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Preface

Cellular lesions are related to macromolecular synthetic processes including the hierarchical flow of genetic information. Heavy metals generate oxidizing radicals through the Fenton and Haber-Weiss reactions leading to metal-induced carcinogenesis mediated primarily by the elevated levels of reactive oxygen species. Heavy metal-induced oxidative stress can lead to different types of cellular damages as a consequence of incomplete reduction of oxygen. Oxidative damage causes changes in DNA structure, the long term effects of which can lead to multiple mutations and malignant transformation. The detection of oxidative damages involves chromatographic, biochemical and immunochemical approaches. Early detection of cytotoxicity at structural and functional level of DNA combined with high sensitivity are the expected benefits of the approaches suggested in this book. The advantages of using cell cultures to measure the cellular toxicity of heavy metals are: controlled cell growth, known concentrations and time of exposure to metal ions.

The book summarizes the cellular effects of metals including in alphabetical order: Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Ta, U, W, Zn with respect to their impact on microbial, plant, yeast, insect and mammalian cells. Cellular effects of heavy metals involve: accumulation, mutagenesis, chromosomal changes, gene expression, activation of signal transduction pathways, apoptosis, transporters, protein binding, folding and degradation. These cellular changes affect not only the fate of cells but also our everyday life. The special website provides vivid performance of cellular movements of individual cells, cell divison and how cellular etology is influenced by the presence of heavy metals.

Cells have evolved sophisticated defense mechanisms to protect themselves agains heavy metal toxicity. At the genomic level many genes and regulatory pathways have been identified, but their implications on the higher order structure of the genetic material have not been investigated. To better define the impact of heavy metals on chromatin structure the effects of cadmium, nickel, chromium and silver in mammalian cells have been examined and compared with earlier studies on mercury and lead. Accumulating data suggest that the chemical properties of metal ions are the primary determinants in their biological effects. The three dimensional structures of heavy metal ions seem to influence their uptake by tranporters into cells and their oxidation potential, mutagenicity and carcinogenicity. As these last two main

properties are different, the genotoxic effects of heavy metal ions are also variable and characteristic to individual metals. To distinguish among morphological changes, data of heavy metal treatments have been converted to graphical presentations allowing the detection of normal behavior, apoptotic or necrotic cell death.

The wealth of information provided in the book and the additional information in the website provide information for a wide spectrum of audience. Besides the experts, universities, schools and students, scientists involved in chemistry and biology, particularly in DNA reserach including cell biology, genetics, biochemistry, molecular biology will find new information in this book, which is expected to have an intellectually stimulating impact on their future research. The book does not go into details regarding the effect of heavy metals on organisms with the notable exceptions of blood lead levels and heavy metal-induced carcinogenicity caused by depleted uranium and heavy-metal tungsten alloy in human and animal populations. The long-term low-grade toxicity is in most of the cases more damaging on the long-term leading to chronic illnes than a single acute heavy metal exposure which is rare. Due to the increasing concern of heavy metals pollutions world-wide, health service employees and non-professional readers will be equally attracted by the book.

Special Website

The reader will find a special Springer website; http://extras.springer.com/ entitled: "Long-term scanning system to visualize the cellular toxicity of heavy metals" orchestrated by Gabor Nagy, Melinda Turani, Kinga Ujvarosi and Gáspár Bánfalvi. This site deals with cellular ethology, and follows the fate of individual cells upon heavy metal treatment. The heavy metal induced cellular changes have been compared with the cellular movement of normal healthy cells.

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Abbreviations

C. albicans	*Candida albicans*
Cr	Chromium
Cr(VI)	Hexavalent chromium
Cr(III)	Trivalent chromium
EPR	Electron paramagnetic resonance
GSH	Glutathione
GSSG	Oxidized glutathione
GRd	Glutathione reductase
GPx	Glutathione peroxidase
ROS	Reactive oxygen species
S. cerevisiae	*Saccharomyces cerevisiae*
S. pombe	*Schizosaccharomyces pombe*
SOD	Superoxide dismutase
8-OHdG	8-oxo-7,8-dihydro-2'-deoxyguanosine
ATP	Adenosine-5'-triphosphate
BiP	Binding immunoglobulin protein
DIC	Differential interference contrast
EGTA	Ethylene glycol-*O,O'*-bis(2-aminoethyl)-*N,N,N',N'*-tetraacetic acid
FCS	Foetal calf serum
FITC	Fluorescein-5-isothiocyanate
Grp	Glucose-regulated protein
HSP	Heatshock protein
IC	Inhibitory concentration
LD	Lethal dose
MeHgCl	Methylmercuric chloride
MK	Modified Kitamura
NADH	Nicotinamide adenine dinucleotide (reduced)
PBS	Phosphate-buffered saline
RER	Rough endoplasmic reticulum
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SER	Smooth endoplasmic reticulum
YE	Yeast extract

EDTA	Ethylenediaminetetraacetic acid
GSH	Glutathione, reduced form
$HP2_{1-15}$	RTHGQ-SHYRR-RHCSR-amide
NTA	Nitrilotriacetate

Contents

Part I
Introduction

Chapter 1
Heavy Metals, Trace Elements and Their Cellular Effects

Gáspár Bánfalvi

Abstract The book starts with the brief review of chapters. In this chapter heavy metals have been redefined as those trace elements that have ≥ 3 g/cm^3 densities and may cause harmful biological effects. The chapter arrived to this definition by clarifying first the light elements on the basis of their electronic configurations and compatibility with those of bioelements (CHNOPS group) in constructing biomolecules. As compatibility criteria the chemical bond formation between *s–p* electrons and *p–p* electrons were taken, allowing the tetrahedral three dimensional construction of biological compounds with four bonding partners. The compatibility range ended at $1s^2 2s^2 2p^6 3s^2 3p^6 4s^2$ electronic configuration corresponding to calcium, which is the 20th element in the periodic table. From element 21 (Sc) the wide range of redox behavior, high reactivity, rich coordination chemistry and complex formation of transition metals is due to the outher *d* and *f* electron subshells and explain their important catalytic role in enzyme reactions and toxicity at higher cellular concentrations. The chapter describes the most important cellular effects of heavy metals. The advantages of changing from *in vivo* to *in vitro* cellular systems have been pointed out. The methods for the detection and determination of heavy metals in cells are summarized.

Introduction

Why Another Book on Heavy Metals?

The short answer to this question is that cellular and subcellular functions of heavy metals have neither been described in any detail nor summarized in a book. To the contrary the effect of heavy metals on organs and organisms has been intensively

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studied. This is indicated by a fast search in PubMed by entering the words "heavy metals" and getting more than 338,000 publications (as of January 2011). After justifying the requirement of a book dealing with the cellular effects of heavy metals one can omit lengthy discussions of general toxic effects, comprehensive reviews of heavy metals dealing with the physiology, including nutrition, intestinal absorption, heavy metal poisoning, excretion, homeostasis and their role in the function of different organs (heart, muscle, liver, lung, brain, kidney etc.).

Brief Review of Chapters

Before going into the details of Chap. 1, a brief outline of Chaps. 2–16 is given.

Chapter 2. The aim of this chapter is the biotechnological evaluation of data accumulated in the last decade on the molecular background of the toxic metal/metalloid tolerance of fungi.

Chapter 3 describes that although, Cr(VI) reduction itself may proceed outside the cell, it is now generally accepted that Cr(VI)-induced DNA damage and genotoxicity takes place intracellularly. The extracellular reduction of Cr(VI) to Cr(III) is regarded as a detoxification process, as Cr(III) crosses the cell membrane at a much slower rate than Cr(VI). There are certain Cr(V) and Cr(III) complexes generated extracellularly that have high permeabilities and consequently may penetrate into the cell and cause intracellular damage. The reduction of Cr(VI) to Cr(III) with different antioxidants is not only a detoxification reaction, but also increases the viability of the budding yeast *Saccharomyces ceravisiae*.

Chapter 4. The arsenic tolerance mechanism of yeast cells is elucidated by reviewing the molecular biology of arsenic tolerance in budding yeast, focusing on arsenic sensing, signalling and detoxification mechanisms and how these pathways are regulated.

Chapter 5. Heavy metal toxicity of methyl-HgCl, $HgCl_2$ and $CdCl_2$ in the *Aedes albopictus* insect cell line is discussed. Short treatment of *Aedes albopictus* cells with sublethal doses of $CdCl_2$ and $HgCl_2$ induced abnormal microtubular polymerization giving the cells a neuron-like appearance, while MeHgCl was not able to induce such neurite-like processes. Viability and proliferation assays showed clear distinction among the toxicities of Cd, Hg and MeHg reflecting differences of their mechanisms of action.

Chapters 6–9. The effect of heavy metal ions (Cd^{2+}, Ni^{2+}, Cr^{6+}, Ag^{+}) on chromatin structure was a neglected field so far. Chromatin distorsions are visualized in these chapters. These chapters confirm the notion that cells similarly to organisms die in many ways and the genotoxic cell death is dependent on the heavy metal or other toxic agent.

Chapter 6. Oxidative DNA damages of Cd have apoptotic biochemical and morphological consequences. Low concentrations of $CdCl_2$ (0.5–5 μM) cause biochemical (strand breaks, carcinogenic indicator, DNA replication, DNA repair) and morphological (chromatin) changes in CHO and murine preB cells.