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Morphological Study of Cadmium-Induced Changes on Root Apex of *Allium cepa*

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Abstract

Cadmium (Cd) is an industrial and environmental pollutant. It has toxic effects on root tip growth and morphogenesis of onion *(Allium cepa L.)*. Onion roots were germinated in 0, 2, 5, 10, 25, 50, 100, 200 ?‡g/ml doses of $CdSO_4$ salt. Then root tips were fixed, dehydrated and paraffin embedded. Afterwards, material was sectioned on a microtom (7 ?‡m thickness) and stained by Acridine orange fluorescent dye and cytochemical methods for DNA and RNA staining. Results show that Cd induces morphological changes such as cell vacuolization, nucleus and cytoplasm condensation and nucleus margination. It also causes decreases nucleoplasmic ratio and cell death induction. It was also observed that Cd toxicity has a threshold level (10-25µg/ml concentrations). In doses higher than this threshold, cells are irreversibly committed to death. Therefore in concentrations lower than 25 ?‡g/ml , the mode of cell death is apoptotic-like cytological features and necrosis happens in higher concentrations.

Key words: *Cadmium toxicity, root tip, cell death, Apoptosis, Necrosis, Allium cepa .*

Introduction

Cadmium is a major industrial pollutant readily taken up by plant roots. It is stored in the apoplast or vacuoles and is known to interact with proteins, influencing protein-protein or protein-DNA interactions (Fojtova & Kovarik 2000). Cd is known to inhibit root growth in plants (Liu, Jiang & Li 1992). It induces chromosomal aberrations and micronuclei formation (Zhang & Xiao 1998). In this report we have described Cd's influences as observed in our microscopic studies on

cell morphology in the morphogenic zones of onion root apex. For this reason we have renewed the scheme of morphogenic zones of root tip and compared it with cadmium-induced changes on morphogenesis patterns. We have also studied Cd-induced cell death and its mode. We have recognized that two types of cell death are induced by Cd^{+2}: apoptotic-like cytological features and necrosis. Necrosis is accidental cell death and apoptosis is a programmed and physiological process that causes cells to die in response to developmental and environmental changes. In animals, Cadmium-induced apoptosis has been described in various cases (Lohmann & Beyersmann 1993; Habeebu, Liu & Klaassen 1998; EL-Azzouzi et al. 1994) . Our paper demonstrated some of the similarities and differences between necrosis and apoptotic-like cytological features in onion root cells and distinguished between these two modes of cell death in different Cd^{+2} concentrations.

Materials and Methods

Onion (*Allium cepa L.*) bulbs were immersed in 0,2,5,10,25,50,100 and 200 ?‡g/ml of $CdSO_4$ salt. Onion roots grew in these concentrations for 72 hours at 25°C. Root tips were fixed in glutaraldehyde (3% in phosphate buffer pH 7.4) for 4 hours or in a solution of 5% formaldehyde: 10% acetic acid: 85% ethanol for 12 hours. Fixed roots were dehydrated through a graded ethanol serried (25, 50, 75 [roots were stored in this step] 95, 100, 100%, each step 1 h). Dehydrated roots were then submitted to a graded toluene serried (25, 50, 75, 100% in ethanol, 1 h each step). Finally, roots were embedded in paraffin by a paraffin serried (25, 50, 75, 100, 100 in toluene, each step 2h, at 57°C) and sectioned longitudinally at a thickness of 7 ?‡m. The material was deparaffinized and stained with acridine orange and cytochemical methods. The sections observed under a Zizze III light microscope or an UV fluorescence microscope and pictures were taken. Statistic and morphometric analyses of various root regions were carried out. Microsoft ExeL-98 was used for diagram drawing .

Cytological Methods

***1- Feulgen staining*:** This method was used for DNA staining. In this assay, deparaffinized material was hydrolyzed in 1 N HCl at 60°C, for 15 min and washed three-times in distilled water at 4°C. The preparations stained with Schiff's reagent for 2 h at room temperature. They were washed three-times in a newly prepared H_2SO_3 solution, dehydrated in ethanol (25, 50, 75, 100% each 1 min) and cleared in toluene (100% three times each 1 min). The slides were mounted in eosin resin.

***2- Methyl-Green Pyronin staining*:** Deparaffinized slides were stained with this dye for 45 min. Then slides were left to dry and dehydrated in ethanol.

3- Acridine Orange Fluorescent Staining: Acridine orange is a fluorescent dye which intercalates with DNA, giving a green staining under UV light. This dye also binds to RNA and it stains orange. Tissue sections were deparaffinized and incubated in 0.2 M glycine (pH 2) for 5 min at room temperature. Sections were stained in acridine orange (1% in distilled water) for 30 min, washed in distilled water and observed under an UV fluorescence microscope. The nuclei are yellow (or light green) and the cytoplasm orange.

Statistic and Morphometric Quantification:

In each experiment five roots were accidentally selected and used for statistic analyses.

1- Measurement of cell dimensions: Photographs were taken from all the morphogenic regions of the root tip (in x500 magnification) and cell dimensions were measured.

2- Computation of nucleoplasmic ratio (NPR): Cytoplasm surface were measured in photographs and NPRs were computed in each cell.

$$NPR = \frac{Nucleus\ Area}{Cytoplasm\ Area}$$

Then average NPR in each region was computed.

3- Computation the Percentage of Dying Cells: We chose nucleus margination and condensation as characteristic hallmarks of dying cells. Nucleus margination is displacement of nucleus in a cell margin.

$$Percentage\ of\ dying\ cells = \frac{Number\ of\ dying\ or\ dead\ cells}{Total\ number\ of\ cells} \times 100$$

Results

Untreated root cells are shown in figure1 (figure1-a,b,c). The scheme of morphogenic zonation of root tip is shown in figure2. In this scheme each cell is determined as a two dimensional point in the Cartesian Coordinate System (Y: symmetric axis of root and X: above the cap). There are five regions in root tip of *Allium cepa,* including: Meristem (M), Central Cylinder (CC), Cortex (CO), Protoderm (PD) and Cap (C). We used this scheme as the basic pattern of root tip morphogenesis and compared it with cadmium-induced changes on morphogenesis. Germination of roots in $CdSO_4$ salt doses causes root growth reduction. With higher doses (more than 100µg/ml) roots do not grow. Reduction of root growth may be linked either to decrease of meristem extent or inhibition of cell enlargement in the elongation zone.

Cell Morphology

General morphological changes caused by Cd in the cells are decrease of cell dimensions (Fig. 3c,d), cell vacuolization, nucleus condensation and margination which leads to nucleus degradation. (Figs. 3c, d, g, h, k, l). These characteristics are observed in 25-50 ?‡g/ml concentrations. In 50 ?‡g/ml doses nucleolus is absent (Figs. 3d, h, l). It may suggest that RNA and protein synthesis has been stopped. The cytoplasm is condensed and shrinks. These events finally lead to cell lysis.

Figure 1. Cytochemical methods for DNA localization using Feulgen staining (a), Methyl green pyronin staining for detection of DNA (nucleus) and RNA (cytoplasm and nucleolus) (b) and Acridine orange fluorescent staining (c).

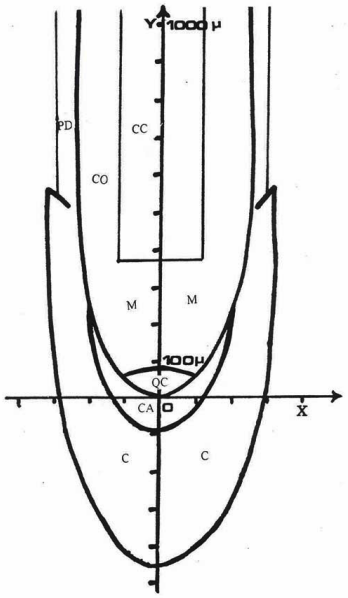


Figure 2 - Morphogenic scheme of onion root tip in the Cartesian coordinate system. Meristem (M), Central cylinder (CC), Cortex (CO), Protoderm (PD) and Cap (C) ,Quiecent center (QC) ,Initial cells of cap or caliptera(CA).

Cell Morphology

Nucleoplasmic ratio (NPR)

Untreated meristem cells are isodiametric and have large nuclei (Fig. 3-a). NPR these cells is higher than in the central cylinder and cortex cells. In the present of 2, 5 ?‡g/ml Cd salt no obvious changes are observed in NPR. With 10, 25, 50 ?‡g/ml , NPR decreases which shows reduction of cell activity. With 2,5 and 10 ?‡g/ml doses central cylinder and cortex cells are similar to the control, but in 25 and 50 ?‡g/ml concentrations NPR is reduced. Therefore NPR decrease appear to be dose-dependent (Fig. 4) .

Percentage of dying cells

Toxic amounts of $CdS0_4$ solutions cause cell death. With 0, 2, 5 ?‡g/ml cell death is not observed. Dead and dying cells are visible on roots with 10, 25, 50 ?‡g/ml. Percentage of dying cells in the meristem is lower than in the central cylinder and cortex. For example, under 50 ?‡g/ml doses of Cd approximately all of central cylinder and cortex cells are dead, whereas dying cells of the meristem range to about 60%. Thus the meristem is the last region to be injured and undergo

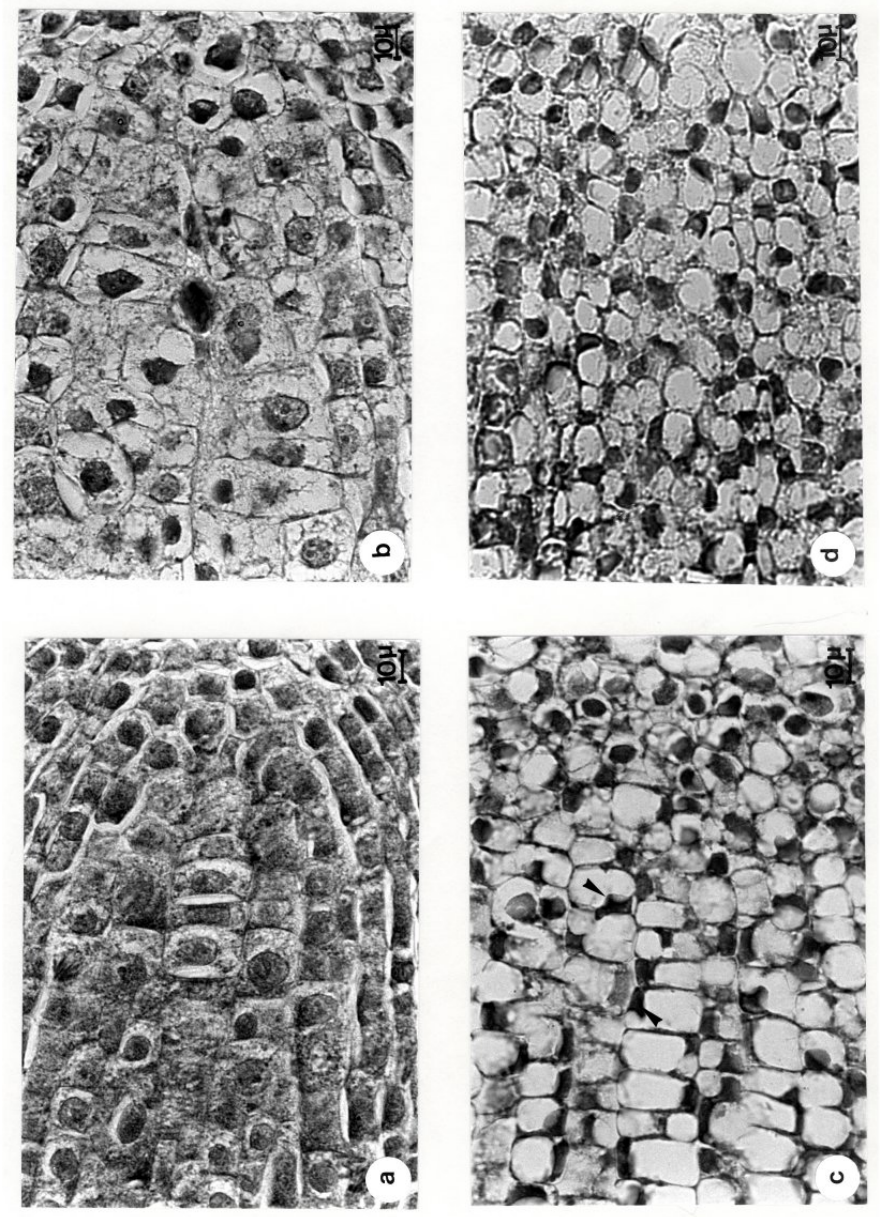
cell death. Under low concentrations cell death is only local. Higher doses cause widespread cell death(Figure 5).

Discussion

We displayed Cd-induced changes on cell morphology. All of these changes lead to cell death. In this case, there is a critical question: Which mode of cell death is observed in metal toxicity? Apoptotic-like cytological features or necrosis.

Necrosis is well established as a feature of Cd effects (Nicotera, Leist & Ferradno-May 1999). In high toxicity of toxicant elements where widespread death is observed, the mode of cell death is necrosis. In low concentration, cell death is local (2, 5, 10, 25 ?±g/ml doses). These observations have been reported in animals too (Habeebu, Liu & Klaassen 1998). Apoptosis is a consequence of a programmed cell death (PCD) process. PCD occurs in physiological states or within environmental interactions (Greenberg 1996). Cadmium-induced apoptosis in plants was recently reported in tobacco cells (Fojtova & Kovarik 2000). It seems that the mode of cell death is dose-dependent and there is a "threshold" level for Cd toxicity. In low doses of Cd ions (between 10-25 ?±g/ml), apoptotic-like cytological features is the prevailing type of cell death and it precedes necrosis. With higher concentrations, cells undergo necrosis. Some of the morphological changes such as cytoplasm condensation and shrinkage, nucleus condensation and margination are known as apoptotic characteristics (Pennel & Lamb 1997 and Wang, Li, Bostock & Gilchrist 1996). We conclude that in toxic amounts of heavy metals such as Cadmium, plants select apoptotic death to preserve themselves against genetic damage. But under condition of high toxicity, necrosis is unavoidable and causes cell lysis and damage.

Figure 3- Methyl-Green Pyronin staining of Different concentrations of $CdSO_4$ salt. Cells of meristem (a,b,c,d); central cylinder (e,f,g,h) and cortex and protoderm (i, j, k, l); control cells without Cd^{+2} treatment (a,e,i); 10 ?±g/ml concentration of Cd^{+2}(b,f,j); 25 ?±g/ml concentration of Cd^{+2} (c,g,k); 50 ?±g/ml concentration of Cd^{+2} (d,h,l).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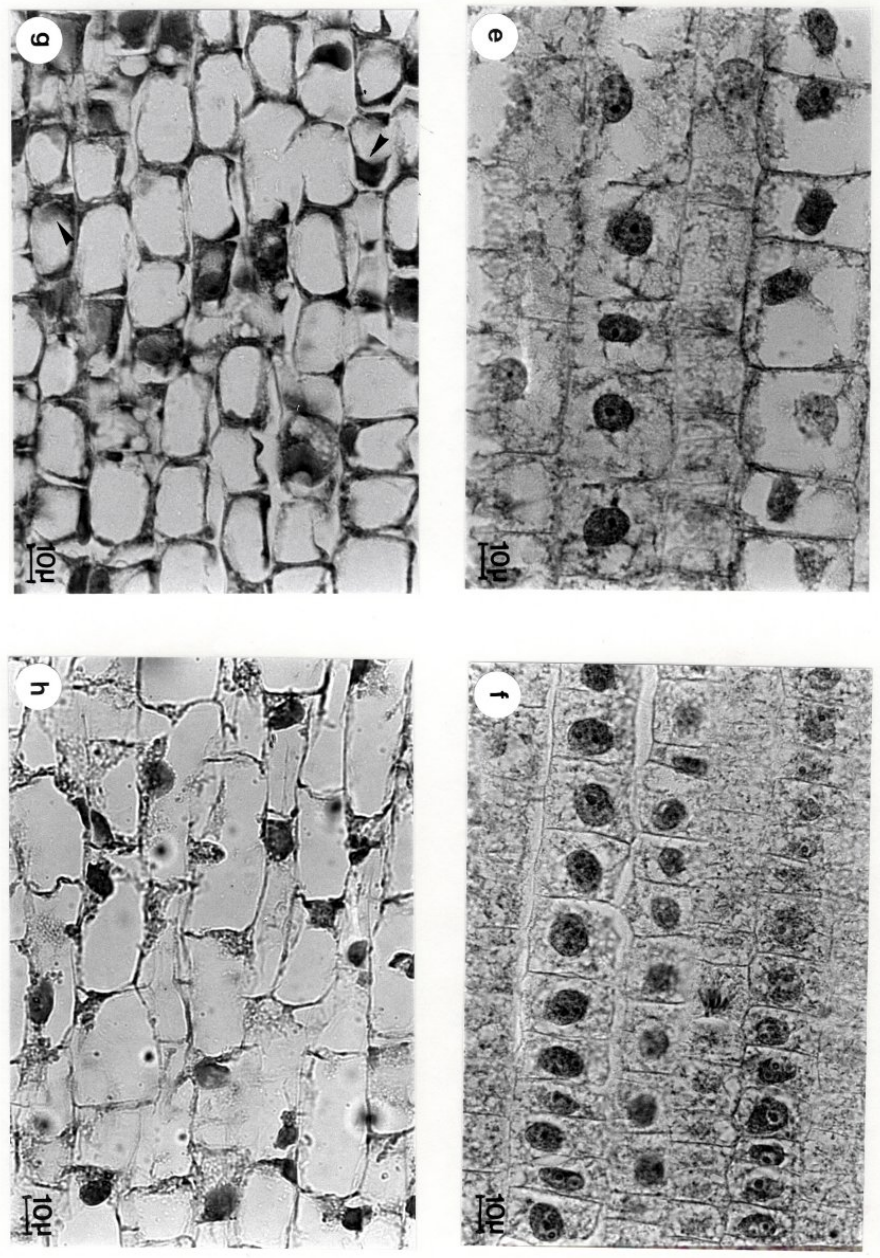
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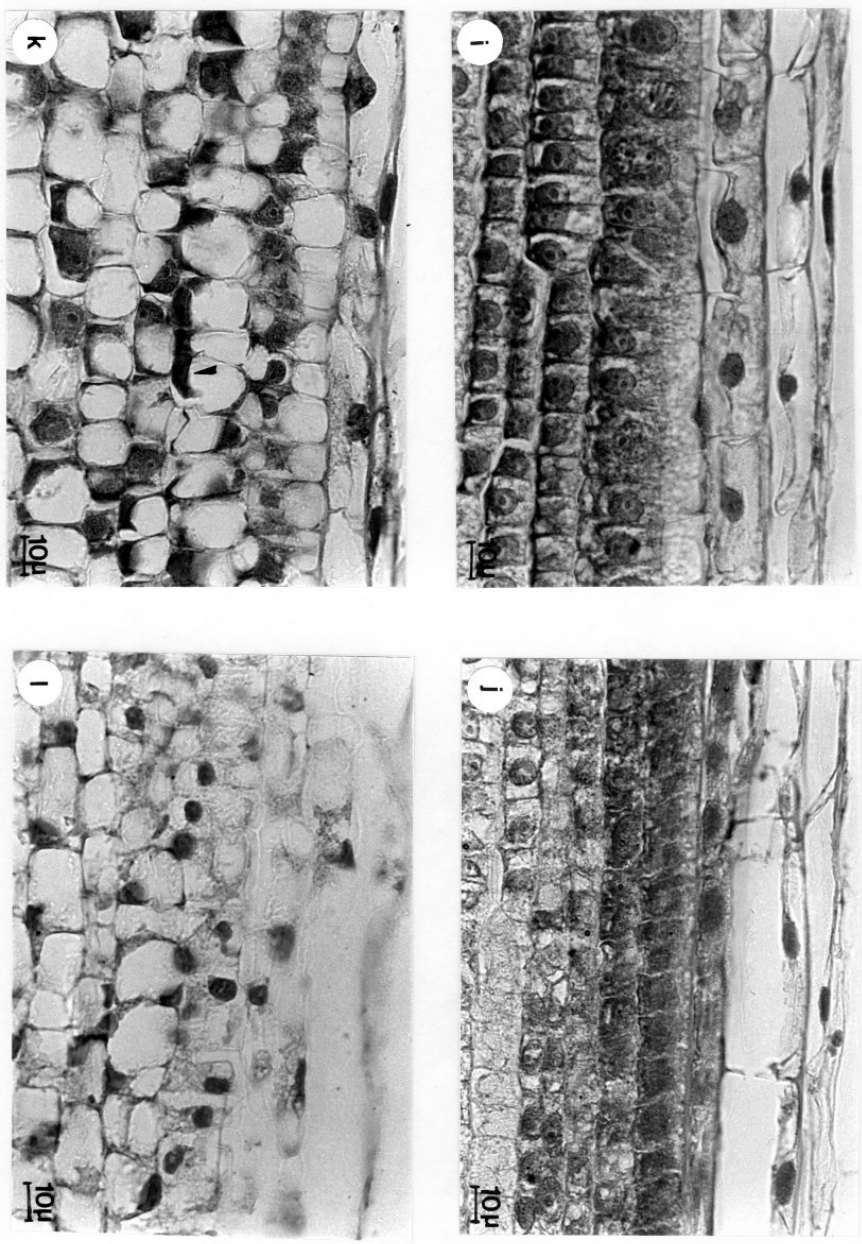
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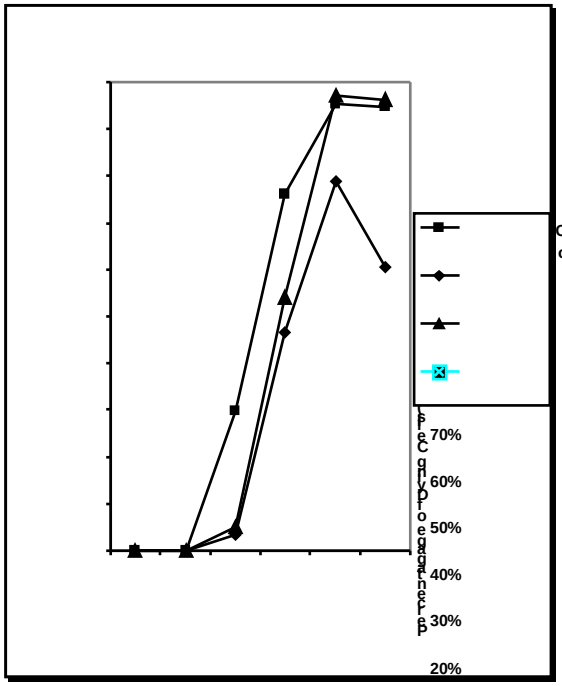


Figure 4- Nucleoplasmic Ratio in morphogenic zones of onion root tip. Diagram shows that decrease of NPR is dose dependent.

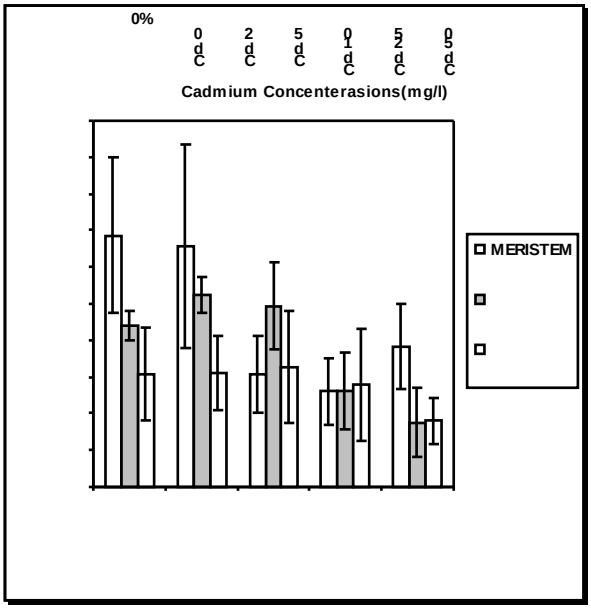
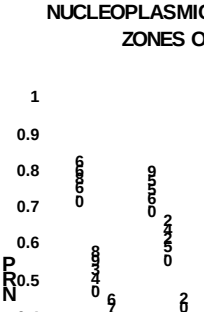


Figure 5- Percentage of dying cells in morphogenic zones of onion root tip. Meristem is the last injured region and maximum of cell death in this region is less than in other regions.



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