6. Patient A [case 2 in reference (3)] had systemic lupus erythematosus and was treated with cyclophosphamide and steroids for over 3 years. She had a 20-month downhill course, with multifocal neurologic signs.
7. L. P. Weiner, R. M. Herndon, O. Narayan, R. T. Johnson, *J. Virol.* **10**, 147 (1972).
8. N. J. Schmidt, in *Diagnostic Procedures for Viral and Rickettsial Infections*, E. H. Lennette and N. J. Schmidt, Eds. (American Public Health Association, New York, 1969), p. 136.
9. Patient B [case 1 in (3)] had a 21-month history of PML (with no apparent underlying process) which ended in death. The serum of this patient showed consistently elevated concentration of SV40 antibody.
10. We thank Drs. W. Flor and K. Shah for assistance. Supported by PHS grants NS 08839, NS 08997, and NS 08963. L.P.W. is the recipient of career development award NS 50274.

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Chromatid Breakage: Differential Effect of Inhibitors of DNA Synthesis during G_2 Phase

Abstract. *The cell cycle specificity of chromatid breakage induced by inhibitors of DNA synthesis depends on the mechanism of drug action. 5-Hydroxy-2-formylpyridine thiosemicarbazone, hydroxyurea, and guanazole, compounds that inhibit ribonucleotide reductase, do not cause chromatid breakage during G_2 phase. In contrast, two active antitumor agents, arabinosylcytosine and 5-azacytidine, which are either incorporated into polynucleotides or affect DNA polymerase, produce chromatid breakage during G_2 phase. All of these agents except guanazole also induce breakage in S phase.*

Because of the demonstration by Benedict *et al.* (*1*) that the antitumor agent arabinosylcytosine (ara-C) induces chromatid breakage in the G_2 phase of the cell cycle, other inhibitors of DNA synthesis were studied for their effect during G_2 phase. These studies indicate that the induction of chromatid breakage during G_2 phase is dependent upon the particular drug employed, and that breakage is not a property of all inhibitors of DNA synthesis. This breakage was the major chromatid aberration produced in G_2 phase by the drugs.

Stationary cultures of the hamster fibroblast line Don-C (T_C, 13 hours; G_1, 3.6 hours; S, 6.2 hours; G_2, 2.2 hours; and M, 0.7 hour) were exposed to ara-C, 5-azacytidine (aza-C), 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP), hydroxyurea, or guanazole; dosages and durations are shown in Table 1. Metaphase cells exposed to drug during G_2 phase were obtained by adding Colcemid (0.06 μg/ml) to synchronous cultures 2.5 hours after the DNA inhibitor was added. Colcemid was added 4.5 hours after the drug if metaphase cells treated in S phase were desired. Metaphase cells were prepared for chromosomal analysis as described (*1*).

The results are tabulated in Table 1 together with some information on the mechanism of action of these inhibitors of DNA synthesis. The compounds ara-C and aza-C both cause chromatid breakage in S and G_2 phases; ara-C primarily affects the activity of DNA polymerases (*2*). Although the target enzyme for aza-C is uncertain, the drug is incorporated into DNA and RNA polynucleotides, replacing 20 to 30 percent of cytidine in bacterial systems (*3*), and it does not affect DNA polymerase (*3*). Hydroxyurea and 5-HP (*4*), both known to be inhibitors of ribonucleotide diphosphate reductase, do not produce chromatid breakage during G_2 phase but do so during S phase. Guanazole (*5*), another inhibitor of ribonucleotide reductase, does not cause breakage in either S or G_2 phase, even at doses that produce inhibition of cell proliferation.

Several kinds of DNA polymerases have been described (*6*). Studies in bacterial (*2*) and animal (*7*) systems have indicated that ara-C does not inhibit DNA polymerase 1, the enzyme probably involved in dark repair of DNA breakage induced by ultraviolet radiation. These data suggest that chromatid breakage induced by ara-C during G_2 phase is related to inhibition of scheduled DNA synthesis. These results are consistent with our previous evidence that such breakage is reduced by treatment with ultraviolet radiation before ara-C is given (*8*). Chromatid breakage induced by aza-C may be the result of incorporation into DNA polynucleotides.

Table 1. Differential effects of inhibitors of DNA synthesis on chromatid breakage.

Target enzyme	Incorporation into DNA (%)	Dose (μg/ml)	Phase exposed	Duration of exposure (hour)	Metaphase cells (%) in which breaks per cell = 0	1–4	5–9	10+
		Control						
		None			98	2	0	0
		Ara-C						
DNA polymerases	0.1	10	G_2	0.5	72	28	0	0
			S		77	23	0	0
		Aza-C						
Unknown	20–30	10	G_2	1.0	72	20	4	4
			S		46	40	6	8
		5-HP						
Ribonucleotide reductase	None	100	G_2	1.0	98	2	0	0
			S		64	24	6	6
		Hydroxyurea						
Ribonucleotide reductase	None	100	G_2	1.0	94	6	0	0
			S		60	30	4	6
		Guanazole						
Ribonucleotide reductase	None	1000	G_2	1.0	96	4	0	0
			S		98	2	0	0

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