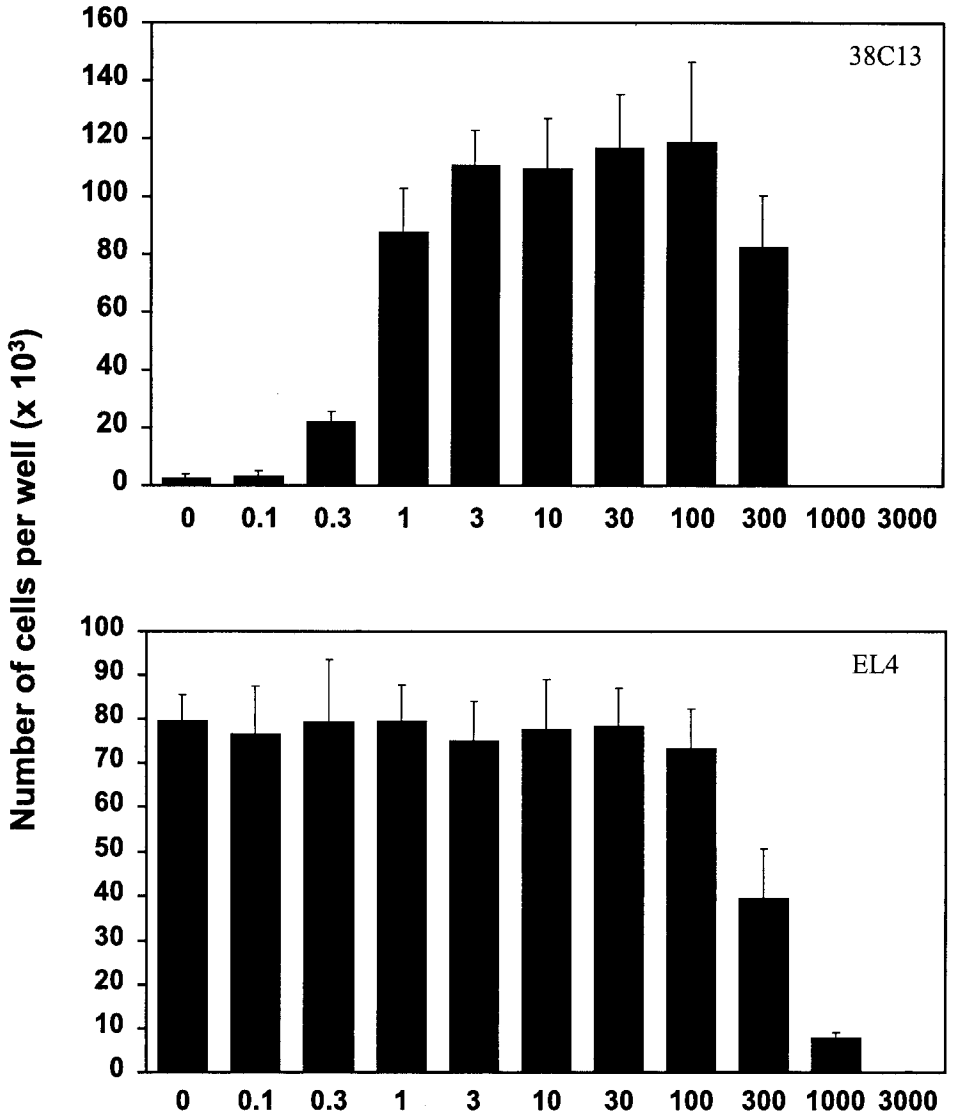

Fig. 1. Effect of 2-mercaptoethanol (0.1–3000 μM) on the growth and survival of 38C13 and EL4 cells. Control cells were incubated without 2-mercaptoethanol, i.e. in the basic medium. Cells were seeded at 40 x 10^3 cells/100 μl of medium in the well. The number of cells in the inoculum is shown as a dotted line. The number of living cells was determined after 24-h incubation. Each column represents the mean of at least four separate cultures ± SEM.

1 mM EDTA, 50% glycerol, 2% SDS, 20% saturated bromphenol blue solution) were added. Samples (20 μl) were run on a 1% agarose gel with ethidium bromide (10 μg/ml) in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA) at about 10 V/cm. DNA was visualized under UV light and photographed.

Indirect immunofluorescence analysis

Cells previously grown in the basic medium supplemented with 50 μM 2-mercaptoethanol were harvested by low-speed centrifugation, washed with the basic medium and seeded at 400 x 10^3 cells/ml of medium into plastic culture flasks. The effect of control conditions (basic medium with 50 μM 2-mercaptoethanol), thiol deprivation (basic medium without 2-mercaptoethanol) and thiol excess (basic medium with 2000 μM 2-mercaptoethanol) was tested. Indirect immunofluorescence according to the modified method of Pollice et al. (1992) was employed to assess the expression of p53, $p21^{CIP1/WAF1}$, Bcl-2 and Bax. After 0, 4, 8, 12 and 16 h of incubation, the cells were harvested by low-speed centrifugation and stained. Briefly, approximately 4 x 10^6 cells per sample were washed with 4 ml of PBS and then fixed in 2 ml of 0.25% paraformaldehyde in the dark for 15 min at room temperature. The cells were spun, washed with PBS and then fixed in 2 ml of 70% methanol for 1 h at 4°C. Fixed cells were centrifuged and washed with PBS. The cell pellet (approximately 1 x 10^6 cells per parallel) was resuspended and incubated in 50 μl of primary antibody (5 μg/ml of PBS) or in 50 μl of non-specific mouse, hamster or rabbit IgG (5 μg/ml of PBS) as a negative control. Mouse monoclonal antibody (IgG) PAb 240 against mouse p53, mouse monoclonal antibody (IgG) HZ 52 against mouse p21, hamster monoclonal antibody (IgG) 3F11 against mouse

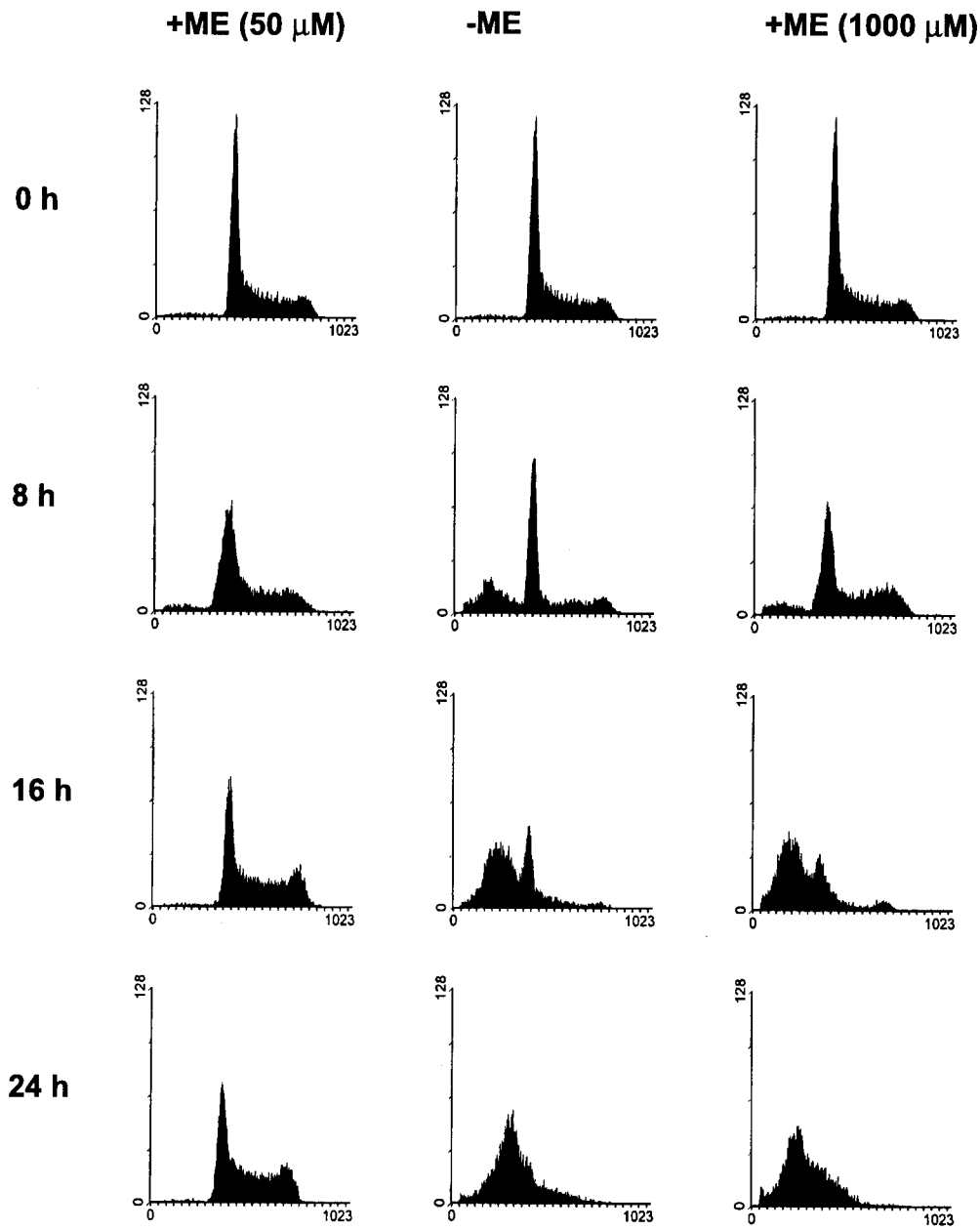

Fig. 2. Effect of availability of thiols, represented by 2-mercaptoethanol (ME), on DNA histograms of 38C13 cells. Control conditions (basic medium with 50 μM 2-mercaptoethanol), thiol deprivation (without 2-mercaptoethanol), and thiol excess (with 1000 μM 2-mercaptoethanol) were tested. After the incubation period (0, 8, 16 and 24 h), the cells were stained with propidium iodide and analysed by flow cytometry.

Bcl-2 and rabbit polyclonal antibody (IgG) Sc-562 against mouse Bax were used as primary antibodies. After 30 min of incubation on ice, 400 μl of PBS were added and cells were resuspended. The sample was underlain with 100 μl of foetal bovine serum (PAN Biotech, Aidenbach, Germany) and spun. The cell pellet was resuspended and incubated in 50 μl of secondary staining reagent (10 μg/ml of PBS). Corresponding (anti-mouse, anti-hamster and anti-rabbit) fluorescein-conjugated goat antibodies were used as the secondary staining reagents. After 30 min of incubation on ice, 400 μl of PBS were added and cells were resuspended. The sample was again underlain with 100 μl of foetal bovine serum and spun. Stained cells were resuspended in 300 μl of PBS and analysed in a FACScan flow cytometer (Becton Dickinson).

Results

Cell growth and survival under differing availability of thiols

We compared the effect of 2-mercaptoethanol in a wide range of concentrations (0.1–3000 μM) on the growth and survival of 38C13 and EL4 cells. Concentrations of 2-mercaptoethanol about 0.3 μM and lower