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Differences in the growth inhibition of cultured K-562 cells by selenium, mercury or cadmium in two tissue culture media (RPMI-1640, Ham's F-10)

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Abstract

Effects of some metals on the growth of cultured human erythroleukemia K-562 cells were investigated when grown in two different types of media based upon RPMI-1640 or Ham's F-10. The study on proliferation, using RPMI-1640 supplemented with sodium selenite, selenomethionine, mercuric chloride, methylmercuric chloride and cadmium nitrate showed no inhibition of growth at concentrations of 2.5, 25, 25, 2.5 and 25 μM, while at 75, 250, 50, 5 and 50 μM toxicity was apparent. Selenite at 5–50 μM and selenomethionine at 50–100 μM inhibited the growth. In Ham's F-10 supplemented with the same compounds no inhibition was found at concentrations of 5, 10, 25, 1 and 50 μM, while at 50, 100, 50, 5 and 75 μM toxic effects were noted. Selenite 10 μM and selenomethionine 25-50 μM inhibited the proliferation. Measurements of trace element levels in pellets of K-562 cells grown in RPMI-1640 or Ham's F-10 unveiled higher cell contents of cadmium and selenium in cells grown in RPMI-1640, being consistent with higher concentrations of these elements in that medium. Manganese and mercury concentrations were higher in cells grown in Ham's F-10 correlating with a higher medium concentration of these elements. The growth responses and cellular uptake differed between the metals and the selenocompounds and although extrapolating the results to humans is difficult the selenium exposures were in approximately the same order of magnitude as in human exposures. The compounds could be ranked according to decreasing toxicity as: methylmercuric chloride > mercuric chloride, cadmium nitrate, sodium selenite > selenomethionine.

Introduction

Selenium is an essential element in human and animal nutrition (McCoy & Weswig 1969, Chen *et al.* 1980) and has been reported to be essential for the normal growth of cells in culture (McKeehan *et al.* 1976; Beilstein *et al.* 1987). It has been found to be a component of the mammalian enzyme glutathione peroxidase (Flohé *et al.* 1973; Rotruck *et al.* 1973), the types I, II and III iodothyronine deiodinase (Valverde-R *et al.* 1997) and human thioredoxin reductase (Gromer *et al.* 1998). Studies on cultured cells have shown that supplemental selenium can inhibit the cell growth (e.g., Caffrey & Frenkel 1992).

Environmental and occupational exposure to different forms of mercury is a hazard to the human health. Inorganic and organic forms of mercury exhibit toxic properties including nephrotoxicity, neurotoxicity and gastrointestinal toxicity with ulceration as well as haemorrhage. Primary exposure occurs through environmental contamination as the result of mining, smelting and industrial discharge including inhalation and ingestion through the food chain (Goyer 1996). Within cells mercury may bind to a variety of en-

zyme systems, including those of microsomes and mitochondria, producing non-specific cell injury or cell death. Mercury has a particular affinity for ligands containing sulph-hydryl groups like glutathione (Goyer 1996). Both mercuric chloride and methylmercury have been shown, among other effects, to alter the calcium homeostasis, but appear to involve different mechanisms (Tan *et al.* 1993).

Cadmium exposure is known to produce damaging effects both to humans and animals. Cadmium has been shown to accumulate in the environment as a result of industrial practices and is used in electroplating, galvanising, as a colour pigment in paints and in batteries. Several studies have indicated that intracellular glutathione provides a first line of defense against cadmium toxicity before induction of metallothionein synthesis occurs (Singhal *et al.* 1987; Kang & Enger 1988; Ochi *et al.* 1988; Chan & Cherian 1992) and depletion of glutathione revealed enhanced toxic effects of cadmium in various tissues and cells (Dudley & Klaassen 1984; Kang & Enger 1990; Chin & Templeton 1993).

The effects of environmental exposure to metals like mercury and cadmium could possibly be counteracted or reduced in part by selenium intake. Several studies using experimental animal models have indicated a selenium protection against toxicity from mercury (Parizek & Ostadalova 1967; Burk *et al.* 1977; Carmichael & Fowler 1979; Chung *et al.* 1982; Lindh *et al.* 1996) or cadmium (Chen *et al.* 1974; Wahba *et al.* 1993; Lindh *et al.* 1996). Even though a protective effect from selenium against mercury and cadmium has been shown, the mechanisms behind the selenium protection are not clearly understood. The present study compares the effects of sodium selenite, selenomethionine, mercuric chloride, methylmercuric chloride and cadmium nitrate on cell growth in human K-562 cells. The effects of metal exposure were investigated in these cells both in RPMI-1640 and Ham's F-10 medium to examine whether different media affected the results. The objective of the work was to assess the response of the cellular model to selenium exposures of interest for human exposure, to acquire basic data pertinent to understanding selenium-cadmium and selenium-mercury interaction and to gather information of the reliability of this human cellular toxicity assay.

Materials and methods

Human erythroleukemia K-562 cells (Lozzio & Lozzio 1975; Klein *et al.* 1976) were optimally grown as stock cultures in two different kinds of tissue culture medium, Ham's F-10 and RPMI-1640 from Biological Industries, Beit Haemek, Israel. The two growth media were both supplemented with 10% fetal calf serum, 2 mmol/l L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 2.5 μg/ml amphotericin B (complete media), all from Biological Industries.

Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$), mercuric chloride ($HgCl_2$) and cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$) were purchased from Merck, Germany. Seleno-L-methionine ($C_5H_{11}NO_2Se$) and methylmercuric chloride (CH_3HgCl) were obtained from (Sigma, USA) and (Alfa, Germany), respectively. Each trace element was dissolved in and diluted with purified water to an original stock solution with a concentration of at least 100-fold the concentration added to the cell cultures. Before use, the original stock solutions were sterile filtered with a 0.2 μm filter (Gelman Sciences, USA). The final concentration was obtained by dilution in culture medium.

In these experiments cells were taken from the optimally growing stock cultures and used according to the following protocol:At first, the cells were exposed to the trace element during a period of 4 days, then the trace element was washed away and the cell growth was studied during a period of about 14 days. In the beginning of the exposure period, the cells were plated at a density of 4×10^4 cells/ml in 25 cm^2 plastic flasks (Bibby, UK) and the trace element was added via the medium to the cell cultures to a total volume of 30 ml in each flask. On the second day of the exposure period, half of the 30 ml medium was changed for fresh medium containing the trace element.

In the beginning of the growth period, on the fourth day of exposure, 15 ml of the medium was removed from each cell flask and the rest was transferred to a 15 ml tube. The cells were centrifuged three times, for 2 min at 230 $\times$ *g*, in phosphate buffered saline (PBS), free of calcium and magnesium. Then, 10 ml of complete medium was added to the tube and the cells were suspended, counted in a cell counter (Model ZM, Coulter Counter, UK), tested for viability by the trypan blue dye exclusion test (Tennant 1964) and plated for cell growth. The controls were treated exactly in the same way as described above with the exception that their medium had no addition of trace elements.

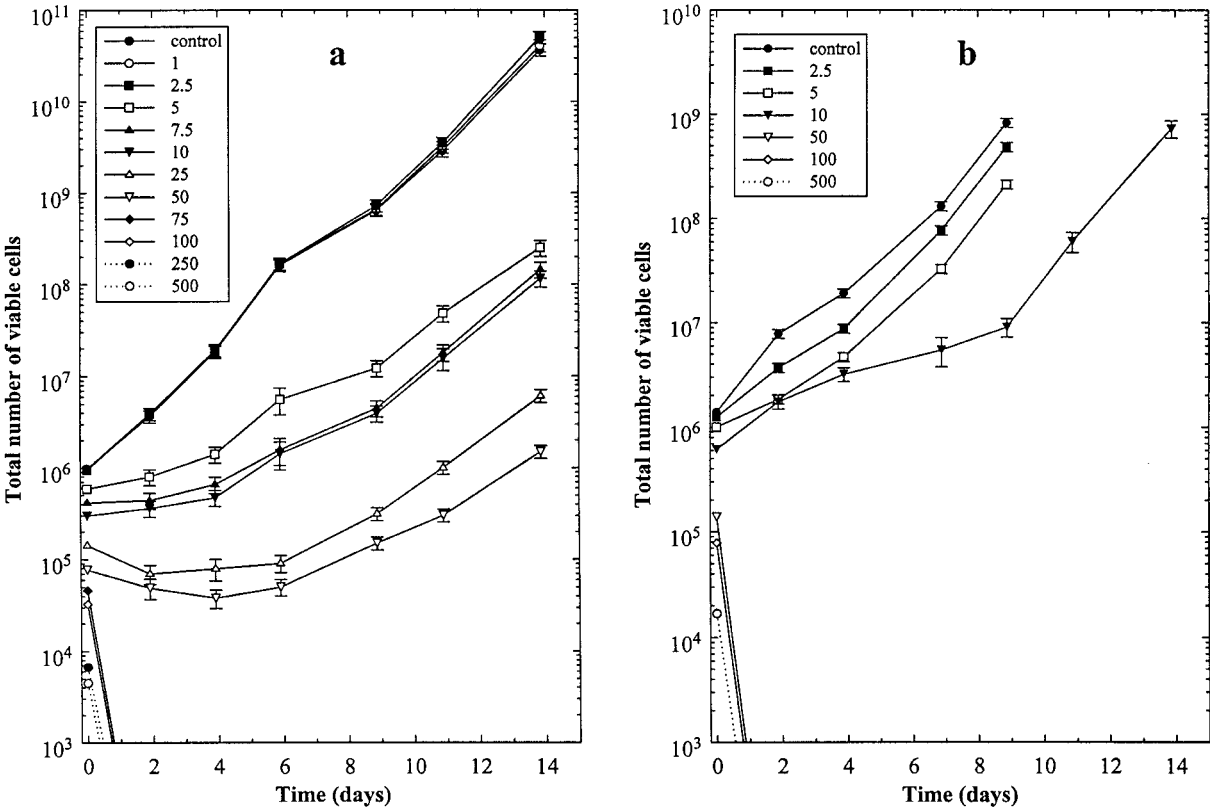

Figure 1. Concentration effects of sodium selenite on cell growth. K-562 cells were treated for 4 days with varying concentrations of sodium selenite before washed and plated for growth in medium based on RPMI-1640 (a) or medium based on Ham's F-10 (b). Each point corresponds to two samples. Mean values and maximal variations are shown.

Growth curves were constructed for each trace element to determine at what concentration the cell growth was affected. Cells were counted and viability tested three times a week and when the cell density reached 1×10^6 cells/ml the cells were replated to a density of 4×10^4 cells/ml. The K-562 cell growth was studied for 14 days at the most, but when the cell growth was unaffected the study was ended already at day 9 or day 11. Growth curves from exposure to sodium selenite, selenomethionine, mercuric chloride, methylmercuric chloride and cadmium nitrate were obtained both for cells grown in RPMI-1640 and Ham's F-10. The concentration ranges of trace element exposures used in RPMI-1640 were more extended than in Ham's F-10. The reason was that the trace element exposures were first performed in RPMI-1640 and when cell pellets grown in RPMI-1640 showed unexpectedly high values of cadmium (*vide infra*) a complementary study on cells grown in Ham's F-10 was initiated. The following ranges of trace element exposures were used in RPMI-1640 and in Ham's F-10 respectively: sodium selenite (1–500 μM) and (2.5–500 μM), selenomethionine (1–1000 μM) and (5–250 μM), mercuric chloride (1–100 μM) and (25–75 μM), methylmercuric chloride (0.01–100 μM) and (1–7.5 μM) and cadmium nitrate (0.1–100 μM) and (10–100 μM).

Determinations of some trace elements in cultured cells and media

Cells in several 25 cm^2 plastic flasks were grown to a high density before they were pooled in a big plastic flask (75 cm^2) with a cell density of 1.5×10^6 cells/ml. The cells were suspended and 10 ml aliquots of cells were transferred to 15 ml tubes. The tubes were centrifuged for 3 min at $470 \times g$, the supernatant removed and two pellets were pooled in one of the tubes and suspended in PBS. Two more centrifugations for 3 min at $470 \times g$, with PBS washings in between, were performed before the trace element content in the pellets (3×10^7 cells) was determined by inductively coupled plasma mass spectrometry, ICP-MS (Houk 1994).

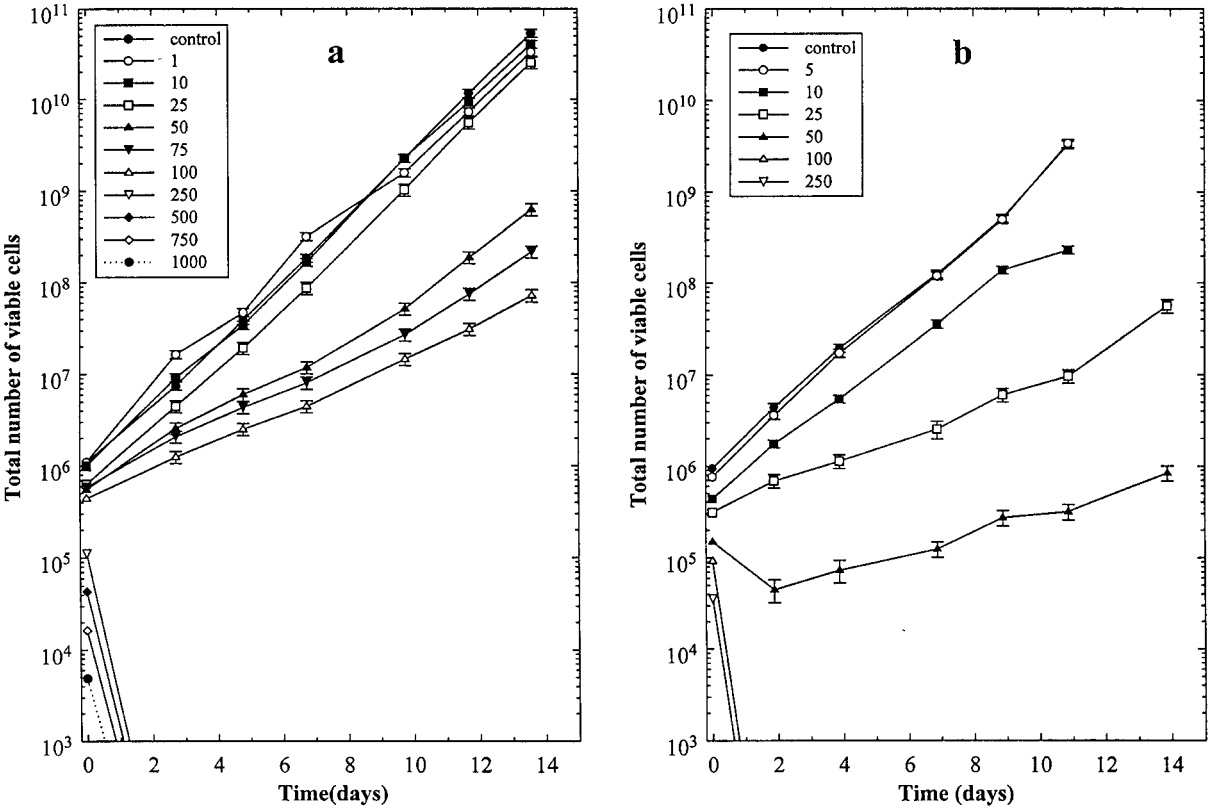

Figure 2. Concentration effects of selenomethionine on cell growth. K-562 cells were treated for 4 days with varying concentrations of selenomethionine before washed and plated for growth in medium based on RPMI-1640 (a) or medium based on Ham's F-10 (b). Each point corresponds to two samples. Mean values and maximal variations are shown.

Measurements were performed both on K-562 cells cultured in RPMI-1640 and in Ham's F-10. Complete medium (based on RPMI-1640 or based on Ham's F-10) was also assessed for the trace element content to provide a comparison to the pellet results. The statistical evaluations were performed by the two-sample Wilcoxon rank sum test.

Results

Growth studies after exposure to Se, Hg or Cd

Figures 1–5 present growth curves when K-562 cells have been exposed to sodium selenite, selenomethionine, mercuric chloride, methylmercuric chloride and cadmium nitrate, respectively. Growth curves for K-562 cells cultured in RPMI-1640 or Ham's F-10 are shown in part a or part b of each figure, respectively. Generally, there is an inverse relation between the concentration of the different compounds and the cell growth.

Exposure to sodium selenite of K-562 cells grown in RPMI-1640 did not lead to inhibition of growth at concentrations as high as 2.5 μM (Figure 1a). At 5–10 μM sodium selenite a slight decrease in viability was noted until day two of growth (85–86%). The viability then started to increase up to day 6 (85–91%). At day 9–14 the viability was at level of the control (90–100%). Exposure to 25 μM and 50 μM resulted in a decrease in viability until day 2 with no direct change in viability at day 4 or 6 (12–16%). From day 9–14 the viabilities began to increase (45–84%). The concentrations 75–500 μM showed toxic to the cells with viabilities ranging between 0–10%. K-562 cells cultured in Ham's F-10 and exposed to sodium selenite showed no growth inhibition at 5 μM (Figure 1b), while the concentration 10 μM showed viabilities at day 2,4 and 6 of 82,70 and 74% respectively. At day 9–14 the viabilities were high (90–100%). The concentrations 50–500 μM were toxic to the cells with low viabilities (0–10%).

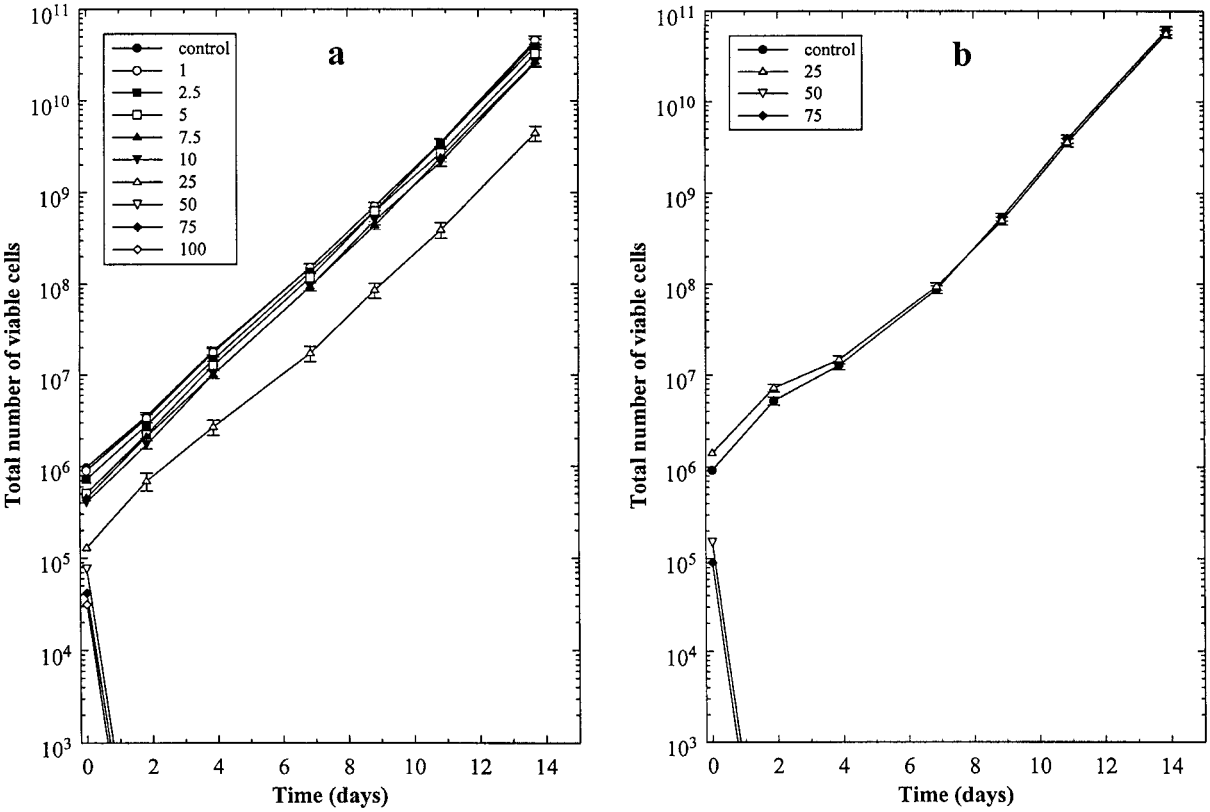

Figure 3. Concentration effects of mercury chloride on cell growth. K-562 cells were treated for 4 days with varying concentrations of mercury chloride before washed and plated for growth in medium based on RPMI-1640 (a) or medium based on Ham's F-10 (b). Each point corresponds to two samples. Mean values and maximal variations are shown.

K-562 cells grown in RPMI-1640 and exposed to selenomethionine showed no growth inhibition at 25 μM (Figure 2a). Cells exposed to 50–100 μM expressed high viabilities (90–100%), but increased population doubling times. The concentrations 250–1000 μM were toxic to the cells with low viabilities (0–10%). Cells which were exposed to selenomethionine and grown in Ham's F-10 showed more inhibition of growth at 25 μM and 50 μM (Figure 2b) compared to when grown in RPMI-1640. The concentration 25 μM showed an increased population doubling time, but high viabilities during the whole growth period (90–100%). The concentration 50 μM caused a decrease in viability until day two of growth (19%), from which time the viability started to increase up to day 6 (40–60%). At day 9–14 the viabilities were in the normal range (90–100%), but one could still notice an increased population doubling time. The concentrations 100–250 μM showed toxic to the cells with viabilities ranging between 0–10%.

When exposed to mercuric chloride and cultured in RPMI-1640, K-562 cells were not growth inhibited at concentrations up to 25 μM (Figure 3a). At concentrations 50–100 μM toxicity was noted with viabilities ranging between 0–10%. Cells which were exposed to mercuric chloride and grown in Ham's F-10 showed no inhibition of growth at 25 μM (Figure 3b), while 50–75 μM showed toxic to the cells with low viabilities (0–10%).

When K-562 were grown in RPMI-1640 and exposed to methylmercuric chloride no inhibition in growth was observed at 2.5 μM (Figure 4a). The concentrations 5–100 μM were toxic to the cells as reflected by low viabilities (0-10%). Cells grown in Ham's F-10 were not growth inhibited at 1 μM of methylmercuric chloride (Figure 4b). At a concentration of 2.5 μM slight growth inhibition was found at day 4 and 7, but the viabilities were high (90–100%) during the whole growth period. The concentrations 5 and 7.5 μM were toxic to the cells with viabilities ranging between 0–10%.

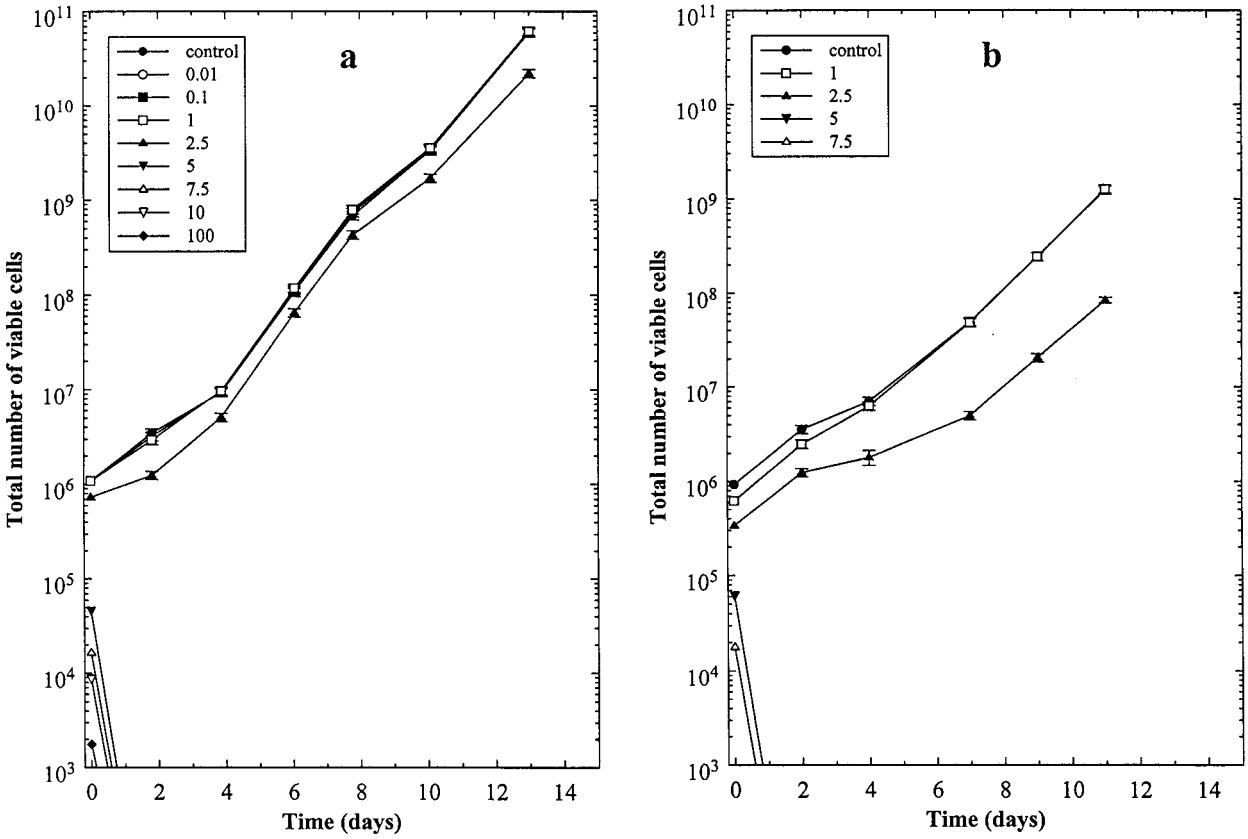

Figure 4. Concentration effects of methyl-mercury chloride on cell growth. K-562 cells were treated for 4 days with varying concentrations of methyl-mercury chloride before washed and plated for growth in medium based on RPMI-1640 (a) or medium based on Ham's F-10 (b). Each point corresponds to two samples. Mean values and maximal variations are shown.

Exposure to cadmium nitrate of K-562 cells cultured in RPMI-1640 did not result in inhibition of growth at concentrations up to 25 μM (Figure 5a), while 50–100 μM were toxic to the cells with low viabilities (0–10%). Cells which were exposed to cadmium nitrate and grown in Ham's F-10 were less affected, with no inhibition of growth at 50 μM (Figure 5b). The concentrations 75 and 100 μM showed toxic to the cells with viabilities ranging between 0–10%.

Trace element analysis on cell pellets and cell media

The calculated concentration (mol/cell) of some trace elements, in K-562 cells grown in RPMI-1640 medium or in Ham's F-10 medium, is displayed in Figure 6. The cell pellet measurements showed that cadmium and selenium values were significantly higher ($P<0.001$) in K-562 cells grown in RPMI-1640 than when grown in Ham's F-10. Lead, copper and nickel values did not differ significantly ($P>0.05$) between K-562 cells grown in RPMI-1640 or Ham's F-10. The cell content of manganese and mercury was significantly higher ($P<0.05$ and $P<0.01$, respectively) when the cells were grown in Ham's F-10 than in RPMI-1640.

Measurements on complete medium, based upon RPMI-1640 or Ham's F-10, have been performed and the concentration of some elements in (mol/l) are given in Figure 7. Cadmium, lead and selenium concentrations were significantly higher ($P<0.01$, $P<0.05$ and $P<0.05$, respectively) in RPMI-1640 medium than in Ham's F-10 medium. The nickel concentration did not differ significantly ($P>0.05$) between the two media. Copper, manganese and mercury showed significantly higher ($P<0.01$, $P<0.01$ and $P<0.01$, respectively) concentrations in Ham's F-10 medium.

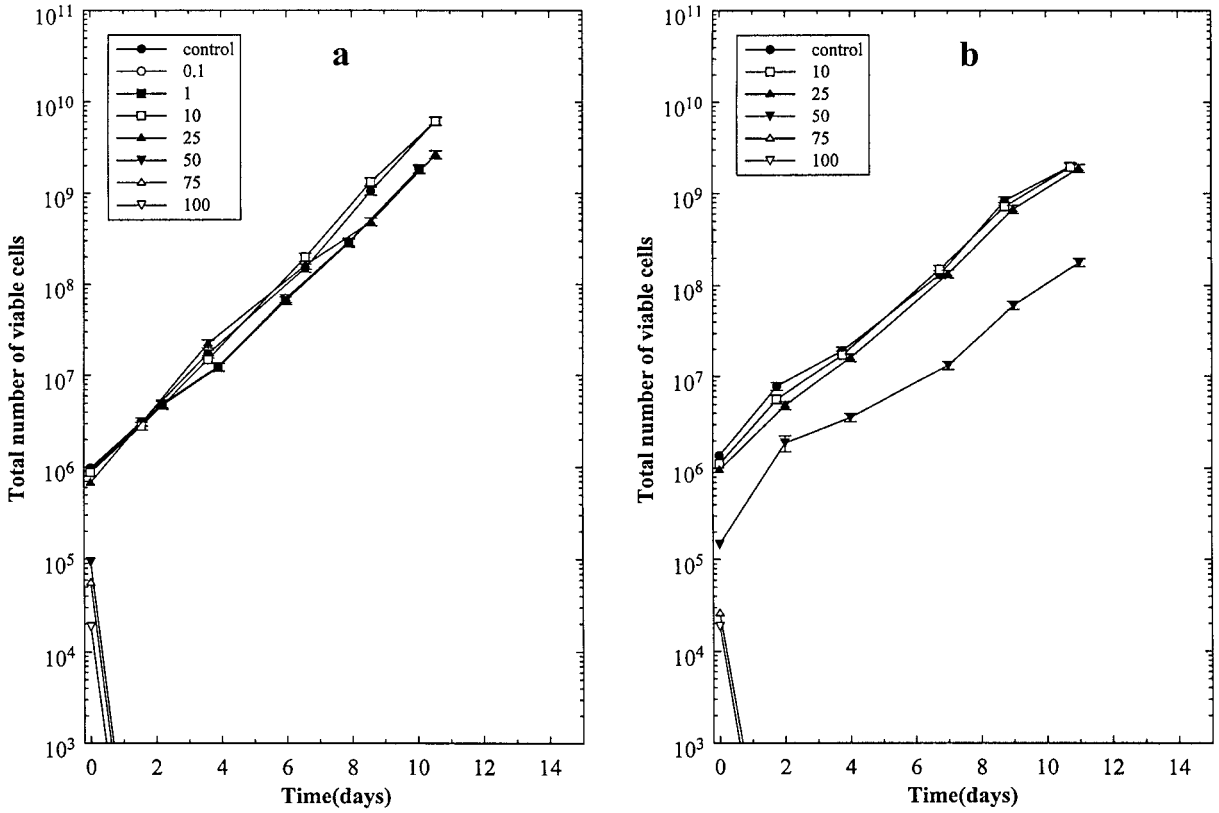

Figure 5. Concentration effects of cadmium nitrate on cell growth. K-562 cells were treated for 4 days with varying concentrations of cadmium nitrate before washed and plated for growth in medium based on RPMI-1640 (a) or medium based on Ham's F-10 (b). Each point corresponds to two samples. Mean values and maximal variations are shown.

Discussion

Exposure of K-562 cells to sodium selenite (50–100 μM) was less toxic in RPMI-1640 than in Ham's F-10 with a strong inhibition of cell growth at 50 μM compared to a toxic effect in Ham's F-10. An inhibition of growth was noticed in both types of media which very well agreed with a decrease in viability. Therefore the cells seem to adapt and protect themselves to survive and continue growing. Several studies have shown that supplemental selenite can inhibit the cell growth, both of cultured mammalian (Medina & Oborn 1984; Kuchan & Milner 1992) and cultured human (Petrie *et al.* 1986; Watson *et al.* 1986; Caffrey & Frenkel 1992) cells. In a study on selenite exposure to murine mammary epithelial cells it was noticed that the cellular response to selenite contains two phases: an early reversible inhibition of cell growth and a late irreversible cytotoxic effect (Morrison & Medina 1988). Our results essentially agree with a study on mouse mammary epithelial cells grown in Dulbecco's Modified Eagle Medium of Medina & Oborn (1984) in that 5 μM selenite inhibited the cell growth while 50 μM was toxic to the cells.

Exposure of the cells to selenomethionine (50–100 μM) in RPMI-1640 resulted in decreased growth compared to in Ham's F-10 showing a strong inhibition of growth at 50 μM and a cellular toxicity at 75–100 μM. Both selenium compounds, especially selenomethionine, were more toxic to the cells when grown in Ham's F-10 medium than in RPMI-1640. A possible explanation is that the concentration of L-methionine is 3.3 times less in Ham's F-10 than in RPMI-1640. A study on K-562 cells, showing that a 3-day exposure to selenomethionine was 1.3–1.8 fold more toxic when the methionine concentration in the medium was decreased 10-fold (Kajander *et al.* 1990), supports this hypothesis.The RPMI-1640 selenomethionine toxicity results could be compared to the study on K-562 cells exposed for 3 days of Kajander *et al.* (1990) which caused a 50% growth inhibition at 93 μM.

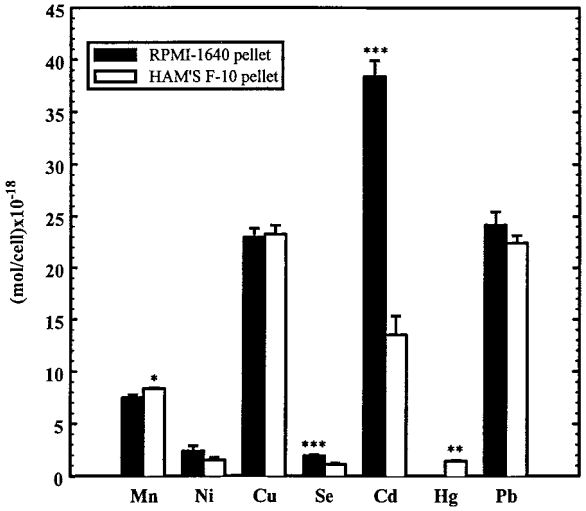

Figure 6. The cell concentration of some elements when grown in RPMI-1640 or Ham's F-10 is expressed as mole of a specific element per cell. The cells were washed thoroughly in PBS before the analysis. Data shown are mean ± SE, $n = 7$. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

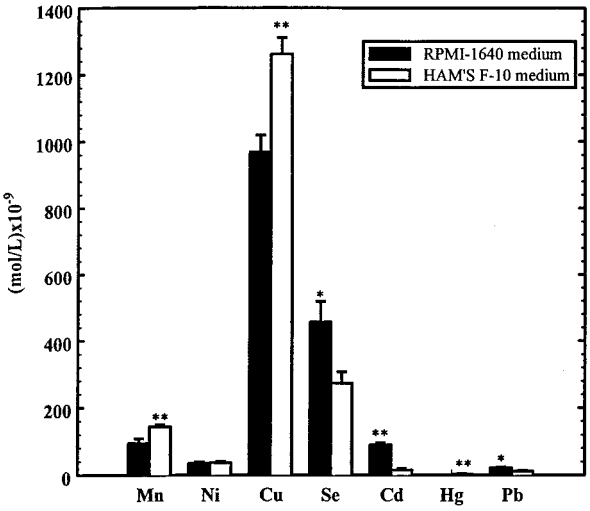

Figure 7. The medium concentration of some elements, expressed as mole per litre of a specific element, in complete RPMI-1640 or Ham's F-10 medium. Note that identical serum and additives were used in both media. Data shown are mean ± SE, $n = 6$. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

One difference in how these two selenocompounds affect the cellular growth is that the growth curves with selenite showed a clear connection between decreased viability and inhibition of growth while the growth curves with selenomethionine showed growth inhibition without any decrease in viability. This phenomenon has been shown before in a study on human mammary tumour cells where selenite affected both cell viability and growth rate of the tumour cells while selenomethionine did not affect the viability but retarded the cell growth (Yan *et al.* 1991). In that study it was noticed that the inhibition of cell proliferation was associated with a loss of intracellular glutathione when the cells were exposed to selenite but not when exposed to selenomethionine. These differences might be explained by different metabolic routes of these selenium compounds. Seko *et al.* (1989) have reported the formation of superoxide anion by the reaction of selenite with GSH. A study on rat hepatocytes indicated that superoxide anion and its reactive metabolites such as the hydroxyl radical might be involved in the cytotoxicity of selenite (Kitahara *et al.* 1993). These generated reactive oxygen species could exert toxic effects such as lipid peroxidation or DNA strand breaks in the cells. In a study with different cultured cell lines the results suggest a direct or indirect involvement of S-adenosylmethionine metabolism in selenomethionine cytotoxicity (Kajander *et al.* 1990). A possible explanation for the decreased population doubling time seen with exposure to selenomethionine could be that the compound affects the cell cycle progression as has been shown earlier (Redman *et al.* 1997).

Exposure to mercuric chloride of K-562 cells cultured in RPMI-1640 or in Ham's F-10 produced no differences in response to the cellular growth. When K-562 cells were exposed to methylmercuric chloride, similar results were observed in both media with almost no growth inhibition at 2.5 μM, while 5 μM showed toxic to the cells. Our findings corroborate with a study on HeLa S3 cells grown in Eagle's minimum essential medium with almost 100% viability for long time exposure to 2 μM methylmercuric chloride while five days of exposure to 3.2 μM resulted in a viability of 0% (Gruenwedel & Friend 1980). The inhibitory effect of methylmercuric chloride was stronger than that of mercuric chloride which is consistent with other cell studies (Nakatsuru *et al.* 1985; Aleo *et al.* 1992). Findings from mercuric chloride and methylmercuric chloride exposure to cells have indicated a GSH depletion as an important event in their cytotoxicity (Shenker *et al.* 1993; Shenker *et al.* 1999). Naganuma *et al.* (1998) have in a study on methylmercury exposure presented results indicating formation of superoxide anions in the mitochondria which might be involved in the mechanism of the cytotoxicity being consistent with a study of Shenker *et al.* (1999).

Exposure to cadmium nitrate of K-562 cells cultured in RPMI-1640 produced toxicity at 50 μM

compared to no growth inhibition at 50 μM when the cells were grown in Ham's F-10. A possible reason to this difference is the much higher amount of cadmium present in RPMI-1640 than in Ham's F-10 (Figure 7). The cadmium toxicity seem to be in accord with a study on human blood cells which showed a LD50 survival at 100 μM when cultured in Cd^{2+} for 40 h (Enger *et al.* 1983). One factor in the cadmium toxicity is that cadmium has been noticed to induce oxidative damage to cell membranes by enhancing lipid peroxidation and by altering the antioxidant capability of cells (Hussain *et al.* 1987; Sarkar *et al.* 1995; Sarkar *et al.* 1997; Yang *et al.* 1997).

The different effects from the RPMI-1640 or the Ham's F-10 medium on the K-562 cells may be better understood by comparing the trace element contents in cell pellets and medium (Figures 6 and 7). The significantly higher amounts of cadmium and selenium in K-562 cells grown in RPMI-1640 than in Ham's F-10 probably is a reflection of the same cadmium and selenium relation seen between the two media. The significantly higher cell content of manganese and mercury for cells grown in Ham's F-10 also reflects significantly higher levels of manganese and mercury present in Ham's F-10 medium.

The K-562 cells tend to accumulate cadmium, mercury and lead effectively (Figures 6 and 7) and these should, thus, be presented to the cells in a biologically available form. A slight increase in medium content of cadmium, mercury and lead directly increases the cellular uptake while this is not the case for the essential trace elements. The reason might be that essential trace elements are under more strict control by homeostatic mechanisms while metals like mercury are not subject to such a control. Studies performed on rat cardiomyocytes and renal cortical epithelial cells have shown that a majority of cadmium enters the cells through transport processes that exist for Ca, Zn and Cu. Furthermore, these transport processes involve membrane sulph-hydryl groups (Endo & Shaikh 1993; Limaye & Shaikh 1999). Methylmercuric chloride has been noticed to accumulate faster in cells than mercuric chloride and this might be explained by differences in membrane permeability and target site. For mercuric chloride the main target seems to be the plasma membrane, while methylmercuric chloride readily crosses this barrier and reacts with intracellular macromolecules (Nakada & Imura 1982; Braeckman *et al.* 1998). Regarding the high uptake of lead by the K-562 cells, several investigations on human red blood cells have indicated that the transport of lead across the erythrocyte membrane is an energy-independent carrier-mediated passive transport (Simons 1986; Sugawara *et al.* 1990).

The selenocompound exposures showed three types of cellular growth responses (a) no inhibition of the cell growth, (b) inhibition of cell growth with or without a reduction in cell viability or (c) toxicity to the cells. The concentrations of 2.5–10 μM of the selenocompounds not affecting the cell growth seem reasonable when compared to an estimated human plasma level of 0.75–5 μM of selenium (Whanger *et al.* 1988). In the metal exposures two types of cellular growth responses were noticed (a) no inhibition of the cell growth or (b) toxicity to the cells. Comparing the studies performed in RPMI-1640 with Ham's F-10, the results indicated no differences in toxicity with the mercuric compounds while cadmium and both selenocompounds differed in toxicity between the two media. Although it is impossible to say which of these two media that best reflects the human plasma situation this cellular assay seems more reliable when performed in Ham's F-10 with regard to the big cadmium uptake noticed when K-562 cells were grown in RPMI-1640 without exposure to metals. Extrapolating these results to the human situation should obviously be done with caution, but it seems as if the different compounds could be ranked according to decreasing toxicity as: methylmercuric chloride $>$ mercuric chloride, cadmium nitrate, sodium selenite $>$ selenomethionine.

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