USE OF A CILIATED PROTOZOA AS A MODEL SYSTEM TO DETECT TOXIC AND CARCINOGENIC AGENTS

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ABSTRACT

The ciliated protozoan Paramecium tetraurelia is proposed for use as a detector of agents hazardous to eukaryotic (true) cells. Two distinctly different bioassays were employed, the mutagenesis and photodynamic assays. The mutagenesis assay identifies genotoxic agents, and the photodynamic assay detects chemical agents which sensitize cells and organisms to lethality when exposed to near-ultraviolet (UV) light.

The mutagenesis assay has been used previously and DNA damaging agents were shown to induce nonsurvivors (lethals), slow growers (detrimentals), and temperature-sensitive cells among the self-fertilization offspring from treated parent cells. In the present study, the carcinogens methylmethanesulfonate and benzo-(a)pyrene after metabolic activation with the induced Ames S-9 microsomal fraction were mutagenic in Paramecium, while the non-carcinogenic naphthalene was nonmutagenic with or without induced S-9. The paramecia mutagenesis assay may be useful as a short-term bioassay which can complement other bioassays for detection of mutagenic and/or carcinogenic agents.

The photodynamic assay (cell immobilization and lysis of paramecia only when exposed to both certain light-sensitizing agents and near-UV light) identifies the photosensitizing chemical agents among certain dyes and polycyclic aromatic hydrocarbons (Epstein et al., 1964). The concentration of the chemical needed for cell immobilization during a given interval and light intensity is an index of the photodynamic activity of the agent. The
significant correlation reported between strong photosensitizing agents and carcinogenicity is improved by recent identification of suspected noncarcinogens as carcinogens. The photodynamic assay may be a rapid, sensitive prescreen detector of potential photomutagens and photocarcinogens. Agents which sensitize cells to light (photodynamic agents) may also represent an environmental hazard since the ecological balance in aquatic surface organisms may be altered by pollutant-mediated increased sensitivity of these organisms to sunlight.

INTRODUCTION

Paramecium and other unicellular eukaryotic organisms occupy a strategically important level of organization, structure, and function between prokaryote (bacteria) and multicellular organisms and as such provide an important research resource (Sonneborn, 1970). Paramecium tetraurelia was utilized as a biological indicator of induced cell damage for both the mutagenesis (genotoxic assay) and the photodynamic assay (photosensitizing chemical assay). P. tetraurelia contains two micronuclei (the germ nuclei) and one macronucleus (the somatic nucleus). The macronucleus contains 400 times as much DNA as the micronucleus, governs growth and cell division, and determines the phenotype of the organism during clonal multiplication (Cummings, 1972; Jurand and Selman, 1974). The fertilization process marks the initiation of the lifespan and the cells undergo a species-specific number of successive cell divisions when the micronucleus mitotically divides (during vegetative growth).

Mutagenesis Assay

The biology of Paramecium tetraurelia is exceptionally favorable for mutagenesis studies since autogamy (self-fertilization) results in the homozygous condition of recessive mutations allowing immediate expression of the phenotype. Another ciliated protozoan Tetrahymena also offers potential for mutagenesis studies since "short circuit" matings and selection techniques also provide recovery and immediate expression of any induced genetic damage (Bruns and Sanford, 1978). During autogamy in P. tetraurelia, the micronuclei undergo meiosis to form haploid gamete nuclei, only one of which is retained and duplicated. The two identical haploid gametes fuse to form a homozygous zygote nucleus or synkaryon. The zygote nucleus subsequently differentiates into the new micronuclei and macronucleus for progeny cells (for review see Beale, 1954; Sonneborn, 1970, 1974). Thus, for the mutagenesis assay, parent cells are treated with the agent, washed free of the agent, and allowed to multiply for several cell divisions. Nonspecific toxicity will kill these parent cells during this interval of parent multiplication. The cells are then starved