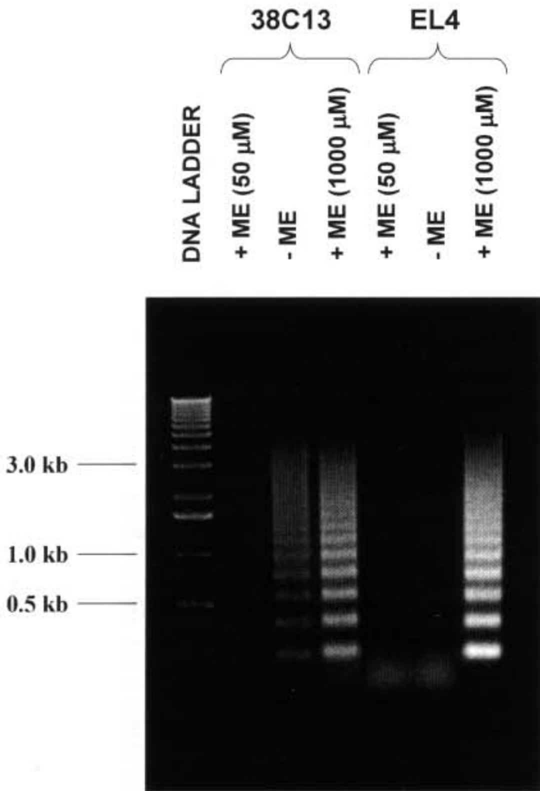


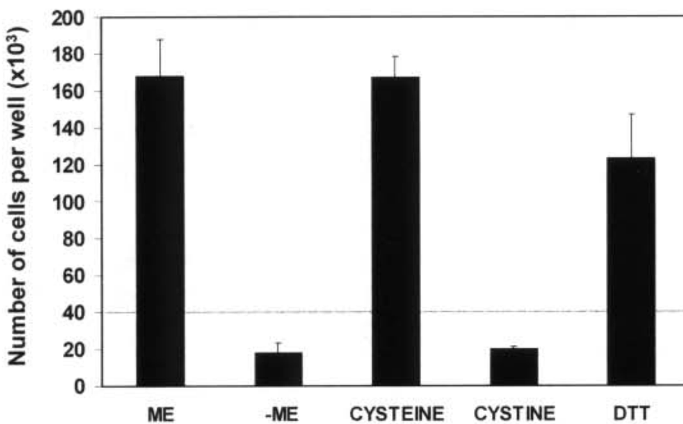
Fig. 3. Effect of availability of thiols, represented by 2-mercaptoethanol (ME), on DNA fragmentation in 38C13 and EL4 cells. Control conditions (basic medium with 50 µM 2-mercaptoethanol), thiol deprivation (without 2-mercaptoethanol), and thiol excess (with 1000 µM 2-mercaptoethanol) were tested. DNA fragmentation was determined after 16 h of incubation. Control DNA ladder is shown.

resulted in the death of 38C13 cells during 24-h incubation. On the other hand, 1000-µM and higher concentrations also resulted in cell death. The cells displayed growth in the presence of 1–300 µM 2-mercaptoethanol with the optimum at about 3–100 µM. In the case of EL4 cells, low 2-mercaptoethanol concentrations or even complete 2-mercaptoethanol withdrawal did not lead to cell death. Also, the growth of the cells under the deprivation of 2-mercaptoethanol was comparable with the growth under such 2-mercaptoethanol concentrations that were near optimal for 38C13 cells. Concentrations of 2-mercaptoethanol higher than 300 µM led to the death of EL4 cells similar to that seen in 38C13 cells (Fig. 1). The effect of another thiol compound, L-cysteine, was similar to the effect of 2-mercaptoethanol. However, effective concentrations were significantly higher (data not shown).

We also tested the effect of thiol deprivation (2-mercaptoethanol withdrawal) and thiol excess (1000 µM 2-mercaptoethanol) on the survival of several other cell lines. Both human Burkitt lymphomas Raji and Jiyoye were sensitive to thiol excess but resistant to thiol deprivation. Similarly, human T-cell leukaemia Jurkat and human cervix carcinoma HeLa were sensitive to thiol excess and resistant to thiol deprivation. All cell lines resistant to thiol deprivation, including EL4 cells, were able to proliferate without the presence of 2-mercaptoethanol permanently.

Apoptosis induction by thiol deprivation and thiol excess

Flow cytometric analysis, after propidium iodide staining, detected accumulation of cells with hypodiploid DNA content, typical for apoptosis, in the population of 38C13 cells after 8 h of incubation under thiol deprivation (2-mercaptoethanol withdrawal) as well as under thiol excess (1000 µM 2-mercaptoethanol). The apoptotic peak of hypodiploid cells increased and the number of cycling



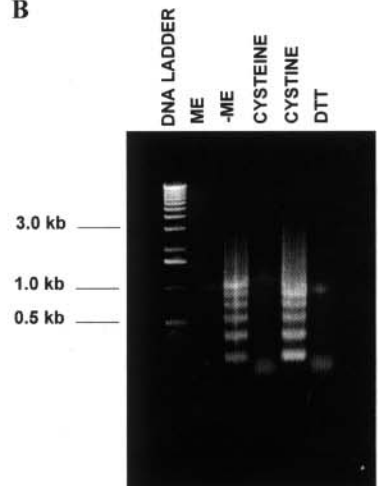


Fig. 4. Effect of 500 µM L-cysteine (CYSTEINE), extra 500 µM L-cystine (CYSTINE), and 500 µM dithiothreitol (DTT) on (A) growth and survival, and on (B) DNA fragmentation of 38C13 cells. Cells incubated with 50 µM 2-mercaptoethanol (ME) represent a positive control and cells incubated without 2-mercaptoethanol (-ME), i.e. in the basic medium, represent a negative control. The basic medium itself contains approximately 200 µM L-cystine. (A) Cells were seeded at 40 x 10^3 cells/100 µl of medium in the well. The number of cells in the inoculum is shown as a dotted line. The number of living cells was determined after 24- h incubation Each column represents the mean of at least four separate cultures ± SEM. (B) DNA fragmentation was determined after 22 h of incubation. Control DNA ladder is shown.