranljivih supstancija potrebnih za rast. Nakon 24 sata rasta u podlozi s kadmijem stanice imaju smanjenu sposobnost formiranja kolonija. Ako se stanice, nakon 24 sata rasta u kadmiju, presade u podlogu bez kadmija, one će se dijeliti normalnim ritmom, ali tek nakon znatno duže *lag*-faze. Prema tome, može se reći da je efekt kadmija na stanice kvasca reverzibilnog karaktera. Prethodna obrada stanica s nižim koncentracijama kadmija omogućava njihov normalan rast u vrlo visokim toksičnim koncentracijama.

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