octan-1-ol phase[16,22]. Although the positive enthalpies and entropies we find for the octan-1-ol/water partition point to a hydrophobic partition mechanism, the non-linearity of the van 't Hoff plots and the poor correlation ($r = 0.972$) with values calculated from fragmental constants raise considerable doubts as to the nature of the effects actually measured.

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Paradoxical effect of 1-β-D-arabinofuranosyl cytosine triphosphate on bleomycin-induced unscheduled DNA synthesis in permeable sarcoma cells[1]

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Summary. 1-β-D-Arabinofuranosyl cytosine-5'-triphosphate (araCTP), an inhibitor of DNA synthesis, paradoxically enhanced unscheduled DNA synthesis (USD) induced by bleomycin in permeable mouse sarcoma cells. A greater enhancing effect of araCTP on bleomycin-induced USD was observed with lower concentrations of dCTP in the assay mixture. USD measured without bleomycin in nuclei isolated from mouse sarcoma cells was not enhanced, but inhibited by araCTP.

1-β-D-Arabinofuranosyl cytosine (araC) is used as an antitumor agent owing to its inhibitory effect on cellular DNA synthesis. The inhibitory effect has been studied in vitro using its active form, araCTP[2]. AraCTP is known to inhibit preferentially replicative DNA synthesis, but USD is also inhibited by araCTP at high concentrations[3]. Bleomycin, which is also an antitumor agent[4], is known to induce USD[5,6]. The present communication concerns the enhancing effect of araCTP on bleomycin-induced USD in permeable cells.

Materials and methods. Mouse ascites sarcoma (SR-C3H/He) cells were permeabilized by treatment with buffer B (0.25 M sucrose, 10 mM Tris-Cl, 4 mM $MgCl_2$, 1 mM EDTA and 6 mM 2-mercaptoethanol, pH 8.0) supplemented with Triton X-100 at 0.0175% (Triton-buffer B)[7]. Nuclei were prepared from SR-C3H/He cells in stationary phase and from livers of adult, male Donryu rats[8]. Permeable cells or nuclei were distributed in assay tubes at 2×10^6 cells or 4×10^6 nuclei per tube. The suspension volume was adjusted to 0.38 ml by adding Triton-buffer B for permeable cells or by adding buffer B for nuclei. For replicative DNA synthesis, 0.2 ml of substrate mixture (0.1 M Tris-Cl, 7 mM $MgCl_2$, 0.24 M NaCl, 7.5 mM ATP, 0.15 mM dATP, 7.5 μM dCTP, 0.15 mM dGTP and 7.5 μM [^{3}H]dTTP, 0.5 Ci/mmole, pH 8.0) was added to the suspension[7]. For USD, ATP was omitted from the above replicase substrate mixture. Bleomycin A_2 and araCTP, both dissolved in distilled water, were added either singly or together to the suspension in a volume of 0.02 ml[9]. The reaction mixture (final volume: 0.6 ml) was incubated at 37 °C for 10 min for replicative DNA synthesis and for 60 min for USD. The radioactivity incorporated into acid-insoluble material was measured by a disc method[10].

Results and discussion. DNA synthesis measured in permeable cells in the presence of ATP, 4 deoxynucleoside triphosphates (dNTPs), Mg^{2+} and a proper ionic environment was S-phase specific, and the DNA synthesis was due largely to the elongation of strands initiated in vivo[7,10]. All available evidence indicated that the DNA synthesis was replicative[7,10]. Replicative DNA synthesis was highly dependent on ATP and was reduced to about 5% by omission of ATP. Bleomycin induced USD, concomitantly inhibiting replicative DNA synthesis[9]. DNA synthesis measured in permeable cells in the presence of bleomycin with an ATP-free assay mixture was unscheduled[9]. USD was also induced without bleomycin in nuclei isolated from SR-C3H/He cells in stationary phase or from rat livers[8,11]. The induction was attributed to DNA damage which occurred in the process of nuclear preparation and to repair of the damaged DNA[8,11]. The unscheduled nature of the DNA synthesis was confirmed by autoradiography. USD in the present systems was clearly distinguished from replicative DNA synthesis by aphidicolin[12]. Aphidicolin inhibited replicative DNA synthesis selectively and almost completely. USD in bleomycin-treated permeable cells and in nuclei isolated from SR-C3H/He cells or rat livers was completely resistant to aphidicolin. Replicative DNA synthesis in permeable cells was highly sensitive to araCTP, whereas USD

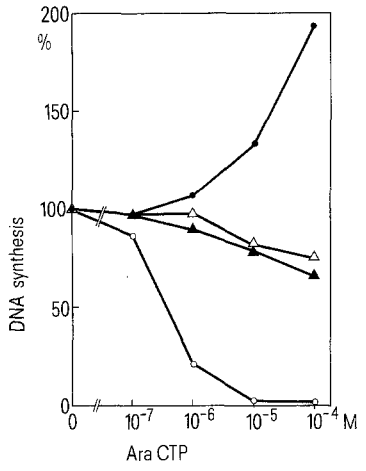

Figure 1. Effects of increasing araCTP concentration on replicative DNA synthesis and USD. Replicative DNA synthesis in permeable SR-C3H/He cells (○), bleomycin-induced USD in permeable cells (●), USD in SR-C3H/He cell nuclei (▲), and USD in rat liver nuclei (△) were measured as described in the text. AraCTP was added to the assay mixture at the concentration indicated. Activity measured in the absence of araCTP was 43.7 pmoles of [^{3}H]dTMP incorporated per 10^7 cells per 10 min for replicative DNA synthesis, 15.9 pmoles per 10^7 cells per 60 min for bleomycin-induced USD in permeable SR-C3H/He cells, 2.41 pmoles per 10^7 nuclei per 60 min for USD in SR-C3H/He nuclei, and 7.08 pmoles per 10^7 nuclei per 60 min for USD in rat liver nuclei. Results are expressed as a percentage of the activity measured in the absence of araCTP.

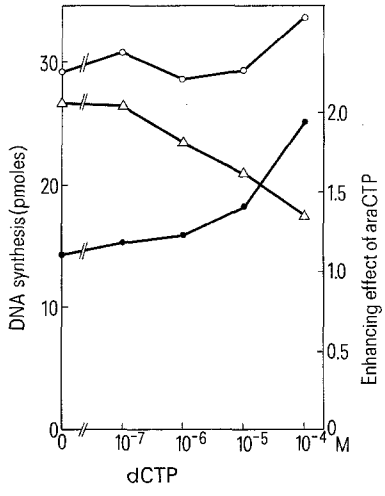

Figure 2. Effects of increasing dCTP concentration on bleomycin-induced USD and araCTP-mediated stimulation of the DNA synthesis. Bleomycin-induced USD was measured in the absence (●) or in the presence (○) of 100 μM araCTP, as described in the text except that the concentration of dCTP was varied as indicated. The activity is expressed as pmoles of [^{3}H]dTMP incorporated per 10^7 cells per 60 min. The enhancing effect of araCTP (△) is expressed by the ratio of DNA synthesis in the presence of araCTP to that in the absence of araCTP.

in nuclei isolated from SR-C3H/He cells and rat livers was much less sensitive (fig. 1). The result is similar to that obtained by Stenstrom et al.[3] using isolated rat liver nuclei. We were surprised to find that bleomycin-induced USD in permeable cells was enhanced, rather than inhibited, by araCTP (fig. 1). Bleomycin-induced USD showed low dependency on all 4 dNTPs[9], and significant USD was observed even when dCTP was omitted from the reaction mixture (fig. 2). Although bleomycin-induced USD measured in the presence, and in the absence, of araCTP increased according to the increment of dCTP concentration in the assay mixture, the relative enhancing effect of araCTP decreased with increasing dCTP concentration (fig. 2). Bleomycin-induction of repair DNA synthesis was attributed to DNA degradation caused by bleomycin, and to repair of the degraded DNA[5,6,13,14]. Some nucleotides (ATP, CTP, GTP, UTP and dNTPs) were shown to enhance bleomycin-induced DNA degradation in vaccinia virus DNA[15]. To distinguish bleomycin-induced DNA degradation from repair DNA synthesis in bleomycin-induced USD, permeable cells were pretreated with bleomycin, washed with Triton-buffer B, then incubated for DNA synthesis in bleomycin-free assay mixture. AraCTP and dCTP markedly enhanced USD when they were added at the time of preincubation with bleomycin, but only slightly at the time of DNA synthesis (table). Considering that some bleomycin might be bound to chromatin in permeable cells even after the washing and that the activity of the remaining bleomycin might be enhanced by araCTP in bleomycin-free assay mixture, the above results suggest that araCTP enhances bleomycin-induced USD not directly but by stimulating bleomycin-induced DNA degradation. Such enhancement of bleomycin-induced USD by araCTP might be worth considering in combined therapy of neoplasms with bleomycin and araC.

USD in permeable cells pretreated with bleomycin, with araCTP and bleomycin, or with dCTP and bleomycin

Additions in pretreatment	Additions in assay	USD
-	-	11.8
araCTP	-	19.1
-	araCTP	14.0
dCTP	-	20.4
-	dCTP	14.9

Permeable cells at 2×10^6 cells/0.6 ml of Triton-buffer B were pretreated at 37 °C for 10 min with 0.22 mM bleomycin and the reagents shown in the table. Preincubated cells were chilled at 0 °C and washed twice with the same volume of Triton-buffer B. USD in bleomycin-pretreated cells was measured in duplicate, as described in the text except that bleomycin was omitted and the reagents shown in the table were added. Where added, the concentration of araCTP and dCTP was 0.1 mM each. USD is expressed as pmoles of [^{3}H]dTMP incorporated per 10^7 cells per 60 min.

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