DIFFERENTIAL SUSCEPTIBILITY OF L CELLS IN THE 
EXPONENTIAL AND STATIONARY PHASES TO 
CADMIUM CHLORIDE

Kazuko OZAWA, Atsushige SATO and Hiroaki OKADA

Department of Pharmacology, Yokohama City University School 
of Medicine, Minami-ku, Yokohama 232, Japan

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Abstract--Comparative studies of the cellular toxicity and uptake of cadmium in L 
cells in the exponential and stationary phases were done. The LD50 of cadmium 
chloride to L cells in the exponential and stationary phases was 5.5 and 30.5 μM, res 
pectively and the cadmium content of L cells exposed to 5.5 μM cadmium chloride 
for 3.5 hr in the exponential and stationary phases was 0.123 and 0.065 μg 10^6 cells, 
respectively. These results suggest that the higher susceptibility of cells in the exponential 
phase to cadmium may be caused by an increased permeability of the cell membrane 
to cadmium.

Cadmium (Cd) has recently attracted attention as a source of environmental pollution, 
and numerous studies have been conducted on its injurious effect on living organisms (1 - 8). 
In studies on the organ distribution and tissue-damaging effect of Cd, Gunn et al. (9) pointed 
out the intense damage inflicted on the testis, despite a small amount of Cd retained by this 
organ. Such findings suggest the severe and selective toxicity of Cd to cells in the proliferative 
phase. Differences in the susceptibility of cells to Cd during exponential and stationary 
phases can be readily demonstrated using cultured cells. Several reports are already available 
on the effect of Cd on cultured cells, and Cd was shown to inhibit proliferation and cause 
cell death (10, 11). As the differences in cell susceptibility at different phases of the cell cycle 
have not been studied, we cultured L cells for extended periods from the logarithmic phase 
to the stationary phase, in order to study the susceptibility to Cd. Cd uptake of the cell at 
each phase of growth was also studied.

MATERIALS AND METHODS

Cells and culture media

L cells derived from mouse subcutaneous connective tissues were grown in Ham's F12 
medium (Nissui Co.) supplemented with 15% (v/v) calf serum (Flow Laboratories), 50 U ml 
Penicillin G and 50 μg ml Streptomycin.

Calculation of LD50

About 2 x 10^4 cells in 2 ml medium were implanted in a series of replicate culture tubes 
(15 x 100 mm) and tubes were incubated at 37 C at 10 ° angle. Cell nuclei were counted by 
hematocytometer (12) over a period of several days and a growth curve constructed. CdCl₂ 
was administered on the 1st, 2nd and 4th days (exponential phase), and the 9th, 11th and
22nd days (stationary phase). CdCl₂ was dissolved in Hank's salt solution at 1/11 ratio and added to the medium to make a final amount of 2.75-88.0 μM. Toxicity was evaluated based on the cytocidal effect of CdCl₂ as measured by the ratio between the number of cell nuclei before CdCl₂ administration and the number of surviving cell nuclei 2 days after administration. The LD50 calculation was based on the dose-response curve.

Morphology

About 1 × 10⁴ cells in 1 ml were implanted in slide chambers (Lab Tek Products, No. 4802) and the chambers incubated in an atmosphere of 3% CO₂ in air at 37°C. In the exponential and stationary phases, the original media were removed and fresh medium containing CdCl₂ were added. After 2 days, cells were fixed in methanol and stained with May-Grünwald and Giemsa.

Measurement of Cd uptake by the cell

About 10⁶ cells were implanted in replicate culture bottles (250 ml). CdCl₂ was added to the medium during the exponential and the stationary phases to give a final concentration of 5.5 μM. After 3.5 hr the medium was discarded, and the cells were washed with cold phosphate buffered saline four times for 40 sec to remove all extracellular Cd. Cells were removed from the glass surface with 2 ml of 0.25% trypsin (Difco 1 : 250). A cell suspension was then prepared and the number of cells was counted. 10⁶ cells were dried then ashed by heating with 1 ml concentrated nitric acid. After redrying, the residue was dissolved in 10% nitric acid and Cd was determined by means of an atomic absorption spectrophotometer (Nippon Jarrell Ash, AA-1).

RESULTS

Cd toxicity in the exponential and stationary phases of cell

Fig. 1 shows the growth curve of L cells. The cells were in logarithmic proliferation on days 1 to 7 during culture. The growth rate dropped thereafter, reaching a plateau after

![Fig. 1. Growth curves of L cells in monolayer culture. Arrows show the administration of CdCl₂ into the medium.](image)

![Fig. 2. Dose-response curves of CdCl₂ in L cells on day 3 and day 24 of cultivation. Vertical lines represent standard error of the mean value of 5 cultures.](image)
day 10, at about $3 \times 10^5$ cells/tube. This represented the stationary phase. On days 1, 2 and 4 of the exponential phase, CdCl$_2$ was added to the medium to make a final concentration of 0.35-11.0 µM. On days 9, 11 and 22 of the stationary phase, CdCl$_2$ was added to the medium at the final concentration of 5.5-88.0 µM. Since the lethal effect of Cd was similar in each phase, representative dose-response curves are shown for day 3 and day 24 in Fig. 2. In the exponential phase, a lethal response to 2.75, 5.5 and 11.0 µM of CdCl$_2$ was 19.3, 64.0 and 90.8% respectively, while 11.0, 22.0 and 44.0 µM of CdCl$_2$ in the stationary phase, killed 7.3, 35.0 and 77.2% of cells respectively. The LD$_{50}$ calculated from Fig. 2 was 5.5 (95% confidence limit, 4.8-6.1) µM in the former, and 30.5 (95% confidence limit, 24.5-36.5) µM in the latter. The susceptibility of cells in the exponential phase of growth was

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**Fig. 3.**
A: Control culture of L cells in exponential phase stained with May-Grünwald and Giemsa
B: Culture of L cells 2 days after exposure to medium containing 5.5 µM CdCl$_2$

The calibration bar is 20 µ long.

**Fig. 4.**
A: Control culture of L cells in stationary phase stained with May-Grünwald and Giemsa
B: Culture of L cells 2 days after exposure to medium containing 5.5 µM CdCl$_2$

The calibration bar is 20 µ long.
thus shown to be about 5.5 times higher than in the stationary phase.

In the control culture, cells were firmly adhered to the slide and formed a sheet-like monolayer (Fig. 3-A and 4-A). In the exponential phase, 5.5 \( \mu \text{M} \) CdCl\(_2\) caused significant cellular degeneration (Fig. 3-B). In the stationary phase, cells exposed to 5.5 \( \mu \text{M} \) CdCl\(_2\) showed no particularly obvious changes in morphology (Fig. 4-B).

**Cd uptake by the cell**

Based on results of measurements of the time course of Cd uptake by the cell contained in a previous report (10), Cd uptake after 3.5 hr was measured. Table I shows the Cd contents of cells cultured in media containing 5.5 \( \mu \text{M} \) CdCl\(_2\) for 3 hr. Uptake in the stationary phase was 1/2 of that in the exponential phase.

| Table 1. Cadmium content of mouse L cells |
|------------------------------------------|
|                                           |
| (\( \mu \text{g}/10^6 \) cells)          |
| Exponential phase: 0.123 ± 0.003 (n = 5)  |
| Stationary phase: 0.065 ± 0.007 (n = 5)   |
| Mean ± S.E.                               |

**DISCUSSION**

According to Norberg and Nishiyama (13), Cd administered to rats is found in the highest concentration in the kidney, liver and pancreas. Lesser amounts were found in the salivary gland and testis. The organ damage, however, was not always proportional to the distribution of Cd. The testis was damaged by a Cd concentration 1/40 to 1/70 that which damaged kidney (8). In the present study, the susceptibility of L cells to Cd was shown to be higher when cells were in the exponential phase rather than in the stationary phase of growth. Such may explain the high susceptibility of the rat testis to Cd.

Drugs which show a metabolic antagonism to DNA precursors are known to exhibit selective toxicity against dividing cells. According to a report by Madoc-Jones and Bruce (14) the susceptibility of L cells in the exponential phase of growth to 5-fluorouracil was 3 times higher than cells in the stationary phase of growth. In our study, the susceptibility of cells to Cd was 5.5 times higher in cells in the exponential phase than in the stationary phase of growth, suggesting the possibility that Cd acts on the mitotic mechanism. The high susceptibility of cells in the exponential phase, on the other hand, is presumably related to Cd uptake. Based upon studies on ultrastructure of plasma membranes of cultured cells, Scott et al. (15) reported discrepancies related to cell density, suggesting differences in drug permeability among cells in the exponential and stationary phases. Thus it would appear that the higher susceptibility of cells in the exponential phase to cadmium may be due to an increased permeability of the cell membrane to cadmium.

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