*Biochemical Pharmacology*, Vol. 35, No. 11, pp. 1841–1848, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00


# INDUCTION OF DIFFERENTIATION IN THE HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60 BY THE CYCLOPENTENYL ANALOGUE OF CYTIDINE

ROBERT I. GLAZER,*† MARVIN B. COHEN,* KATHLEEN D. HARTMAN,* MARIAN C. KNODE,* MU-ILL LIM‡ and VICTOR E. MARQUEZ‡

*Applied Pharmacology Section and the ‡Drug Design and Chemistry Section, Laboratory of Medicinal Chemistry and Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20892, U.S.A.

(*Received* 27 *April* 1985; *accepted* 22 *November* 1985)

**Abstract**—The effects of the cyclopentenyl (cCyd) and cyclopentyl (carbodine) analogues of cytidine on differentiation, and nucleic acid and nucleotide biosynthesis, were examined in the human promyelocytic leukemia cell line HL-60. Continuous exposure for 5 days to $10^{-8}$ to $10^{-6}$ M cCyd or $10^{-6}$ to $10^{-5}$ M carbodine produced progressive inhibition of cell growth. During this exposure interval, pronounced differentiation to mature myeloid cells occurred wherein 95% of the cell population reduced nitroblue tetrazolium 4 days after exposure to $10^{-7}$ M cCyd or $10^{-5}$ M carbodine. Preceding differentiation was the inhibition of DNA synthesis which reached 10% of control levels 24 hr after exposure to $10^{-7}$ M cCyd or $10^{-5}$ M carbodine, while RNA synthesis was inhibited to a lesser extent. The induction of mature myeloid cells by cCyd was preceded by the inhibition of c-*myc* mRNA levels which was more pronounced than the reduction in total cellular RNA synthesis. During the interval of cCyd treatment, there was a rapid and pronounced inhibition in the level of CTP, but not of UTP, ATP or GTP, where the half-life for the disappearance of CTP was 1.5 to 2 hr. Following drug removal, cells treated with cCyd showed a sustained reduction in CTP levels, whereas cells treated with carbodine showed almost complete recovery of CTP levels within 48 hr. These results indicate that the reduction in CTP levels leads to rapid inhibition of DNA synthesis and reduction in c-*myc* mRNA levels which precede the appearance of differentiated cells.

Carbocyclic analogues of nucleosides have been studied for a number of years as both anticancer and antiviral drugs [1]. Among these compounds is the cyclopentyl analogue of cytidine, termed carbodine, which possesses inhibitor activity against some RNA viruses [2] and prolongs significantly the life span of mice bearing L1210 leukemia [3]. Carbodine is more resistant to deamination than cytidine and is metabolized to the triphosphate, which is believed to be the active metabolite responsible for inhibiting CTP synthesis in L1210 cells *in vitro* [3]. Recent interest has centered on another carbocyclic analog of cytidine, the cyclopentenyl derivative termed cCyd§ [4, 5]. This compound exhibits cytotoxic activity against KB [4] or L1210 [5] cells in culture, as well as human colon carcinoma cell line HT-29 [6]. Cytotoxicity appears to be related to the ability of cCyd to reduce intracellular CTP concentrations [6]. The antiproliferative activity of cCyd against human tumor cells prompted us to investigate whether this compound might also possess another therapeutic activity, viz. the ability to induce myeloid differentiation in the human promyelocytic leukemia cell line HL-60. This tumor line possesses the ability to express many of the phenotypic characteristics of either mature myelocytic or monocytic cells when subjected to the appropriate stimulus such as retinoic acid or phorbol esters, respectively [7]. HL-60 cells also possess the amplified cellular oncogene, c-*myc* [8, 9], whose reduced expression appears to be related to the action of differentiation-inducing agents such as 1,25-dihydroxy vitamin $D_3$ [10], retinoic acid [11] and dimethyl sulfoxide [11]. Thus, in this investigation we have assessed the ability of cCyd to induce morphologic differentiation and changes in c-*myc* mRNA expression in HL-60 cells in relation to its capacity to inhibit CTP biosynthesis.

## MATERIALS AND METHODS

*Materials.* [5-$^3$H]Urd (30 Ci/mmole) and [*methyl*-$^{14}$C]dThd (53 mCi/mmole) were purchased from New England Nuclear. cCyd was synthesized as reported previously [5, 12]. Carbodine was provided by Dr. Y. F. Shealy, Southern Research Institute, Birmingham, AL. Cyd, Urd and dCyd were obtained from the Sigma Chemical Co. The [$^{32}$P]v-*myc* DNA probe was nick-translated to 2–3 × $10^8$ dpm/$\mu$g and was purchased from Oncor, Inc.

† To whom reprints request should be addressed at: National Cancer Institute, Building 37, Room 5D02, Bethesda, MD 20892.

§ Abbreviations: cCyd, cyclopentenyl cytidine analogue, 1-[(1*R*,2*S*,3*R*)-4-hydroxymethyl-2,3-dihydroxy-4-cyclopenyten-1-yl]cytosine; Hepes, 4-(2-hydroxyethyl)-1-piperazinethane sulfonic acid; SDS, sodium dodecyl sulfate; NBT, nitroblue tetrazolium; SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0; carbodine, cyclopentyl cytidine analogue, (±)-1-[1$\beta$,2$\alpha$,3$\alpha$,4$\beta$)-4-hydroxymethyl-2,3-dihydroxycyclopentyl]cytosine; and PBS, phosphate-buffered saline (6.6 mM $Na_2HPO_4$, 0.8 mM $KH_2PO_4$, 0.154 M NaCl, pH 7.4).

*Tissue culture.* HL-60 cells were obtained from Dr. Theodore R. Breitman, National Cancer Institute. Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 40 mM Hepes (pH 7.4) and 50 μg/ml of gentamicin at 37° under an air atmosphere containing 5% $CO_2$. Cell inocula were $5 \times 10^6$ cells/10 ml of medium in 25-$cm^2$ flasks or $5 \times 10^7$ cells/100 ml of medium in 175-$cm^2$ flasks. Cell number was determined with a model ZH Coulter counter.

*NBT assay.* Cells were analyzed for their ability to reduce NBT to formazan as described by Breitman *et al.* [13].

*DNA and RNA synthesis.* Cells ($5 \times 10^6$/10 ml) were labeled with 1 μCi of [$^{14}$C]dThd (53 mCi/mmole) and 2 μCi of [$^3$H]Urd (555 mCi/mmole after dilution with unlabeled Urd) during the last hour of drug treatment. Cells were washed twice with cold PBS, precipitated with cold 10% trichloroacetic acid onto glass fiber filter discs, and the radioactivity was determined by scintillation spectrometry.

*Ribonucleotide determinations.* Following drug treatment, cells were harvested and washed once with cold PBS. The cell pellet was then extracted with 0.2 ml of cold 10% trichloroacetic acid and immediately neutralized with 2 vol. of 0.5 M trioctylamine in trifluorotrichloroethane. The aqueous phase was chromatographed by anion-exchange high pressure liquid chromatography (HPLC) as described previously [14].

*c*-myc *mRNA determinations.* *c-myc* RNA was measured by the "quick-blot" procedure of Bresser *et al.* [15], except that 1/20 vol. of Brij-35 was added during the last 20 min of incubation with proteinase K to ensure adequate digestion. Filters were prehybridized for 16–18 hr at 37° in 50% formamide, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 5x SSC, 50 mM $Na_2HPO_4$ (pH 6.5), 100 μg/ml sonicated salmon sperm DNA and 0.1% SDS. Hybridization was for 16–18 hr at 37° in the above solution made 10% in dextran sulfate and containing $10^6$ dpm of [$^{32}$P]v-*myc* DNA per ml. After hybridization, the filter was washed three times with 1x SSC/0.1% SDS for 5 min at room temperature and three times with 1x SSC/0.1% SDS for 30 min at 50°. Filters were dried and autoradiographed on Kodak X-Omat XAR-2 film with two intensifying screens. mRNA levels were quantitated by densitometry of the autoradiographs and expressed as a percentage of control levels.

## RESULTS

*Cell growth and differentiation.* The growth of HL-60 cells following continuous exposure to cCyd or carbodine is depicted in Fig. 1. Both cCyd and carbodine inhibited the growth of HL-60 cells in a similar manner over the course of 5 days except that the relative potency of cCyd was approximately 10-fold greater than carbodine.

The differentiation-inducing activities of cCyd and carbodine were assessed by the NBT reduction assay (Fig. 2). At growth inhibitory concentrations, cCyd (Fig. 2A) and carbodine (Fig. 2B) produced a concentration-dependent increase in NBT positive cells whose appearance resembled mature myeloid cells. The effects of these analogues on differentiation were readily apparent after 3 days of continuous drug exposure and reached a maximum by 4 days. Concurrent treatment with $10^{-4}$ M Urd and Cyd completely reversed the ability of $10^{-7}$ M cCyd to increase the number of NBT positive cells over 4 days as well as to inhibit cell growth (results not shown). No reduction in cell viability (trypan blue exclusion) occurred following treatment for 3, 4 or

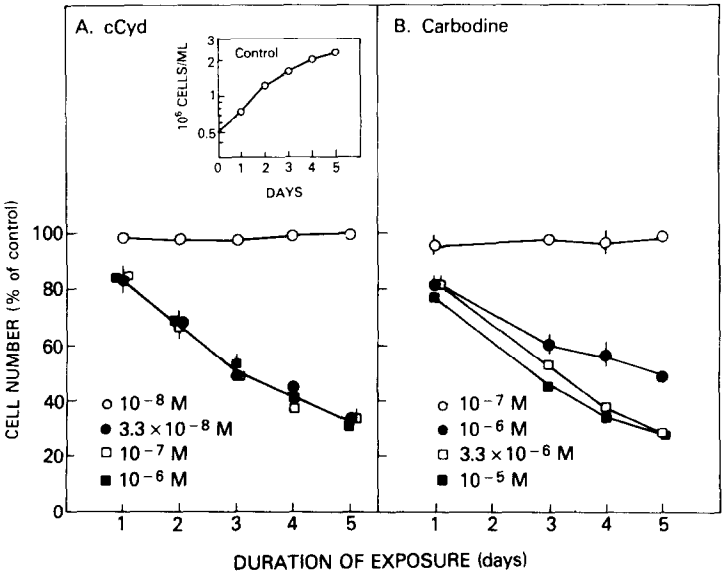


Fig. 1. Growth of HL-60 cells after treatment with cCyd or carbodine. Cells were treated continuously for the indicated time intervals with $10^{-8}$ to $10^{-6}$ M cCyd (A) or $10^{-7}$ to $10^{-5}$ M carbodine (B), and cell counts were determined. Each value is the mean ±S.E. of three to five experiments or the mean of two experiments.

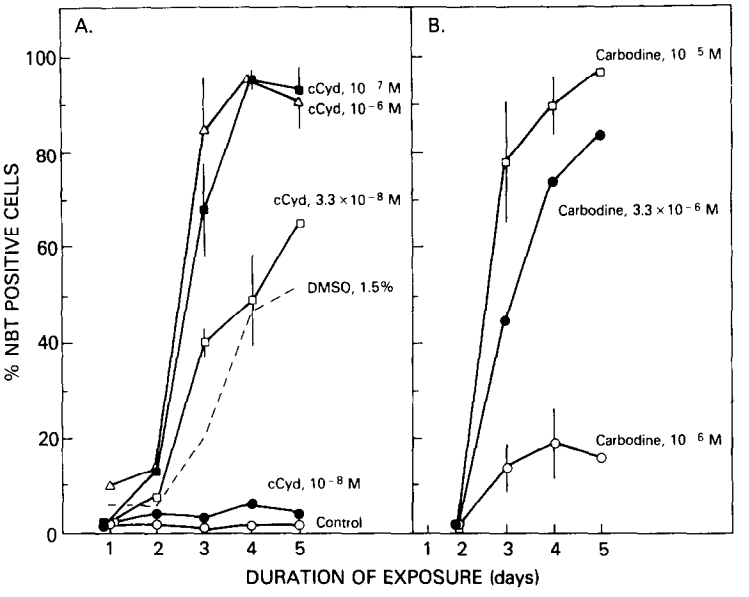


Fig. 2. Differentiation of HL-60 cells following treatment with cCyd or carbodine. Cells were treated continuously for the indicated time intervals with cCyd (A) or carbodine (B), and NBT positive cells were determined. Each value is the mean ±S.E. of three to seven experiments or the mean of two experiments.

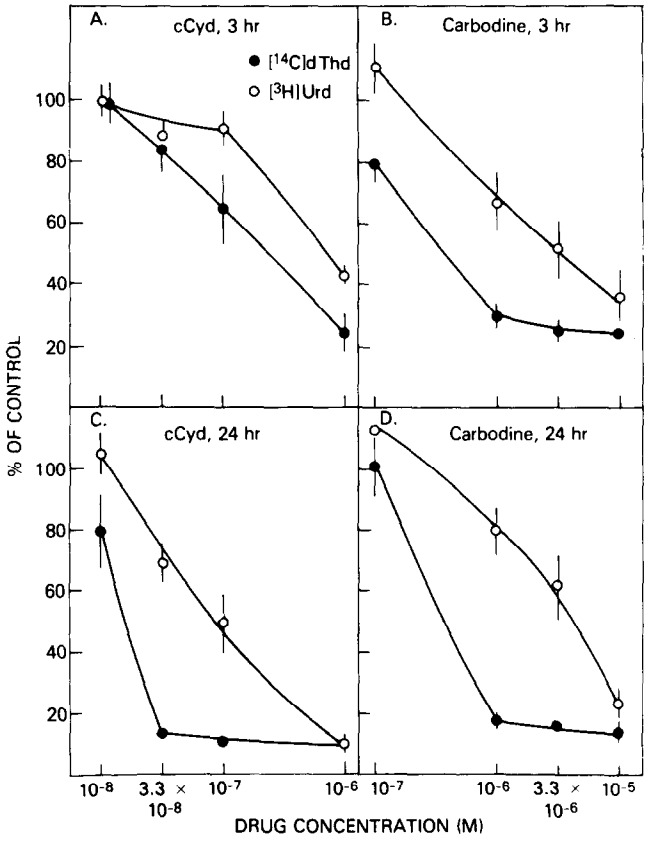


Fig. 3. DNA and RNA synthesis following treatment with cCyd or carbodine. Cells were treated for either 3 hr (A, B) or 24 hr (C, D) with cCyd (A, C) or carbodine (B, D), and the incorporation of [$^{14}$C]dThd and [$^{3}$H]Urd into trichloroacetic acid-precipitable radioactivity was determined. Each value is the mean ±S.E. of three experiments.

Table 1. Inhibition of CTP and UTP synthesis following treatment with cCyd and Carbodine

| Treatment | | Specific radioactivity (% of control) CTP | UTP |
|---|---|---|---|
| 3 hr: | | | |
| cCyd, | $10^{-8}$ M | 92 ± 14 | 102 ± 3 |
| | $3.3 \times 10^{-8}$ M | 85 ± 4 | 95 ± 3 |
| | $10^{-7}$ M | 48 ± 4 | 107 ± 2 |
| | $10^{-6}$ M | 3 ± 1 | 70 ± 5 |
| Carbodine, | $10^{-7}$ M | 69 ± 12 | 107 ± 1 |
| | $10^{-6}$ M | 31 ± 10 | 99 ± 3 |
| | $3.3 \times 10^{-6}$ M | 11 ± 5 | 67 ± 2 |
| | $10^{-5}$ M | 9 ± 4 | 45 ± 3 |
| 24 hr: | | | |
| cCyd, | $10^{-8}$ M | 61 ± 13 | 103 ± 11 |
| | $3.3 \times 10^{-8}$ M | 11 ± 3 | 110 ± 9 |
| | $10^{-7}$ M | 4 ± 1 | 96 ± 12 |
| | $10^{-6}$ M | 2 ± 1 | 21 ± 3 |
| Carbodine, | $10^{-7}$ M | 66 ± 7 | 89 ± 7 |
| | $10^{-6}$ M | 14 ± 5 | 93 ± 13 |
| | $3.3 \times 10^{-6}$ M | 7 ± 2 | 55 ± 5 |
| | $10^{-5}$ M | 2 ± 1 | 21 ± 4 |

Cells were treated for 3 or 24 hr with cCyd or carbodine and labeled during the last hour of drug exposure with 0.5 $\mu$Ci of [$^3$H]Urd. CTP and UTP were separated by HPLC as described under Materials and Methods. Each value is the mean ±S.E. of three experiments. The control values (dpm/nmole) for CTP and UTP were 2981 ± 570 and 15,070 ± 1,490, respectively, at 3 hr, and 10,140 ± 1,140 and 38,830 ± 1,870, respectively, at 24 hr.

5 days with $10^{-6}$, $10^{-7}$ or $3.3 \times 10^{-8}$ cCyd (results not shown).

*DNA and RNA synthesis.* Cells were pulse-labeled with [$^{14}$C]dThd and [$^3$H]Urd during the last hour of either a 3- (Fig. 3, A and B) or 24- (Fig. 3, C and D) hr exposure to cCyd or carbodine. cCyd and carbodine produced a greater and more rapid inhibitory effect on DNA vs RNA synthesis at both exposure intervals wherein inhibition of DNA synthesis was virtually complete within 24 hr. The two drugs differed qualitatively in that the incorporation of precursor was inhibited more after 24 hr of exposure to cCyd than after a 3-hr treatment, whereas the effects of carbodine did not differ at the two treatment intervals.

*Specific radioactivities of UTP and CTP.* The specific activities of UTP and CTP were measured under the same conditions as used for determining DNA and RNA synthesis (Table 1). cCyd did not affect markedly the specific activity of [$^3$H]UTP except at the highest concentration following 24-hr treatment. This effect was reflected in a lower uptake and incorporation of [$^3$H]Urd into UTP. A similar effect by carbodine was noted except that its effect on lowering the specific activity was more apparent at the two highest concentrations. The incorporation of [$^3$H]Urd into CTP was inhibited markedly by both drugs at concentrations which did not affect the incorporation of [$^3$H]Urd into UTP. These data suggest that the synthesis of CTP was preferentially impaired by both cytidine analogues. Moreover, the apparent reduction of RNA synthesis occurring at the highest concentration of drug was actually a result of an alteration in the specific activity of UTP.

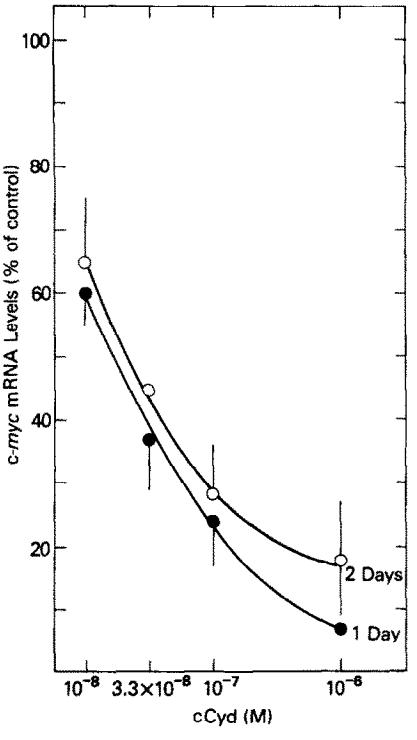


Fig. 4. c-*myc* RNA levels following treatment with cCyd. Cells were treated with various concentrations of cCyd for 1 or 2 days, and the level of c-*myc* RNA was determined by hybridization as described under Materials and Methods. Each value is the mean ±S.E. of three to five experiments.

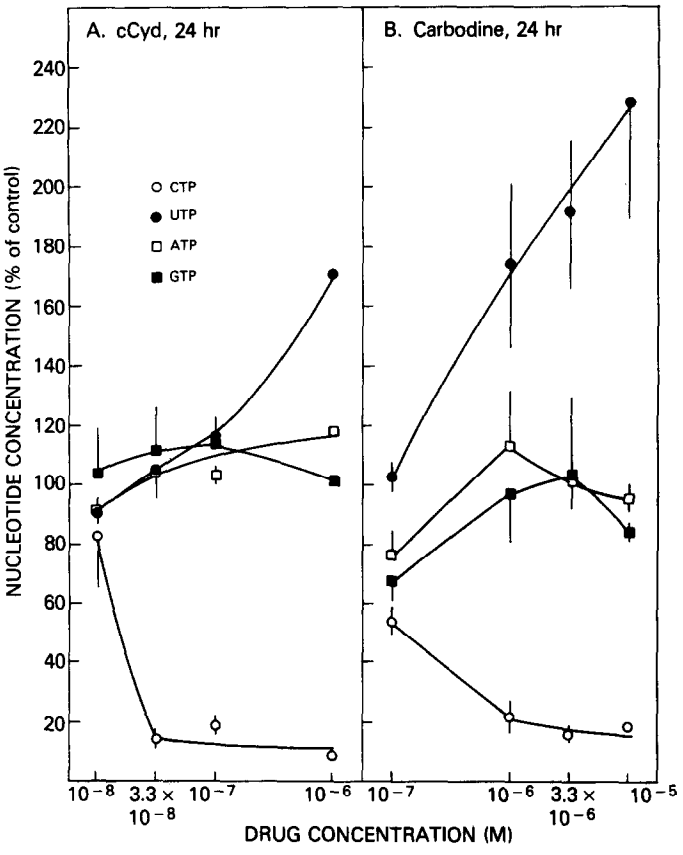


Fig. 5. Ribonucleoside triphosphate levels following treatment with cCyd or carbodine. Cells were treated for 24 hr with various concentrations of cCyd (A) or carbodine (B), and nucleotide levels were determined as described under Materials and Methods. Each value is the mean ±S.E. of three experiments.

c-myc *mRNA levels*. Since HL-60 cells possess the amplified cellular oncogene, c-*myc*, which appears to decrease before the onset of differentiation [10], c-*myc* mRNA levels were measured 1 and 2 days after treatment with cCyd (Fig. 4). A pronounced decrease in c-*myc* mRNA expression was readily apparent after both treatment intervals. Reduction in c-*myc* mRNA levels was detectable at a concentration of $10^{-8}$ M cCyd. The concentration-dependent reduction in c-*myc* RNA was inversely related to the differentiating capacity of this drug.

*CTP synthesis*. Both carbodine and cCyd affect the biosynthesis of CTP [3, 6]. Therefore, we determined whether the rapid decrease in DNA synthesis and, to a lesser extent, total RNA and c-*myc* mRNA synthesis could be accounted for by inhibition of this nucleotide. cCyd and carbodine produced pronounced and specific reductions in CTP synthesis after a 24-hr exposure interval (Fig. 5). ATP and GTP were marginally, or not, affected by drug treatment, whereas UTP levels were elevated at $10^{-6}$ M cCyd and at $10^{-6}$ to $10^{-5}$ M carbodine. The latter effect was not proportional to the ability of cCyd to inhibit CTP or its differentiating capacity but was dose-related in the case of carbodine.

The inhibitory effect of cCyd or carbodine on CTP synthesis was rapid and the reduction of CTP levels to 50% of control values occurred 1.5 to 2 hr after exposure to $10^{-6}$ M drug (Fig. 6). CTP levels reached a nadir after 4 hr of exposure to both analogues. The time course of recovery of nucleotide levels was also assessed by removal of cCyd and carbodine after treating the cells for 4 hr (Fig. 7). CTP levels remained reduced and UTP levels remained elevated after removal of cCyd (Fig. 7A), whereas, following removal of carbodine, CTP levels returned to 80% of control concentrations within 48 hr and the other nucleoside triphosphates remained within the normal range.

## DISCUSSION

HL-60 cells can be induced to differentiate along either the myelocytic or monocytic pathways by a diverse number of compounds [7]. Although, it is not known what triggers the cells to undergo morphologic changes, there is evidence that it may be related to a decline in DNA synthesis and the arrest of cells in the $G_1$ phase of the cell cycle as shown in the human myeloblastic cell line ML-1 [16]. A number of drugs which produce rapid inhibition of nucleotide biosynthesis also induce rapid maturation of HL-60 cells. Tiazofurin and mycophenolic acid, inhibitors of GTP biosynthesis by virtue of their

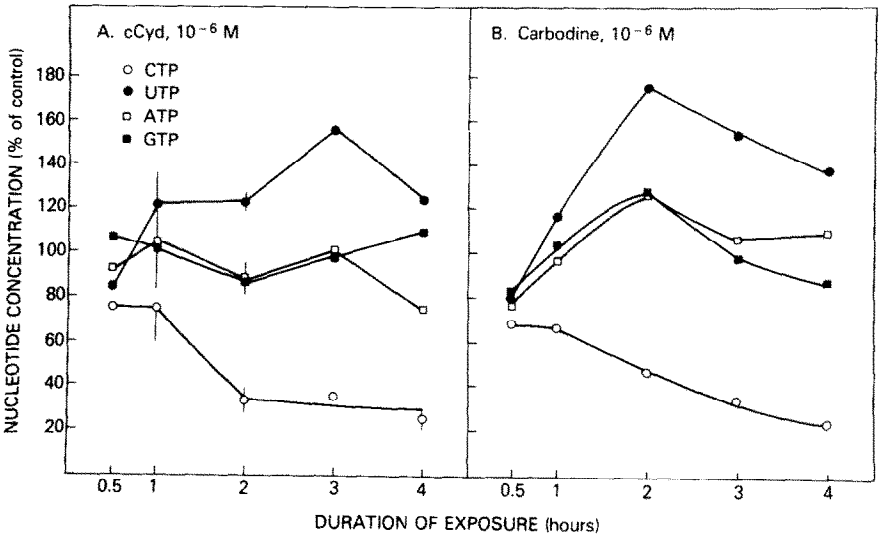


Fig. 6. Time course of inhibition of CTP following treatment with cCyd or carbodine. Cells were treated with $10^{-6}$ M cCyd (A) or $10^{-6}$ M carbodine (B), and nucleotide levels were determined at the indicated time intervals. Each value is the mean ±S.E. of three experiments or the mean of two experiments.

inhibitory action on IMP dehydrogenase [17–19], induce maturation of HL-60 cells [20]. The greater effect of mycophenolic acid in promoting myeloid differentiation vs tiazofurin may be related to its greater specificity for inhibiting DNA vs RNA synthesis [17–19]. Particularly intriguing is the ability of 3-deazauridine, a less potent inhibitor of CTP synthetase than either cCyd or carbodine, to induce myeloid differentiation of HL-60 cells [21]. The present results with the CTP synthetase inhibitors, cCyd

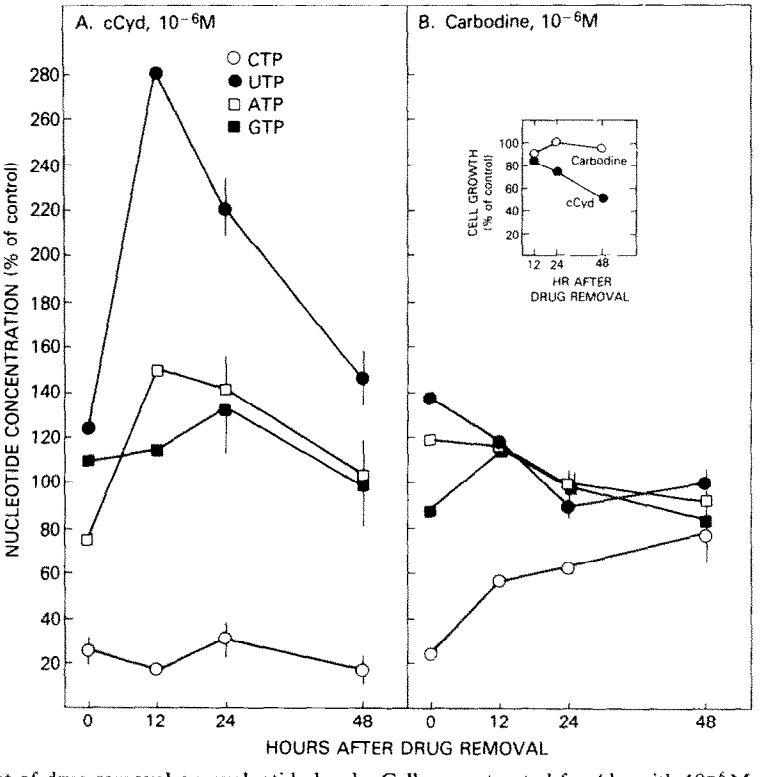


Fig. 7. Effect of drug removal on nucleotide levels. Cells were treated for 4 hr with $10^{-6}$ M cCyd (A) or $10^{-6}$ M carbodine (B) after which the cells were washed free of drug. Cells were resuspended in fresh medium and incubated for 12, 24 and 48 hr at which times nucleotide levels were determined. Each value is the mean ±S.E. of three experiments or the mean of two experiments. The inset indicates the cell growth at these time intervals as a percentage of control cell number.

and carbodine, confirm and extend these findings in that reduction of CTP levels elicits one of the most rapid and complete morphologic responses by this cell line along the myeloid pathway. These cells showed a striking increase in the ability to reduce NBT but did not exhibit an elevation in nonspecific esterase (results not shown), a monocyte marker. In contrast, the cyclopentenyl analogue of adenosine, neplanocin A, an inhibitor of RNA methylation and protein synthesis [22], produced an incomplete maturation of HL-60 cells over a very narrow concentration range [23].

The kinetics of inhibition of CTP synthesis were similar with cCyd and carbodine but the recovery of CTP levels after drug removal differed. cCyd, by virtue of the potentially reactive double bond in the carbocyclic portion of its structure, might be expected to undergo covalent attachment to its intracellular target, viz. CTP synthetase. In contrast, the reduced carbocyclic moiety present in carbodine would preclude any reactivity of this nature with the target enzyme. The data on recovery of CTP levels following drug removal confirm these hypotheses and suggest that cCyd is an irreversible inhibitor of CTP synthetase. The reversal of differentiation by high exogenous levels of Cyd or Urd suggests that this effect is mediated via increased CTP levels and the resulting commencement of cell cycling rather than by interference with cCyd uptake or its phosphorylation since a 1000-fold excess of Urd or Cyd was required.

The significance of the amplified c-*myc* gene in HL-60 cells is not known since it is not present in other human myeloid leukemia cell lines and primary promyelocytic leukemia [8, 9] but is present in human breast carcinoma cell lines [24]. Several studies have established that cellular oncogene expression is reduced markedly preceding the onset of morphologic differentiation. Treatment of the human myeloblastic cell line ML-1 with phorbol ester leads to a macrophage-like phenotype and a reduction in c-*myb* RNA in concert with reduced DNA synthesis [25]. Similarly, treatment of human neuroblastoma cells with retinoic acid leads to a reduction in N-*myc* expression which precedes decreased cell-cycle transit and neurite outgrowth [26]. Exposure of HL-60 cells to 1,25-dihydroxyvitamin $D_3$ results in reduced c-*myc* RNA before the onset of the monocyte phenotype [10], and the antiproliferative effect of Ig antiserum on the murine lymphoma cell line WEHI results in a precipituous drop in c-*myc* RNA which precedes inhibition of DNA synthesis and cell growth [27]. On the other hand, there is some evidence that the initial rapid drop in c-*myc* RNA is an early manifestation of an antiproliferative effect. Inhibition of proliferation of human lymphoma (Daudi) cells with interferon-$\beta$ results in a reduction of c-*myc* RNA which coincides with inhibition of cell growth [28]. In the present study, c-*myc* RNA levels were affected to a lesser extent than CTP and DNA synthesis, but to a greater extent than total RNA synthesis, particularly when the specific activity of UTP is taken into account. Low levels of c-*myc* expression preceded growth inhibition and the onset of morphologic changes which began to occur 2 days after treatment with cCyd and carbodine. The nuclear location of the translation products of the v-*myc* and c-*myc* mRNA [29,30], as well as the cell cycle specific induction of the c-*myc* gene in response to growth factors and mitogens [31], suggests that this gene may regulate the ability of HL-60 cells to traverse the cell cycle and enter the $G_1$ phase where the events leading to differentiation commence. Whether the changes in c-*myc* expression are responsible for, or a consequence of, the onset of morphologic changes cannot be assessed from our data and will have to await further studies.

## REFERENCES


1. J. A. Montgomery, *Mednl Res. Rev.* **2**, 271 (1982).
2. Y. F. Shealy and C. A. O'Dell, *J. heterocyclic Chem.* **13**, 1353 (1976).
3. W. M. Shannon, G. Arnett, L. Westbrook, Y. F. Shealy, C. A. O'Dell and R. W. Brockman, *Antimicrob. Agents Chemother.* **20**, 769 (1981).
4. M. Arita, K. Adachi, H. Sawai and M. Ohno, *Nucleic Acids Res.* (Symposium Series) **12**, 25 (1983).
5. M-I. Lim, J. D. Moyer, R. L. Cysyk and V. E. Marquez, *J. med. Chem.* **27**, 1536 (1984).
6. R. I. Glazer, M. C. Knode, M-I. Lim and V. E. Marquez, *Biochem. Pharmac.* **34**, 2535 (1985).
7. H. Hemmi and T. R. Breitman, in *Developments in Cancer Chemotherapy* (Ed. R. I. Glazer), pp. 247–80. CRC Press, Boca Raton, FL (1984).
8. R. Dalla-Favera, F. Wong-Staal and R. C. Gallo, *Nature, Lond.* **299**, 61 (1982).
9. I. Collins and M. Groudine, *Nature, Lond.* **298**, 679 (1982).
10. P. H. Reitsma, P. G. Rothberg, S. M. Astrin, J. Trial, Z. Bar-Shavit, A. Hall, S. L. Teitelbaum and R. C. Gallo, *Nature, Lond.* **306**, 492 (1983).
11. E. H. Westin, F. Wong-Staal, E. P. Gelman, R. Dalla-Favera, T. S. Papas, J. A. Lautenberger, A. Eva, E. P. Reddy, S. R. Tronick, S. A. Aaronson and R. C. Gallo, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2490 (1982).
12. M-I. Lim and V. E. Marquez, *Tetrahedron Lett.* **24**, 4051 (1983).
13. T. R. Breitman, S. E. Selonick and S. J. Collins, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2936 (1980).
14. R. I. Glazer and L. S. Lloyd, *Biochem. Pharmac.* **31**, 3207 (1982).
15. J. Bresser, H. R. Hubbell and D. Gillespie, *Proc. natn. Acad. Sci. U.S.A.* **80**, 6523 (1983).
16. R. W. Craig, O. S. Frankfurt, H. Sakagami, K. Takeda and A. Bloch, *Cancer Res.* **44**, 2421 (1984).
17. J. K. Lowe, L. Brox and J. F. Henderson, *Cancer Res.* **37**, 736 (1977).
18. M. B. Cohen, J. Maybaum and W. Sadee, *J. biol. Chem.* **256**, 8713 (1981).
19. M. F. Earle and R. I. Glazer, *Cancer Res.* **43**, 133 (1983).
20. D. L. Lucas, H. K. Webster and D. G. Wright, *J. clin. Invest.* **72**, 1889 (1983).
21. A. J. Bodner, R. C. Ting and R. C. Gallo, *J. natn. Cancer Inst.* **67**, 1025 (1981).
22. R. I. Glazer and M. C. Knode, *J. biol. Chem.* **259**, 12964 (1984).
23. J. Linevsky, M. B. Cohen, K. D. Hartman, M. C. Knode and R. I. Glazer, *Molec. Pharmac.* **28**, 45 (1985).
24. D. Kozbor and C. M. Croce, *Cancer Res.* **44**, 438 (1984).
25. R. W. Craig and A. Bloch, *Cancer Res.* **44**, 442 (1984).
26. C. J. Thiele, C. P. Reynolds and M. A. Israel, *Nature, Lond.* **313**, 404 (1984).

27. J. E. McCormack, V. H. Pepe, R. B. Kent, M. Dean, A. Marshak-Rothstein and G. E. Sonensheim, *Proc. natn. Acad. Sci. U.S.A.* **81**, 5546 (1984).
28. G. J. Jonak and E. Knight, Jr., *Proc. natn. Acad. Sci. U.S.A.* **81**, 1747 (1984).
29. K. Alitolo, G. Ramsay, J. M. Bishop, S. O. Pfeiffer, W. W. Colby and A. D. Levinson, *Nature, Lond.* **306**, 274 (1984).
30. H. D. Abrams, L. R. Rohrschneider and R. N. Eisenman, *Cell* **29**, 427 (1982).
31. K. Kelly, B. H. Cochran, C. D. Stiles and P. Leder, *Cell* **35**, 603 (1983).