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# EFFECT OF DIMETHYLSULFOXIDE AND DIMETHYLFORMAMIDE ON THE GROWTH AND MORPHOLOGY OF TUMOR CELLS



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## Introduction

The morphological pattern in which cells grow in culture has often been taken as an indication either of their malignancy or non-tumorigenicity. Unlike their normal counterpart, tumor cells grow in culture in disarray, pile up and migrate past each other in such a manner as to resemble their behavior in tumors. We have been interested to see whether reversal of the pattern of growth back to that characteristic of non-tumor cells could be achieved and whether this would also be accompanied by loss of malignancy.

The reported decrease in the malignancy of Friend erythroleukemic cells and the marked enhancement of their differentiation along the erythroid pathway following their treatment by the simple solvent dimethylsulfoxide (DMSO)[1-3] stimulated our study of the effect of this agent, as well as the closely related solvent dimethylformamide (DMF), on various malignant and normal cell lines used in our laboratory. An alteration in the pattern of growth of tumor cells resulted following treatment with these agents. This effect is mimicked by the totally unrelated agent 5-bromodeoxyuridine (BrdU)[4-6].

## Materials and Methods

Cell Lines. The mouse tumor cells used included the melanoma line Mel B16, established in our laboratory, Ehrlich ascites cells adapted to grow *in vitro*,

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3T3 cells transformed by polyoma virus (3T3-Py) and TCMK-$SV_{40}$, a virus-transformed mouse kidney cell line obtained from the Tissue Type Culture Association. Most of the experiments were carried out with QUA, a cell line established by Dr. June Biedler (Sloan-Kettering Institute) from a tumor induced in C57/Bl/6 mice by methylcholanthrene[7]. Cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, streptomycin and penicillin and maintained in a humidified atmosphere of 5% $CO_2$-95% air at 37°. Cultures were frequently monitored for the presence of mycoplasma and found to be free of contamination.

Chemicals

Reagent-grade dimethylsulfoxide and dimethylformamide were obtained from Fisher Scientific Company, N.Y.; thymidine and bromodeoxyuridine from Schwarz/Mann, Orangeburg, N.J.; cycloheximide from Upjohn, N.Y., N.Y.; ethidium chloride from May and Baker, Dagenhew, England; conconavalin A and wheat germ lipase from Calbiochem, San Diego, Calif.; puromycin from Lederle Lab, Pearl River, N.Y.; actinomycin from Merck Sharp and Dohme, Rahway, N.J. Fucose $^{3}H$ (1.8 Ci/mM) and fucose $^{14}C$ (160 mCi/mM) were purchased from Amersham/Searle, Arlington Heights, Ill. and 2-deoxyglucose-$^{3}H$ (7.2 Ci/mM) from New England Nuclear, Boston, Mass.

Assays

An inoculum of 250 cells was seeded onto 60 mm plastic petri dishes and the various agents were added to the medium 24-48 hrs later. Concentrations of DMSO varied from 1-2%, DMF from 0.5-1% and BrdU from 1-3 µg/ml depending on the sensitivity of the cell lines studied. In other experiments, chemicals

were added only after the typically randomly piling individual colonies had been established. For mass culture studies, $2 \times 10^5$ cells were inoculated either into milk dilution bottles or plastic petri dishes to which media containing the agents was then added.

Studies involving incorporation of radioactive fucose into surface glycoproteins were carried out according to the procedure described by Buck, et al[8] (see legend to Fig. 1), and the digested "trypsinates" were chromatographed on Sephadex G-50 columns. Radioactivity in chrmatographic fractions was determined in a Packard-Tri-Carb liquid scintillation counter. Agglutination studies with wheat germ lipase and conconavalin A were carried out as described by Burger and Goldberg[9] and Inbar and Sachs[10]. Growth curves and replication cycles were determined by seeding a series of replicate dishes and counting cells at given time intervals. Viability was assessed by the neutral red exclusion test.

To measure 2-deoxyglucose uptake, duplicate 60 mm dishes containing $1 \times 10^6$ cells were washed with pre-warmed glucose-free Hanks solution and incubated for 10 min at 39° with 2.0 ml 0.25 µC 2-deoxyglucose-$^3$H per ml (7.2 Ci/mM) Hanks without glucose as described by Martin et al[11]. After four rapid washes with ice-cold glucose-free Hanks, cells were scraped into 0.5 ml water, disaggregated in a vortex mixer, and aliquots taken for scintillation counting in a toluene-triton mixture and for protein determination[12].

Results

A marked alteration in the randomly piling, non-contact inhibited growth pattern of the QUA tumor cell line occurred when the normal growth medium was

supplemented by DMSO at final concentrations of 1-2%, DMF at 0.5-1% and BrdU at 1-3 µg/ml. Instead of the piling pattern, monolayers of cells in regular parallel orientation formed which were typical of non-malignant fibroblasts (Fig. 1). The same drastic changes were observed when the agents were added even after colonies of piled-up cells, characteristic for this tumor line, had been allowed to form. Accordingly, this reversion of colonial morphology was not due to a selection of cells from within the total population. This was borne out by a study of the replication cycle, which showed that although there was an increase in generation time, there was no cell death. The doubling time was about 30% longer in the presence of the agents.

Incubation with DMF or DMSO for about 3 days, and with BrdU for about 5-6 days, was required before morphological changes were manifested. These changes could be maintained indefinitely by growth in the continuous presence of the chemical agents. Reversion to the original growth pattern could be effected in about 3 days after removal of DMSO or DMF, or 6 days after withdrawal of BrdU from the medium. Thymidine (but not uridine) prevented the BrdU-induced effect if added to the growth medium at 5 times the molar concentration of BrdU, but no such interference with the observed DMF or DMSO effect occurred. 5-Bromouridine at concentrations of 1-10 µg/ml did not mimic the effect induced by BrdU. The tumor lines QUA, Mel B16, 3T3-Py, or TCMK-$SV_{40}$ behaved similarly when treated with the agents under investigation.

The changes described above could not be induced by treatment of the tumor cells with dibutyryl cyclic AMP, with or without theophyllin,

as had been observed by other investigators with different cell lines[13,14] or by cytochalasin B, a mold metabolite which interferes with cell movement and microfilament function. To study the mechanism of action of DMSO, DMF and BrdU, cell cultures containing these agents were treated with inhibitors of protein, cytoplasmic RNA or mitochondrial DNA biosynthesis. The inhibitors had to be used at sub-toxic concentrations, since several days of growth in the presence of the agents are required before morphological changes are manifested. Under these conditions puromycin (0.2 μg/ml), cycloheximide (0.05 μg/ml) and ethidium chloride ($10^{-7}$ molar) failed to interfere with the observed effects.

Since the membrane properties of the cells appeared to be affected after incubation with the agents under investigation, their effect on the agglutinability of the cell with wheat germ lipase and concanavalin A was examined. We observed a considerable decrease in agglutinability after the cells were grown in the presence of DMSO, DMF or BrdU. This prompted us to examine the surface glycoproteins by a double-label experiment in which we exposed cells to $^{14}C$ or $^{3}H$ fucose before and after incubation with the chemical agents. Chromatographic analysis of pronase-digested trypsinates of glycoproteins of the surface membranes showed that treatment with DMSO or DMF brought about a decrease in the high molecular weight fucose-containing fraction and increased the low molecular weight fraction of the tumor cells when compared with those of the untreated tumor cells (Fig. 2). However, incubation with BrdU did not significantly alter the elution profile of the glycopeptide fractions of these cells.

Several laboratories have reported an increased uptake of 2-deoxyglucose

by virus-transformed cells, as compared to their normal counterparts[11,15]. This is presumably due to increased phosphorylation of the sugar by the tumor cells[16]. Uptake of 2-deoxyglucose by the untreated parent tumor cell line was not significantly altered after treatment with DMSO, DMF or BrdU. Similar results were obtained regardless of whether the agents were present in the cultures for 30 min or for several days (Table 1). Those findings are at variance with a report of a decrease in the 2-deoxyglucose uptake after treatment of 3T6 fibroblasts with BrdU[17].

Discussion

It has been shown that DMSO, DMF, and BrdU affect the morphology and growth pattern of tumor cells in culture inducing a drastic change from the randomly piling non-contact inhibited growth characteristic of tumor cells to one more closely resembling the parallel, oriented growth of normal cells. This effect was readily reversed after withdrawal of the chemical from the medium. Despite a similarity in action, the three agents exert their effects by different mechanisms and on different targets within the cell. BrdU has been reported to suppress cytodifferentiation[4,5], melanin production and tumorogenicity[6,18] of cells in vitro and to inhibit preferentially synthesis of tyrosine amino-transferase in hepatoma cells[19]. These effects of BrdU as well as those observed in our studies could be reversed with excess thymidine; this was not so for the DMSO- or DMF-treated cultures. BrdU might interact directly with the genetic material. DMSO and DMF on the other had seem to affect membrane components, or their assembly, thereby leading to structural changes or spatial rearrangements of the glycoproteins at the cell surface.

The observations by Buck et al[8] and Warren et al[20] of the presence of larger amounts of high molecular weight fucose-containing glycopeptides at the surfaces of virus transformed cells as compared with their normal controls and those of Bosman et al[21] of a marked increase of glycosyl and galactosyl transferases in virus transformed cells suggest that DMSO and DMF may exert their effects on cellular differentiation. This is exemplified by the DMSO-induced erythroid differentiation of tumor cells[1], the increase of hydroxyproline concentration in human fibroblasts[22] and the extrusion and budding of virus particles from virus-infected cells[23,24]. DMSO also reversibly inhibits neurite extension by mouse neuroblast cells in vitro[25].

DMSO and DMF which are both very stable, highly polar substances, with dielectric constants greater than water, are known to enhance cell permeability[26,27]. It is possible that these properties and the influence these solvents may exert on the hydration and solvation shells around the membrane components may account for the various effects on cells, including increased uptake of RNA isolated from mengo virus[28].

An enhanced rate of uptake of 2-deoxyglucose by transformed cells has been reported from a number of laboratories[15,16,29]. This could either reflect membrane alterations during the process of transformation or to enhanced phosphorylation of the sugar by intracellular kinases[16]. Whatever the effects of DMSO or DMF on cell membranes might be, they did not significantly affect the uptake of 2-deoxyglucose by the tumor cells in our studies.

It is tempting to speculate that BrdU might act at the gene level, where due to its incorporation into DNA, it exerts an influence on transcription, and thus on the synthesis of some of the gene products characteristic of the

state of differentiation of these cells[30,31]. DMSO and DMF on the other hand might stimulate tumor cells to differentiate. The observed inhibition by BrdU of the DMSO-induced erythroid differentiation of Friend leukemia cells[32] suggests that the use of other agents which affect cells may provide further support for the argument that cancer is a disease of differentiation[33,34].*

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*Although the QUA tumor cells used in these studies uniformly grew as tumors upon inoculation into mice[7,35], pretreatment of the cells with DMSO failed significantly to influence their tumorgenicity. However, the time needed for the appearance of tumors would have been quite sufficient to restore the malignant growth pattern if the cells had been kept in culture in the absence of DMSO for this protracted period. Recipient mice were therefore pretreated for several days with DMSO and maintained on this agent after the DMSO-treated cells were injected. Although there was inhibition of tumorgenicity in a few instances, the results were quite variable (unpublished results).

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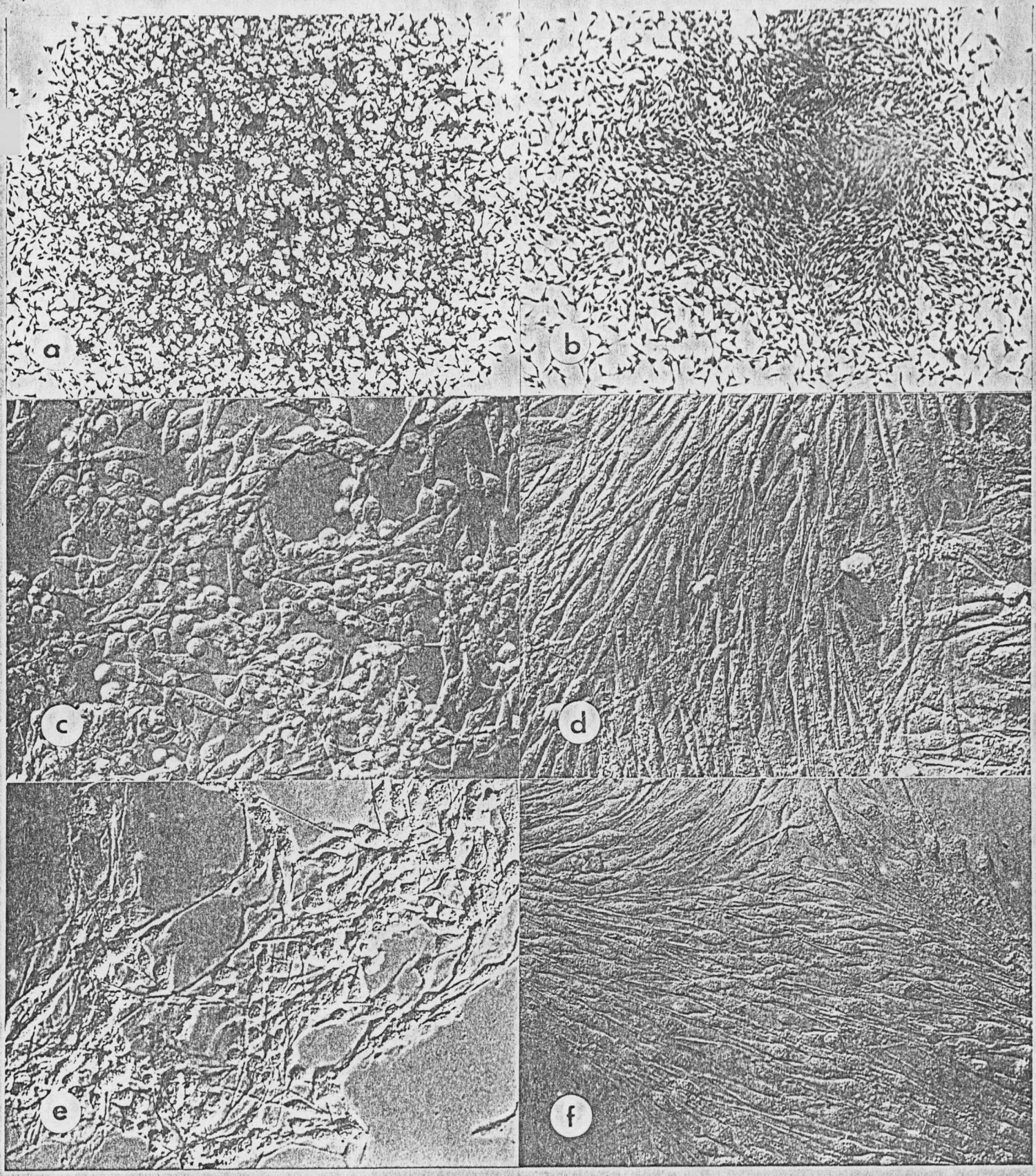
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Legend to Figure 1: The effect of dimethylsulfoxide and dimethylformamide on the *in vitro* growth pattern of tumor cells.

a) Colony of QUA tumor cells; X32; Giemsa.

b) Colony of QUA tumor cells grown for 8 days in presence of 2% DMSO; X32; Giemsa.

c) Colony of QUA tumor cells.

d) Colony of QUA tumor cells grown for 7 days in presence of 1% DMF.

e) Colony of Melanoma B12 cells.

f) Colony of Melanoma B12 cells grown for 10 days in presence of 0.5% DMF.

[c to f: X200, photographed in living state, Nomarski optics]

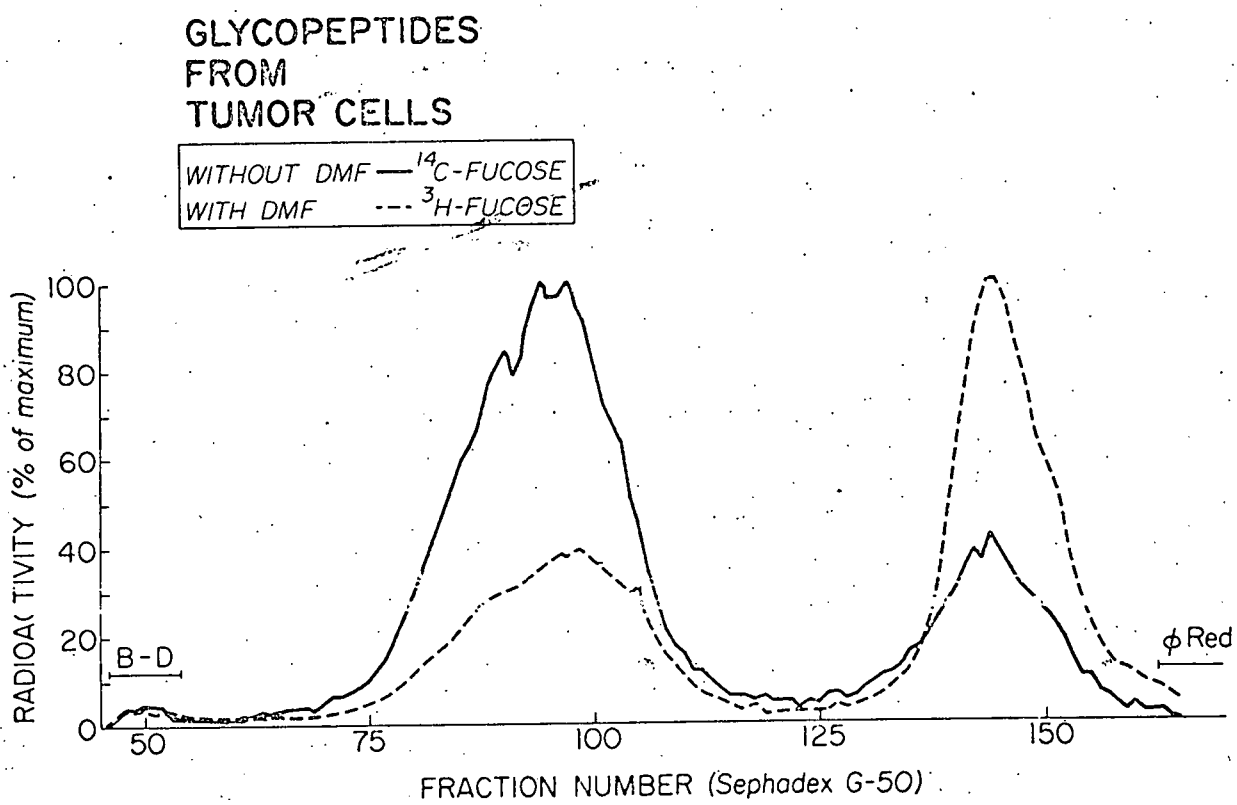
GLYCOPEPTIDES
FROM
TUMOR CELLS
WITHOUT DMF —— $^{14}$C-FUCOSE
WITH DMF --- $^{3}$H-FUCOSE
RADIOACTIVITY (% of maximum)
100
80
60
40
20
0
B-D
ϕ Red
50
75
100
125
150
FRACTION NUMBER (Sephadex G-50)

Figure 2

Legend to Figure 2: $2 \times 10^6$ QUA cells were grown in each of 7 Blake flasks with 50 ml Eagle's minimum essential media with Hank's salts, supplemented with 10% fetal bovine serum and fucose $^{14}C$ to a final concentration of 0.2 µC/ml (160 mCi/mM). After 24 hours, 25 ml of medium (without additional fucose) was added to each flask and cells grown for an additional 48 hours. Cells which had been grown in the presence of DMSO, DMF, or BrdU were incubated in media containing the respective agents and fucose $^{3}H$ 0.4 µC/ml media (1.8 Ci/mM). After incubation, cells were washed 4 times with 0.01 M Tris buffer pH 7.4, trypsinized, digested with pronase and chromatographed on Sephadex G-50 as described[8]. Fractions were counted in a Packard-Tri Carb liquid scintillation counter.

TABLE I

UPTAKE OF 2-DEOXYGLUCOSE[$^3$H] BY TUMOR CELLS AFTER INCUBATION WITH DIMETHYLSULFOXIDE, DIMETHYLFORMAMIDE OR 5-BROMODEOXYURIDINE

| Treatment | Length of Treatment | Counts/µg Protein |
|---|---|---|
| Control | 0 | 707 |
| DMSO, 1% | 30 min. | 791 |
| DMF, 1% | 30 min. | 694 |
| BrdU, 1 µg/ml | 30 min. | 561 |
| Control | 0 | 1,195 |
| DMSO, 1% | 42 days | 982 |
| DMF, 1% | 13 days | 1,158 |
| BrdU, 1 µg/ml | 27 days | 919 |

After short term or long term incubation of the tumor cells with the agents studied, the monolayers were washed with glucose-free Hanks, then incubated with 0.25 µC/ml 2-deoxyglucose-$^3$H (7.2 Ci/mM) for 10 min. at 39° (see text for details). Aliquots were removed for counting and protein determination.

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