Europ. J. Cancer, Vol. 17, pp. 271–278

0014-2964/81/030271-08 $02.00/0

Tritiated Thymidine and Deoxycytidine Suicide of Mouse Hemopoietic Colony Forming Cells (CFC)*

EDWIN M. UYEKI, KONSTANTY WIERZBA and TERESA U. BISEL

Department of Pharmacology, University of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City, KS 66103, U.S.A.

Abstract—*Significant enhancement of tritiated dCyd suicide occurred when unlabelled dThd was added to cultures of mouse monocytic colony-forming cells. Incorporation experiments supported the suicide experiments in that incorporation of tritiated dCyd into DNA was significantly increased. One hundred micromolar dCyd significantly reduced the radiotoxicity of* 0.3 μCi *of tritiated dThd; incorporation experiments indicated a dose-related reduction in the incorporation of tritiated dThd into DNA with the addition of* 1–100 μM *unlabelled dCyd. The addition of* 1 μM *aminopterin reversed the effect of* 100 μM *deoxycytidine; viz., incorporation of dThd into DNA was* 90% *of controls. Aminopterin had a similar effect on deoxyuridine reversal of tritiated dThd incorporation into DNA. Aminopterin had no effect on the reduction of tritiated dThd incorporation into DNA due to the addition of* 100 μM *unlabelled thymidine. Unlabelled ribonucleosides, Urd and Cyd, did not significantly affect the suicide pattern of tritiated dThd or dCyd when they were added to CFC cultures. Unlabelled deoxyribonucleosides, dThd or dCyd, did not significantly affect the suicide pattern of either tritiated Cyd or Urd when they were added to cultures containing tritiated ribonucleosides. Unlabelled Urd or Cyd was effective in reversing the suicide due to tritiated Urd or Cyd.*

INTRODUCTION

RADIOACTIVELY labelled nucleosides or bases are widely used in cell biology, mainly to label nucleic acids and to detect the occurrence of DNA or RNA synthesis. The incorporation of tritiated thymidine (^{3}H-dThd) into DNA is also the basis of methods used to measure the stages of the cell cycle. It is also known that the incorporation of tritiated compounds into the cell nucleus can cause radiation damage, resulting in reproductive death to the cell [1, 2]. The ^{3}H-dThd suicide technique has been used to detect 'cycling' cells in antibody producing cells [3] and in CFC [4].

A major difficulty in the analyses of metabolic pathways in CFC of primary bone marrow cultures is that a direct biochemical analysis is not feasible. Assuming that morphological identification is possible, although this has been questioned by McCulloch [5], the infrequency of these cells in the bone marrow (about one out of 500 cells by our assay), even after enrichment procedures, would make a direct biochemical analysis inconclusive since cellular heterogeneity would obscure any conclusions about the CFC.

In several recent studies [6–9] we extended the tritiated thymidine suicide technique to include the radiotoxic effects of other tritiated nucleosides on monocytic, lymphocytic and tumor CFC. In the present study, the suicide technique was used to analyse deoxypyrimidine metabolism by analysing whether the addition of unlabelled nucleosides could alter the suicide pattern of tritiated thymidine or deoxycytidine. The *prima facie* assumption was that, if the non-radioactive nucleoside inhibited the transport, metabolism or incorporation of a tritiated nucleoside into CFC, by isotopic dilution or by feedback mechanisms, the biological consequence would be a reduction in the suicide of CFC. Additionally, if no alteration in the suicide pattern was observed with the co-administration of the unlabelled with the labelled nucleoside, the *prima facie* assumption was that there was no

Accepted 4 September 1980.
*This work was supported by National Institutes of Health Grant ES-2046.

metabolic interaction between the unlabelled and labelled nucleoside in the colony-forming cell.

MATERIALS AND METHODS

BDF_1 (C57BL/6 × DBA/2) female mice were used as sources of bone marrow cells (Jackson Laboratory, Bar Harbor, ME) for the clonal cultures. Unlabelled nucleosides and aminopterin were obtained from Sigma Chemical Co., St. Louis, MO. The following tritium-labelled compounds were obtained from New England Nuclear (Boston, MA): Methyl-tritiated thymidine (sp. act. 20–26 Ci/mmole), 6-tritiated deoxyuridine (sp. act. 30 Ci/mmole). Methyl-tritiated dThd (sp. act. 47 Ci/mmole) and deoxy-1′,2′-^{3}H-uridine (sp. act. 35 Ci/mmole) were obtained from Amersham/Searle Radiochemicals, Arlington Heights, IL. The following were obtained from ICN Pharmaceuticals, Irvine, CA: 5-tritium deoxycytidine (24.7 Ci/mmole), 5-tritium cytidine (19.7 Ci/mmole) and 5-tritium deoxyuridine (16 Ci/mmole).

Unwashed studies

Bone marrow cells (1×10^5 cells per dish) were plated in 0.5 ml McCoy's 5a media (modified) with 10% fetal calf serum (FCS) and 5% horse serum in 35 mm tissue culture dishes. Tritiated and unlabelled nucleosides were added to these cell suspensions. An agar–media admixture was then added to make 1.0 ml of semi-solid media, such that the final concentration was 0.3% of Bactoagar. Bone marrow cells were cultured for monocytic CFC according to our modification [7] of the technique of Metcalf and Moore [10]. The source of colony stimulating factor was conditioned medium from L-929 cell cultures. Cultures were examined for clonal growth after 7 days incubation at 37°C.

Washed out studies

In cases where radioactive and unlabelled agents were incubated for short periods, washed out studies were conducted. Bone marrow cells were usually added at 2×10^5 cells per well in 1.0 ml of fluid media. Costar 6-well cluster tissue culture plates were routinely employed for these studies. Cells were then chased with unlabelled nucleosides and centrifuged in the same cluster plates in order to avoid cell suspension transfer steps; after two washes (using Hank's balanced salt solution plus 5% serum) and centrifugations, the cells were resuspended in 1.0 ml semi-solid media and cultured as was described for the unwashed cultures. A Cooke carrier was used to hold the cluster plates during the centrifugation.

Incorporation studies

Bone marrow cells ($2–5 \times 10^5$ cells) were cultured in 35 mm dishes using McCoy's 5A media (modified) plus serum, as employed in clonal cultures. After a period of incubation (usually 4–8 hr), 1.0 microcurie of tritiated dThd or dCyd plus unlabelled nucleosides were added to the cultures. After a 6–8 hr incubation period at 37°C, cultures were chased with the appropriate unlabelled nucleosides. The cells were then transferred to one inch square filter paper (Whatman No. 1) supported on plastic hair brushes and prepared for scintillation counting by established procedures [11].

HAP (hydroxyapatite) separation of nucleic acids

The technique of Britten and co-workers, using HAP columns for the separation of nucleic acids directly from cultured cells, was modified by us. A similar procedure was recently published by Shoyab and Sen [12]. Because of the small amounts of tissue available from each cultured dish (about 1×10^6 cells), we modified existing HAP techniques for the separation of DNA from RNA and protein. Briefly, after washing cells with media, cells were disrupted with a lysing solution containing 8 M urea, 0.001 M PB (phosphate buffer, pH = 8.0), 1% sodium dodecyl sulfate and 0.001 M EDTA. Cell lysates were placed onto HAP columns, which were prepared by suspending 200 mg of HAP (HTP grade, Biorad Laboratories, Richmond, CA) in 8 M Urea–0.001 M PB. The columns were eluted with 0.001, 0.06, 0.12, 0.18, 0.24 and 0.4 M PB. For liquid scintillation counting, DNA aliquots were taken from fractions eluting with 0.18 and 0.24 M PB and RNA fractions were taken from aliquots eluting with 0.12 M PB.

RESULTS

Usually, a radioactive dose of a tritiated nucleoside was chosen so that cultures were inhibited to values which were 10–20% of control cultures. Hence, we optimized our ability to detect any reduction of suicide due to the addition of an unlabelled nucleoside. In a preliminary study,

we found that addition of unlabelled nucleosides (except guanosine derivatives) inhibited the colonization of cells at millimolar concentrations. Hence, the highest concentration of unlabelled nucleosides that could remain for the duration of the culture was 100 μM. Routinely, for unwashed cultures, we added 1, 10 and 100 μM amounts of the unlabelled nucleosides. In all of the studies we conducted with admixtures of tritiated and unlabelled nucleosides, 1 μM amount of any unlabelled nucleoside did not significantly alter the suicide of any tritiated nucleoside (except in one case). Hence, even though 1 μM of unlabelled dThd was added with 0.01 μM tritiated dThd (which is equivalent to 0.3 μCi per ml with a specific activity of 30 Ci/mmole), the 100-fold isotopic dilution of the labelled dThd with the unlabelled dThd was inadequate to influence the suicide (Fig. 1).

Figure 1 shows the alteration of the suicide pattern of 0.3 μCi per culture of either dThd (top) or Urd (bottom) when unlabelled nucleosides were co-administered with tritiated pyrimidine nucleosides. Only unlabelled dThd reversed the suicide of 0.3 μCi of dThd, whereas unlabelled dUrd and Cyd did not affect the suicide of tritiated dThd. One hundred micromolar Urd did significantly reduce tritiated dThd suicide although the reduction was not as prominent as with unlabelled dThd. Washout studies confirmed the unwashed studies above. However, because approximately a 10-fold increase in radioactive nucleosides was required as well as it being a more time-consuming analysis, we did not routinely perform washout studies. Co-cultivation of unlabelled dThd or dCyd with 0.3 μCi of tritiated Urd did not significantly alter the suicide pattern of tritiated Urd (Fig. 1). On the other hand, addition of either unlabelled Cyd or Urd (10 and 100 μM) markedly reduced the suicide of tritiated Urd (Fig. 1).

Similarly, the addition of unlabelled Urd or Cyd (at 10 and 100 micromolar) markedly reduced the suicide of 0.5 μCi of tritiated Cyd (Fig. 2). Co-cultivation of unlabelled Cyd or Urd did not influence the suicide pattern brought about by tritiated dCyd; on the other hand, 100 μM unlabelled dCyd completely reversed the suicide of its tritiated counterpart.

Figure 3 presents the results of studies using the combination of tritiated dCyd and unlabelled dThd in culture; in this study, radioactive doses of 0.3 and 0.5 μCi were

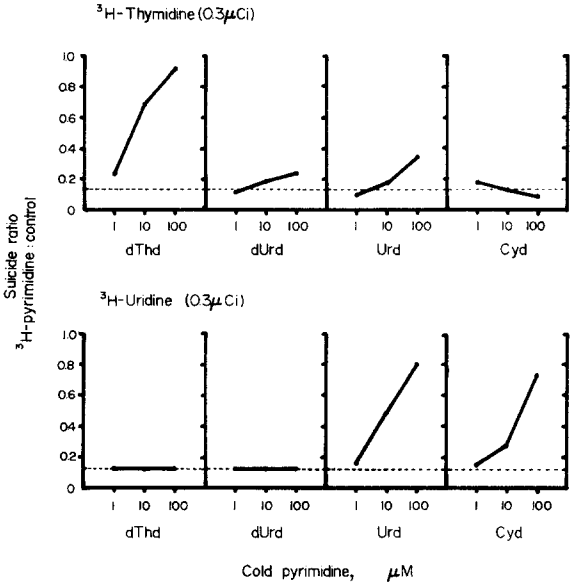

Fig. 1. Effect of unlabelled pyrimidines on ^{3}H-dThd and ^{3}H-Urd suicide of CFC in vitro. *Each value represents the average of 2 experiments.*

$$\textit{Suicide ratio} = \frac{\textit{colony counts with } ^3\text{H-nucleoside}}{\textit{colony counts of controls}}.$$

Bone marrow cells (1×10^5) *were cultured for* 7 days *with and without nucleosides.*

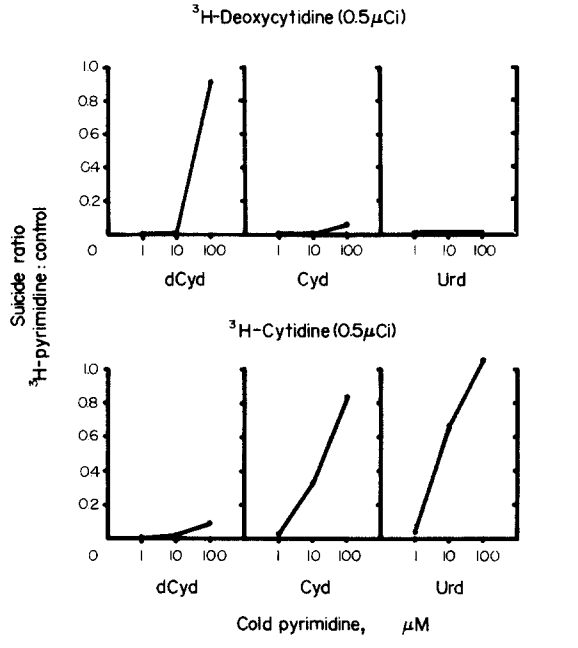

Fig. 2. Effect of unlabelled pyrimidines on ^{3}H-dCyd and ^{3}H-Cyd suicide of CFC in vitro. *Each value represents the average of two experiments. Culture conditions similar to those in Fig. 1.*

chosen and suicide ratios of 0.6 and 0.3, respectively, were obtained (Figs. 3a and b). An unexpected finding was that the addition of unlabelled dThd actually enhanced the suicide of tritiated dCyd. Hence, the suicide

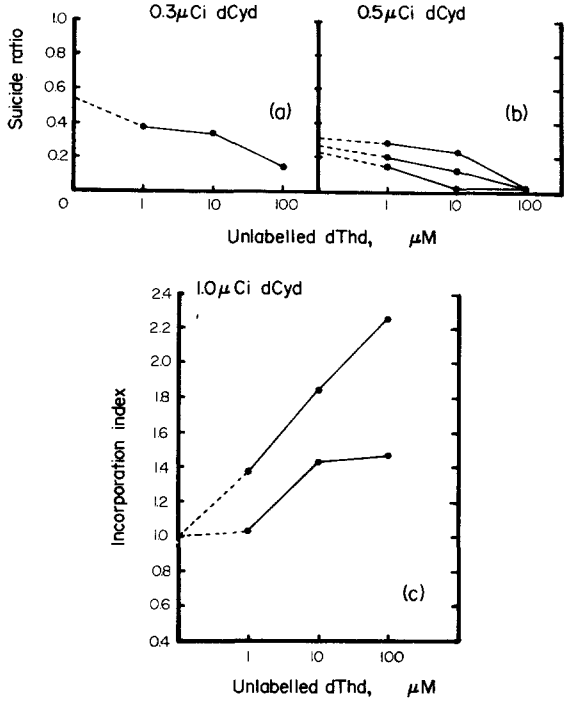

Fig. 3. The effect of unlabelled dThd on ^{3}H-dCyd suicide of CFC and on DNA synthesis of mouse bone marrow cells in vitro.

$$\textit{Suicide ratio} = \frac{\textit{colony counts with } ^3\text{H-}dCyd + \textit{cold dThd}}{\textit{colony counts with } ^3\text{H-}dCyd \textit{ alone}}.$$

Each line represents one experiment in which triplicate samples were used for each value.
Incorporation index =

$$\frac{\textit{cpm of cultures containing } ^3\text{H-}dCyd + \textit{cold dThd}}{\textit{cpm of cultures containing } ^3\text{H-}dCyd \textit{ alone}}.$$

ratio dropped from 0.6 to less than 0.2 (Fig. 3a), indicating that the suicide of CFC was enhanced to values which were one-third that of tritiated dCyd alone. These studies were corroborated in studies using 0.5 μCi of dCyd per culture (Fig. 3b). At this radioactive concentration, the suicide ratio was reduced from 0.3 to 0. Thus, at 100 μM dThd, colony formation was completely inhibited by tritiated dCyd.

To further support this finding, we performed some simple incorporation experiments in which 3×10^5 bone marrow cells were incubated for 6 hr in the presence of 1 μCi of dCyd and unlabelled dThd. After incubation the cells were prepared for liquid scintillation counting (described in Materials and Methods) and the data were expressed as the incorporation index. The incorporation index was defined as the following ratio: cpm of cultures containing ^{3}H-dCyd + unlabelled dThd divided by cpm of cultures containing ^{3}H-dCyd alone. Figure 3(c) indicates that the addition of unlabelled dThd does, indeed, enhance the incorporation of tritiated dCyd into DNA. HAP (hydroxyapatite) chromatography, used to isolate DNA in small amounts of tissue, confirmed that the major portion of tritium was incorporated into DNA. Thus, in two consecutive experiments (indicated by separate lines in Fig. 3c), there was a dose-related increase in tritiated dCyd incorporation into DNA with the addition of unlabelled dThd. Hence, the enhanced suicide was consistent with the enhanced incorporation into DNA, both brought about by the addition of 10–100 μM unlabelled dThd.

Figure 4 presents the results of studies in which a combination of tritiated dThd and unlabelled dCyd was added to the culture. Alteration in the suicide pattern was opposite to that observed with tritiated dCyd–unlabelled dThd combination. At 0.5 μCi of dThd, 1–100 μM dCyd did not significantly affect the dThd suicide pattern (Fig. 4b). On the other hand, 100 μM dCyd did significantly reduce the radiotoxicity of 0.3 μCi of dThd (Fig. 4a) in two consecutive experiments. Furthermore, corroboration of this sui-

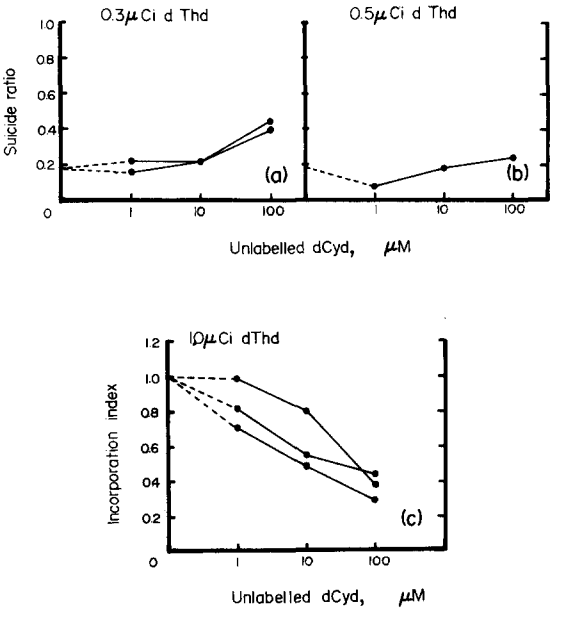

Fig. 4. The effect of unlabelled dCyd on ^{3}H-dThd suicide of CFC and on DNA synthesis of mouse bone marrow cells in vitro. *Each line represents one experiment in which triplicate samples were used for each value.*

$$\textit{Suicide ratio} = \frac{\textit{colony counts with } ^3\text{H-}dThd + \textit{cold dCyd}}{\textit{colony counts with } ^3\text{H-}dThd \textit{ alone}}.$$

Incorporation index =

$$\frac{\textit{cpm in cultured containing } ^3\text{H-}dThd \textit{ plus cold dCyd}}{\textit{cpm in cultures containing } ^3\text{H-}dThd \textit{ alone}}.$$

Table 1. Effect of aminopterin (AMN), unlabelled deoxycytidine, deoxyuridine and thymidine on the incorporation of tritiated thymidine into DNA of mouse bone marrow cells, in culture

Addition	cpm per culture	Percentage of control
1. ^{3}H-dThd*	2560	100
2. ^{3}H-dThd + 1 μM AMN	2220	87
3. ^{3}H-dThd + 100 μM dCyd	960	38
4. ^{3}H-dThd + 1 μM AMN + 100 μM dCyd	2334	90
5. ^{3}H-dThd + 100 μM dUrd	480	19
6. ^{3}H-dThd + 1 μM AMN + 100 μM dUrd	1080	42
7. ^{3}H-dThd + 100 μM dThd	320	12
8. ^{3}H-dThd + 1 μM AMN + 100 μM dThd	365	14

*Two microcuries of tritiated dThd were incubated with 1.0×10^6 bone marrow cells in 16 mm wells (Costar 24-well plates). The cells were incubated in a total volume of 0.5 ml at 37°C for a period of 14–18 hr. Each value represents the average of two experiments in which triplicate samples were used for each treatment.

cide reversal came from our incorporation experiments. In three consecutive experiments (indicated by different lines in Fig. 4c), there was a dose-related reduction of tritiated dThd with the addition of 1, 10 and 100 μM amounts of unlabelled dCyd. Hydroxyapatite chromatography verified that there was decreased radioactivity in the isolated DNA from cultures in which unlabelled dCyd was added.

Results of incorporation experiments shown in Table 1 supported the notion that the reduced incorporation of tritiated dThd into DNA resulting from exogenously supplied 10–100 μM, unlabelled dCyd is due to the conversion of dCyd to dThd nucleotides. One micromolar AMN, itself, does not affect tritiated dThd incorporation into DNA. In the presence of either unlabelled dUrd (group 5) or unlabelled dCyd (group 3) plus tritiated dThd, there is a substantial reduction in tritiated dThd into DNA. However, the addition of AMN to either of these conditions (groups 4 and 6) resulted in a return toward control values. These results are consistent with the notion that the reduced incorporation of tritiated dThd into DNA, resulting from the addition of unlabelled dUrd or dCyd, is due to isotope dilution.

DISCUSSION

Henderson *et al.* [13] and Masters [14] emphasized the importance of studying metabolic pathways in intact cells as compared with broken-cell preparations. They point out that because these broken-cell preparations are more specific and quantitative, there is a tendency to extrapolate observations from these studies to operation of intact cells. "So long as these extrapolations are qualitative in nature, i.e., identity of enzymes or metabolites that are present, the extrapolations are justified..." Therefore, it is important that any differences between intact- and broken-cell preparations, should they arise, be carefully analysed with their comments in mind.

In the present study, we feel that the suicide technique, along with cold chases, identifies the preferred pathway of the intact precursor cell for the exogenously supplied NAP (nucleic acid precursor). The most obvious discrepancy between broken-cell experiments and ours is the effect of isotope dilution (addition of chase with the pulse) on suicide. If one assumes that tritiated dThd has a specific activity of 25 Ci/mmole and that 0.25 μCi per ml results in a marked degree of suicide (see Fig. 1), this amounts to a concentration of 0.01 μM. Addition of 1.0 μM unlabelled dThd does not significantly reduce the degree of suicide. This observation is also true for tritiated Urd, dCyd or Cyd chases with their unlabelled counterparts (see Figs. 1 and 2). In most cases, not until 100 μM concentration of unlabelled nucleoside is added do you obtain a significant reversal of suicide. Because broken-cell preparations demonstrate the well known isotope dilution, absence of isotope dilution in our suicide experiments was unexpected. In the studies above, the labelled was diluted by the unlabelled nucleoside 100- to 1000-fold.

In light of the results above, we re-evaluated the literature regarding pool sizes of NAP in various mammalian cells. Because of cell heterogeneity difficulties, as noted in the Introduction, a rigorous analysis of cellular pool sizes in precursor cells is infeasible and

unprofitable. However, literature citations of the concentration range of nucleosides and nucleotides facilitate the interpretation of our data. Using Plagemann's [15] data that one million cells occupy a volume of 2.5 μl, we calculated Henderson *et al.'s* data [13]; data from both studies showed that the nucleotide concentration is in the millimolar range. The ribotide triphosphates are the most abundant species of NAP in the cell; the deoxytide levels constitute about 1% of the ribotide levels. On the other hand, a 10% serum concentration in the culture media would supply dThd and dCyd in the range of 0.4–1.6 μM [16]. Nucleosides constitute only a minor portion of the total NAP pool of the cell. Hence, the concentration of nucleotides, in the millimolar range, is the important concentration in interpreting our suicide studies. A recent study of Plagemann *et al.* [17] supports this contention; after the first 5–10 sec, phosphorylation, rather than transport, is the rate-limiting step in the incorporation of uridine into Novikoff hepatoma cells.

One hundred thousand cells per ml, which we normally use for our clonal analysis, occupy a volume of 0.25 μl, based on Plagemann's data [15]; if the millimolar nucleotide content in cells were diluted to 1 ml, the resulting concentration would be 0.25 μM. Under these circumstances, it would be easy to see that the isotope dilution by 1 and 10 μM, unlabelled nucleoside would occur in these broken-cell concentrations. By contrast, the intact cell, having a concentration of nucleotides in the millimolar range, 'resists' isotope dilution until 0.1 mM (100 μM) concentrations of cold nucleosides are added. Hence, the simplest, though certainly not the only, explanation for the absence of isotope dilution is that the endogenous nucleotide concentration in the intact cells is in the millimolar range.

Our studies with either tritiated Cyd or Urd revealed that their unlabelled counterparts can effectively abolish the suicide of the other tritiated nucleoside, indicating that exogenously supplied Urd and Cyd share a common metabolic pathway. (Although Cyd-Urd effects are related, this is not the purpose of this report.) In the view of this easy substitution of Urd-Cyd to chase their tritiated counterparts, it was surprising that the relationship between the deoxyside pyrimidines was so different.

Observed differences between the ribosides and the deoxysides in their ability to modify the suicide pattern by isotope dilution clearly point to the usefulness of the suicide technique in 'informing' us of the preferred metabolic pathway of exogenously supplied NAP in intact cells. Thus, we see that the addition of unlabelled dThd clearly enhances the suicide of tritiated dCyd (Fig. 3b). On the other hand, the presence of unlabelled dCyd showed a reversal of tritiated dThd suicide, although the effect was not prominent. These results were in reasonable accord with our experiments dealing with the incorporation of nucleosides into DNA of bone marrow cells in short term cultures. The correspondence observed between the incorporation studies in heterogeneous cells and suicide studies using clonal assays was interpreted to mean that this dThd–dCyd interaction is likely to be a general phenomenon, not only in precursor cells (CFC), but occurring in the population of proliferating cells. Thus, we see that the addition of 10 and 100 μM amounts of unlabelled dThd enhanced the incorporation of tritiated dCyd into DNA. On the other hand, the presence of unlabelled dCyd in the media, 10 and 100 μM, reduced the incorporation of tritiated dThd into DNA. Using HAP chromatography, we observed that the incorporation of tritiated dUrd into DNA was markedly inhibited by 1 μM AMN (not shown). Our results, in Table 1, support the view of isotope dilution, i.e., that the exogenously-supplied, unlabelled dCyd is converted to unlabelled dThd nucleotides and, hence, dilutes the incorporation of the tritiated dThd.

How widespread the interplay of dThd–dCyd is among the set of proliferating cells will be decided by future experiments. Since much of the literature regarding dThd–dCyd interplay deals with BrdUrd effects, it is appropriate here to discuss the biological effects of BrdUrd on cultured mammalian cells. Bromodeoxyuridine incorporation into cells has been used to modify biological functions of cells in culture [18–22]. Hence, inhibition of differentiation, tumorigenicity, pigmentation and induction of Friend leukemia virus are a consequence of BrdUrd addition. Strom and Dorfman [18] showed that the BrdUrd-induced suppression of chondrogenesis in chick embryo limb bud cultures was related to the incorporation of BrdUrd into moderately repetitive DNA sequences. In most studies, the BrdUrd effects are compatible with the enhanced incorporation of BrdUrd into DNA.

A new approach to the study of molecular mechanisms of BrdUrd action was suggested by Meuth and Green [19] who showed that BrdUrd induced a dCyd-less state in cultured

cells, and that added dCyd can reverse the toxic effects of BrdUrd in some mammalian cell lines (confirmed by Horn and Davidson [20]). Further, the presence of dCyd in culture results in decreased BrdUrd incorporation into nuclear DNA. Horn and Davidson showed that dCyd inhibited the effects of BrdUrd on pigmentation, contact inhibition, cell morphology and tumorigenicity and this effect corresponded to a drop in BrdUrd substitution into DNA. Bick [21] showed a similar correlation of the BrdUrd effects on the induction of Friend leukemia virus. Subsequent results suggested that the reversal of BrdUrd toxicity of dCyd was due to the intracellular conversion of dCyd to dThd nucleotides.

Davidson and Kaufman [22], on the other hand, demonstrated that dCyd reversed the suppression of pigmentation by BrdUrd without changing the amount of BrdUrd in DNA. Their notion of dCyd reversal, however, requires the intracellular conversion of exogenously supplied dCyd to dThd nucleotides. In sum, BrdUrd-induced alterations of biological function is compatible with BrdUrd substitution of dThd into DNA, and dCyd–dThd interactions play a prominent role in altering the BrdUrd-induced effects.

Regarding the interplay of deoxynucleosides in affecting DNA synthesis in mammalian cells, Reichard [23] remarked: (1) although DNA synthesis requires an equal supply of all four bases, very large differences in pool sizes exist; (2) dCTP pools most closely reflect the rate of DNA synthesis. Our studies, as well as others cited above, are in reasonable accord with these statements.

REFERENCES

1. J. S. Bedford, J. B. Mitchell, H. G. Griggs and M. A. Bunder, Cell killing by gamma rays and beta particles from tritiated water and incorporated tritiated thymidine. *Radiat. Res.* **6,** 531 (1975).
2. P. C. Chan, E. Lisco and S. J. Adelstein, The radiotoxicity of iodine-125 in mammalian cells. II. A comparative study on cell survival and cytogenetic responses to 125 IUdR, 131 IUdR and 3HTdR. *Radiat. Res.* **67,** 332 (1976).
3. R. W. Dutton and R. I. Mishell, Cell populations and cell proliferation in the *in vitro* response of normal mouse spleen to heterologous erythrocytes. *J. exp. Med.* **126,** 443 (1967).
4. N. N. Iscove, J. E. Till and E. A. McCulloch, The proliferative states of mouse granulopoietic progenitor cells. *Proc. Soc. exp. Biol. (N.Y.)* **134,** 33 (1970).
5. E. A. McCulloch, In *Haemopoietic Stem Cells.* Ciba Foundation Symposium Vol. 13, p. 68. Elsevier, Amsterdam (1973).
6. E. M. Uyeki, T. Nishimura, S. Strom and T. U. Bisel, Suiciding of hematopoietic and tumor clonal cells by tritiated nucleosides, *in vitro. J. nat. Cancer Inst.* **59,** 1031 (1977).
7. E. M. Uyeki, T. Nishimura, T. N. Miller and T. U. Bisel, Effect of methotrexate and aminopterin on tritiated nucleoside suicide of colony forming cells, *in vitro. Europ. J. Cancer* **14,** 263 (1978).
8. E. M. Uyeki, T. Nishimura and T. U. Bisel, Suiciding of lymphocytic precursor cells by tritiated nucleosides, *in vitro. Europ. J. Immunol.* **8,** 141 (1978).
9. S. Strom and E. M. Uyeki, A suicide technique to study purine antimetabolites on bone marrow and tumor colony forming cells. *Europ. J. Cancer* **15,** 415 (1979).
10. D. Metcalf and M. S. Moore, *Haemopoietic Cells, Frontiers of Biology*, Vol. 24. Elsevier, Amsterdam (1971).
11. E. M. Uyeki, Effect of antibiotics on the development of antibody-produing cells, *in vitro. Biochem. Pharmacol.* **21,** 59 (1972).
12. M. Shoyab and A. Sen, The isolation of extrachromosomal DNA by hydroxyapatite chromatography. In *Methods of Enzymology*, (Edited by S. P. Colowick and N. O. Kaplan) Vol. 68, p. 199. Academic Press, New York (1979).
13. J. F. Henderson, J. K. Lower and J. Barankiewicz, Purine and pyrimidine metabolism: pathways, pitfalls and perturbations. In *Purines and Pyrimidine Metabolism*, Ciba Foundation, Symposia Vol. 48, p. 3. Elsevier, Amsterdam (1977).
14. C. J. Masters, Metabolic control and the microenvironment. In *Current Topics in Cellular Regulation.* (Edited by B. L. Horecker and E. R. Stadtman) Vol. 12. p. 75. Academic Press, New York (1977).

15. P. G. W. PLAGEMANN, Nucleotide pool in Novikoff rat hepatoma cells growing in suspension cultures. III. Effects of nucleosides in medium on levels of nucleotides in separate nucleotide pools for nuclear and cytoplasmic RNA synthesis. *J. Cell Biol.* **52,** 131 (1972).
16. D. A. SJOSTROM and D. R. FORSDYKE, Isotope-dilution analysis of rate-limiting steps and pools affecting the incorporation of thymidine and deoxycytidine into cultured thymus cells. *Biochem. J.* **138,** 253 (1974).
17. P. G. W. PLAGEMANN, R. MARZ and R. M. WOHLHUETER, Uridine transport in Novikoff rat hepatoma cells and other cell lines and its relationship to uridine phosphorylation and phosphorolysis. *J. Cell Physiol.* **97,** 49 (1978).
18. C. H. STROM and A. DORFMAN, Amplification of DNA sequences during chick cartilage and neural retina differentiation. *Proc. nat. Acad. Sci. (Wash.)* **75,** 4451 (1978).
19. M. MEUTH and H. GREEN, Induction of a deoxycytidineless state in cultured mammalian cells by bromodeoxyuridine. *Cell* **2,** 109 (1974).
20. D. HORN and R. L. DAVIDSON, Inhibition of biological effects of bromodeoxyuridine by deoxycytidine: correlation with decreased incorporation of bromodeoxyuridine into DNA. *Somat. Cell Genet.* **2,** 469 (1976).
21. M. D. BICK, Bromodeoxyuridine inhibition of Friend leukemia cell induction. *Biochim. biophys. Acta* **476,** 279 (1977).
22. R. L. DAVIDSON and E. R. KAUFFMAN, Bromodeoxyuridine mutagenesis in mammalian cells is stimulated by thymidine and suppressed by deoxycytidine. *Nature (Lond.)* **276,** 722 (1978).
23. P. REICHARD, From deoxynucleotides to DNA synthesis. *Fed. Proc.* **37,** 9 (1978).