

cells was little affected. However, in PTDS-treated cells, incorporation of [^3H]-thymidine was about 20% of that in control cells. These results suggest that PTDS can block DNA synthesis, and that this effect is likely the primary cause of its antiproliferative effect.

We also examined the effect of PDTC and PTDS on the ability of HT29 cells to form clones when plated at low seeding densities. HT29 cells were plated and treated with 100 μM PDTC or PTDS for 14 days, and then cells were fixed, stained, and quantified. The result is shown in Fig. 4. PTDS was effective in reducing the clonogenic capacity, whereas PDTC was just a little effective in reducing the clonogenic capacity; the reducing capacity of DMSO was almost negligible. These experiments demonstrate that treatment with PTDS leads to a dramatic reduction in linear or clonogenic cell growth.

PDTC and PTDS Do Not Induce Differentiation

HT29 cells treated with PTDS manifest unique morphological changes, become unattached to the culture dish, and achieve cell growth quiescence (data not shown). These results can be associated with, if not

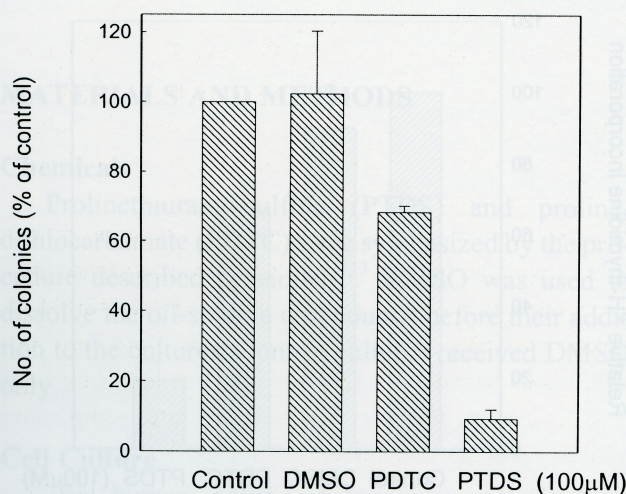


Fig. 4. Clonogenic formation in PDTC- and PTDS-treated cells. HT29 cells were seeded in 6-well plates and grown for 14 days in the presence of 0.1% DMSO, or 100 μM PDTC or PTDS. The number of colonies was quantified as described in Materials and Methods. Values are the mean $\pm$ SD of 3 different experiments.

caused by, the induction of cell differentiation. Sodium butyrate (NaBT) is a differentiation agent in the HT29 colon adenocarcinoma cell line.^{15,16} Alkaline phosphatase is a well-established differentiation marker in colon cancer cells.^{16,17} To determine whether PDTC

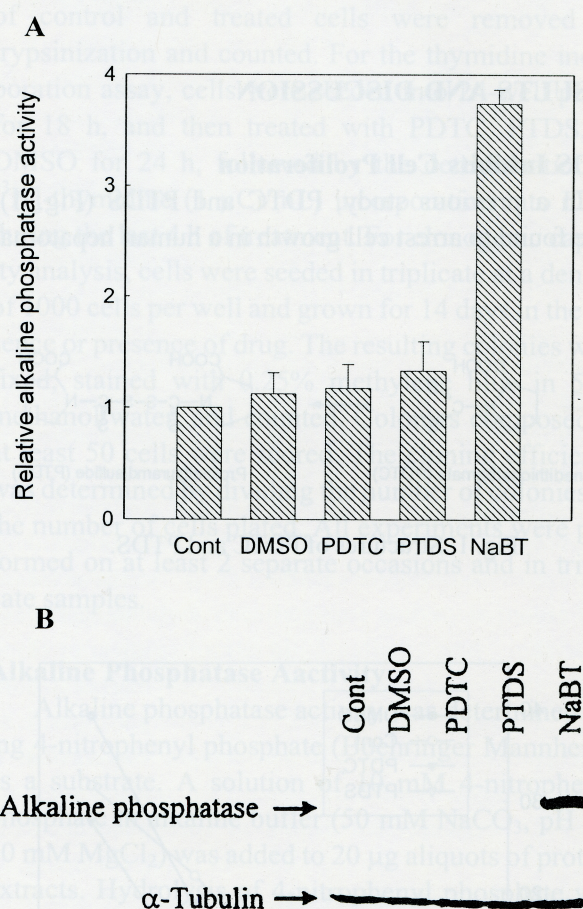


Fig 5. Western blot of alkaline phosphatase in PDTC- and PTDS-treated cells. HT29 cells were treated with 100 μM PDTC, PTDS, 5 mM NaBT, or 0.1% DMSO for 96 h. (A) Alkaline phosphatase activity assay was performed as described in "Materials and Methods". Values are the mean $\pm$ SD of 3 different experiments. (B) Proteins were extracted and fractionated on 10% SDS-PAGE gels; then Western blots were performed as described in "Materials and Methods". An anti-placental alkaline phosphatase antibody was used at a dilution of 1:1000. Alkaline phosphatase was appreciably expressed only in NaBT-treated cells. This experiment was repeated 2 additional times, and similar results were obtained each time.