**In Vitro Metabolism of Deoxycoformycin in Human T Lymphoblastoid Cells**

PHOSPHORYLATION OF DEOXYCOFORMYCN AND INCORPORATION INTO CELLULAR DNA*

(Received for publication, November 7, 1983)

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The biochemical and metabolic effects of deoxycoformycin, a potent inhibitor of adenosine deaminase, were investigated using two human T lymphoblastoid cell lines. A dose-response analysis demonstrated that the concentration of deoxycoformycin at which there was 50% inhibition of growth was greater than $1 \times 10^{-3}$ M in lymphoblastoid cells. Uptake of deoxycoformycin was biphasic and occurred much more slowly than for natural nucleosides, and lower saturation levels were reached. The intracellular concentration of deoxycoformycin achieved was 0.4 to 0.5 $\mu$M when the extracellular concentration was 1 $\mu$M. At 10 $\mu$M extracellular concentration, the intracellular concentration was 3–4 $\mu$M. Although deoxycoformycin at very low concentrations (1 or 10 $\mu$M) did not have any detectable effects on the growth of these cells, the nucleoside was found to be metabolized, and was phosphorylated to give the mono-, di-, and triphosphate derivatives. The triphosphate derivative was incorporated into cellular DNA with little incorporation into cellular RNA. Metabolism of deoxycoformycin in several mutant lymphoblastoid cells deficient in adenosine kinase and/or deoxyctydine kinase was found to be unchanged from wild-type cells, indicating that these major nucleoside kinases do not play a significant role in the phosphorylation of deoxycoformycin. These results may account, at least in part, for the differences that are observed between the pharmacologic inhibition of adenosine deaminase, and the inherited deficiency of adenosine deaminase.

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*dCF* (Fig. 1, Miniprint) (1) is a potent inhibitor of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4). This nucleoside is an interesting and unique drug with wide clinical potential because it causes selective lysis of lymphocytes (desirable in lymphoproliferative diseases) or specific impairment of normal immune function which is not accompanied by cellular lysis (desirable in autoimmune diseases and graft versus host disease following organ transplantation). The action of *dCF in vivo* in humans is concentration dependent. Administration of high levels of *dCF* in humans results in 1) abnormally high ratios of dATP/ATP, 2) massive lymphocyte lysis, and 3) acute clinical toxicity (2–5). Intracellular deoxynucleotide metabolism at high *dCF* concentrations mimics that observed in children with hereditary deficiency of adenosine deaminase coupled with severe combined immunodeficiency disease (6, 9). However, pharmacologic and disease patterns differ in that although intracellular ATP is severely depleted at high concentrations of the drug, ATP is not severely depleted in children with adenosine deaminase deficiency even after long-term therapy with erythrocyte infusion (6, 7). Moreover, these children fail to exhibit any of the acute systemic toxicity observed at high *dCF* concentrations.

Administration of moderate doses of *dCF* in humans fails to cause 1) alteration in dATP/ATP ratios in lymphocytes, 2) acute clinical toxicity or 3) rapid lymphocyte lysis (3, 8). However, its ability to impair the immune system is retained. When low concentrations of *dCF* are incubated with human lymphocytes, both antibody-dependent and non-antibody-dependent cellular cytotoxicity are irreversibly impaired with no alteration in dATP/ATP (4). The lymphotoxic action of *dCF* under conditions where dATP/ATP pools are not altered suggests that a mechanism may exist which does not involve perturbed nucleotide pools or inhibition of adenosine deaminase per se, but metabolized products of *dCF* itself.

Deoxycoformycin has heretofore been considered a relatively inert nucleoside analogue which binds principally to adenosine deaminase. The possibility that *dCF* might be used as a substrate by any normal cellular pathways has not been widely investigated. However, previous studies have shown that *dCF* is not a substrate for purine nucleoside phosphorylase, a major degradative enzyme of purine salvage (10). As a consequence, it does not seem likely that this nucleoside analogue enters the major degradative pathways for purines. Therefore, it seemed logical to us to explore anabolic pathways of phosphorylation. In this context, one study has shown that *dCF* can be phosphorylated by L1210 cells in vitro (11). However, in this study only the monophosphate form was detected.

Participation in major anabolic pathways requires formation of the triphosphorylated derivative of *dCF*. We therefore examined two cell lines treated with *dCF* for the presence of higher phosphorylated forms. After modifying standard procedures of nucleotide extraction to take into account the lability of these compounds, we did indeed find significant levels of mono-, di-, and triphosphorylated derivatives of *dCF* in lymphoblastoid cells. Moreover, we have evidence that *dCF* is incorporated into cellular DNA. These results support the hypothesis that the action of *dCF* is not limited to inhibition of adenosine deaminase.
EXPERIMENTAL PROCEDURES AND RESULTS

Dose Response to Deoxycoformycin—The response of CEM and Molt-4 cells to dCF was compared with the response of these cells to the growth-limiting nucleosides, dAdo, Ado, and araA. The effects of each of the nucleosides on cell growth was not readily apparent until 72 h. At that time, dCF was far less cytotoxic than Ado, dAdo, or araA. The concentrations at which there was 50% inhibition of cell growth were $3 \times 10^{-6}$ M (araA), $7 \times 10^{-7}$ M (dAdo), $1 \times 10^{-5}$ M (Ado), and $1.0 \times 10^{-3}$ M (dCF). A substantial contributor to a lack of cytotoxicity of the nucleosides is intracellular deamination which occurs via adenosine deaminase. As has been shown by others, when adenosine deaminase was inhibited by dCF (10 μM), dAdo (10 μM) and araA (10 μM) were particularly toxic to cells (see Fig. 2, Miniprint).

Accumulation of dATP in Cells Incubated with dAdo in the Presence of dCF—The amount of dATP accumulation in CEM and Molt-4 cells cultured in the presence of dCF was investigated. The level of dATP in the normal nucleotide pools of CEM and Molt-4 cells was found to be about 26.3 pmol/10⁶ cells and 16.2 pmol/10⁶ cells, respectively (Table I, Miniprint). When incubated with 10 μM dCF alone, these cells did not exhibit any significant changes in their intracellular dATP levels. However, cells incubated with 100 μM dAdo in the presence of 10 μM dCF were found to accumulate large amounts of dATP in their cells. After 24-h incubation at 37 °C, the amount of dATP in CEM and Molt-4 cells incubated with 100 μM dAdo in the presence of 10 μM dCF was found to be around 614 pmol/10⁶ cells and 460 pmol/10⁶ cells, respectively. Lower levels of dATP accumulate in cells that were incubated with 100 μM dAdo alone. This was really not surprising since it is known that dAdo undergoes rapid deamination by adenosine deaminase to form dino in the absence of any adenosine deaminase inhibitors. This experiment serves to demonstrate that, when its deamination is inhibited by dCF, dAdo can be phosphorylated to form dATP.

Cellular Uptake of Deoxycoformycin—CEM and Molt-4 cells were found to exhibit similar kinetics in their uptake of dCF with respect to time (Fig. 3). It was found that the uptake of dCF initially proceeded rapidly and was linear for about an hour before slowly leveling off until a steady intracellular level was reached after about 10 h. At 1 μM extracellular concentration, the level reached after 24 h for CEM and Molt-4 cells was 1.5 pmol/10⁶ cells and 2.0 pmol/10⁶ cells, respectively. At 10 μM extracellular concentration, this level was 14 pmol/10⁶ cells and 16 pmol/10⁶ cells, respectively. The intracellular concentration of dCF that was taken up in these cells (assuming a volume of 4.1 μl/10⁶ cells) would be 0.4 to 0.5 μM when the extracellular concentration was 1 μM. At 10 μM extracellular concentration, the intracellular concentration was between 3 and 4 μM. In contrast, the uptake of the adenosine analog, araA, or the natural nucleoside, dAdo, was found to be more rapid, with higher saturation levels being reached at much earlier times. The level of araA or dAdo taken up into these cells was 100-fold higher (intracellular concentration of 400-500 μM when the extracellular nucleosides were 10 μM) than that for dCF, undoubtedly due to the fact that araA and dAdo are rapidly metabolized inside the cell and the experiments described here reflect both uptake and metabolism. The concentration dependence of the uptake of dCF into CEM and Molt-4 cells was examined at various extracellular concentrations ranging from 1 to 500 μM. The uptake of dCF was found to be a biphasic process. Deoxycoformycin was taken into the cells in a linear fashion only at very low concentrations (up to 50 μM), but slowly began to level off at higher concentrations. From the double reciprocal plots of 1/v (v is picomoles/10⁶ cells/min) versus 1/[dCF] (μM⁻¹), it was apparent that there were two (additive) uptake mechanisms, one operating more efficiently at low concentrations, and the other at high concentrations. The apparent $V_{\text{max}}$ and $K_m$ values associated with these two mechanisms for CEM and Molt-4 cells are listed in Table II.

Metabolic Fate of Deoxycoformycin—Experiments to investigate the metabolic fate of dCF in in vitro cultures of CEM...
and Molt-4 cells were carried out by incubating cells with radioactive dCF and isolating labeled nucleotides using HPLC on an anion-exchange column.

A representative HPLC chromatogram of the absorbance at 254 nm of extracted nucleotides from CEM or Molt-4 cells incubated with [3H]dCF is shown in Fig. 4A. At the bottom of Fig. 4 are profiles of the radioactivity associated with the various absorbance peaks. A major peak, corresponding to that of authentic dCF, was seen at about 2 min elution time. Three other major peaks were seen at 10, 24, and 43 min elution time. These radioactive peaks were representative of those peaks we predicted for the mono-, di-, and triphosphate derivatives of dCF. Five to seven other minor peaks were also seen. From the results of seven experiments, it was apparent that there was some variability in the distribution of radioactivity that was associated with each of the peaks (Table III). The peak containing unmodified [3H]dCF (peak I) varied from as low as 32.1% (in Experiment 4) to as high as 92.3% (in Experiment 2) of total radioactivity for CEM cells. With Molt-4 cells, [3H]dCF extracted varied from as low as 22.6% (in Experiment 3) to as high as 94.9% (in Experiment 2). It appeared likely that the variation in amount of dCF present in phosphorylated forms was partially reflective of the instability of the compounds. When the isolation, extraction, and identification of nucleotides was carried out rapidly, most of the intracellular dCF was converted to phosphorylated forms. Four major peaks (peaks I, IV, VII, and IX) were always detected, along with five to seven other minor peaks. We postulated, based on their retention times, that peaks I, IV, VII, and IX represented dCF, dCFMP, dCFDP, and dCFTP. The identities of the minor peaks were not known, but they may represent the phosphorylated derivatives of the ring-opened or degraded nucleoside. However, confirmation was hampered by the lack of authentic standards of dCFMP, dCFDP, and dCFTP.

To confirm peak identities, a portion of the nucleotides extracted from cells was treated with alkaline phosphatase to degrade all the nucleotides to nucleosides, which were subsequently fractionated on a Bondapak C18 (reverse-phase) column. Degradation of all the putative dCF nucleotides resulted in only two peaks (Fig. 4B). The top part shows absorbance at 254 nm and the bottom shows radioactivity present in the same fractions. The two radioactive peaks were thought to represent those of dCF (peak 2) and a breakdown product of dCF (peak 1).

Confirmatory tests were also done by performing enzymatic shift reactions with nucleosides in peaks 1 and 2 using adenosine deaminase and purine nucleoside phosphorylase. Authentic [3H]dCF was also subjected to adenosine deaminase and purine nucleoside phosphorylase treatment, under the same experimental conditions as that used for the CEM and Molt-4 samples. Authentic [3H]dCF, when treated under these conditions, gave rise to the same two peaks (peaks 1 and 2) observed on the C18 column after alkaline phosphatase treatment of the CEM and Molt-4 samples (Fig. 5A). Peak 2 had UV absorbance at 254 nm, and was that due to intact dCF. However, peak 1 did not have any detectable UV absorbance at 254 nm, and we have postulated that it represents a breakdown product of dCF that may result from a degradation of the highly unstable compound under low pH conditions. These experiments also confirmed that authentic dCF was not a substrate for adenosine deaminase or purine nucleoside phosphorylase (Fig. 5A). Peaks 1 and 2 isolated from CEM and Molt-4 cells were also not altered by adenosine deaminase or purine nucleoside phosphorylase (Fig. 5, B and C, respectively). Based on the results of these experiments,
to determine whether dCF was phosphorylated by the same column, under the conditions as described under "Experimental Procedures." The products were then analyzed on the CIS (reverse-phase) of alkaline phosphatase, adenosine deaminase, and purine nucleoside phosphorylase. The results of these analyses are shown in Table IV and confirmatory tests were also carried out by similarly treating their component nucleosides by DNase I, RNase A, bacterial alkaline phosphatase, and snake venom phosphodiesterase I. The nucleosides products were then analyzed by HPLC on a Bondapak 

![Fig. 5. Results of enzymatic shift reactions using adenosine deaminase and purine nucleoside phosphorylase. Authentic [\textsuperscript{3}H]dCF (A), and the 60% methanol-extracted nucleotide pools from CEM (B), and Molt-4 (C) were treated sequentially with alkaline phosphatase, adenosine deaminase, and purine nucleoside phosphorylase. The products were then analyzed on the CIS (reverse-phase) column.](image)

**TABLE IV**

Distribution of radioactivity among dCF, dCFMP, dCFDP, dCFTP, and other minor peaks

Calculations were based on an average of the seven experiments in Table III from the HPLC analyses of the 60% methanol-extracted nucleotide pools. These analyses on the Partisil-10 SAX column were carried out as described under "Experimental Procedures." Fractions of 0.5 ml were collected and counted. The results of alkaline phosphatase, adenosine deaminase, and purine nucleoside phosphorylase treatment are shown at the top, middle, and bottom, respectively.

| Cell line | dCF | dCFMP | dCFDP | dCFTP | Others |
|-----------|-----|-------|-------|-------|--------|
| CEM       | 25.3| 1.9   | 1.6   | 4.8   | 2.6    |
| Molt-4    | 26.8| 1.1   | 0.5   | 0.5   | 0.7    |

From these data, we have concluded that neither Ado kinase nor dCyd kinase is responsible for dCF phosphorylation. Furthermore, dCF does not appear to compete with dAdo in enzymatic reactions which lead to the formation of monophosphate derivatives of the cells. The enzymes tested here for dCF phosphorylation do not represent an exhaustive list. Other candidates would include thymidine and uridine kinases, viral or mitochondrial deoxyribonucleoside kinases; however, these have not yet been tested. Studies in our laboratory with procaryotic cells suggest that a phosphotransferase efficiently converts dCF to its monophosphate derivative and a similar enzyme may be present in eucaryotic cells.3

**Incorporation of Deoxycoformycin into Cellular Nucleic Acids—Approximately 15–20% of the radioactivity in cells incubated with [\textsuperscript{3}H]dCF was not extractable by 60% methanol. To account for this loss in radioactivity, we investigated the incorporation of dCF into cellular nucleic acids. CEM and Molt-4 cells were incubated with [\textsuperscript{3}H]dCF for varying time periods. Following purification, the nucleic acids were treated with formamide, heated, and fractionated by CsCl gradient centrifugation. DNA and RNA banded at densities between 1.42–1.48 g/ml and 1.62–1.68 g/ml, respectively. In control experiments, [\textsuperscript{3}H]dCF was incubated with cellular nucleic acids which were subsequently carried through the same purification and fractionation experiments as those described above. Trichloroacetic acid-precipitable radioactivity was found only to be associated with DNA (or minor amounts of RNA) isolated from CEM or Molt-4 cells incubated with [\textsuperscript{3}H]dCF (Fig. 7). The apparent amount of dCF incorporated into cellular DNA was 1–1.5 pmol/10⁶ cells after 72 h (>90% of total radioactivity).**

To investigate the nature of the tritium label that had been incorporated, total cellular nucleic acids, were degraded to their component nucleosides by DNase I, RNase A, bacterial alkaline phosphatase, and snake venom phosphodiesterase I. The nucleosides products were then analyzed by HPLC on a Bondapak C₈ column. Degradation of total cellular nucleic acids gave rise to four radioactive peaks (Fig. 8; peaks A–D). When the nucleosides in peaks A and B were incubated with adenosine deaminase or purine nucleoside phosphorylase, no change in the elution profile of either was observed. The material in peak B when incubated with pure adenosine deaminase efficiently inhibited the catalytic activ-

3 M. F. E. Siaw and M. S. Coleman, unpublished data.
Fig. 6. HPLC profiles of the nucleotide pools from four hypoxanthine-guanine phosphoribosyl transferase-deficient, kinase mutant CEM cells. Cells were incubated with 1 μCi/ml of [3H]dCF for 72 h at 37 °C, under conditions described under "Experimental Procedures." Profile of the absorbance at 254 nm (top), and the radioactivity-associated peaks (bottom) are shown for the following kinase mutant cell lines: A, BU-CEM-Tub4-M10-2 (AK-, dCK+); B, BU-CEM-AraC-8D (AK+, dCK-); C, BU-CEM-AraC-8D-MMPR-10-5 (AK-, dCK-); D, BU-CEM-HGPRT+ (AK+, dCK+).

The commercial preparations of [3H]dCF we used in these experiments do not contain detectable levels of Ado or dAdo (limit of detection was <0.02%). The data from the mutant cell lines coupled with the absence of radioactive phosphorylated derivatives of Ado or dAdo in intracellular extracts indicates that the label transfer to Ado or dAdo may occur when the dCF is incorporated into DNA.

To compare the extent of incorporation of dCF into DNA with other nucleoside analogues, CEM cells were incubated with [3H]araA or [3H]araC and analyzed. The results are shown in Table V. After 24 h, araA was incorporated into...
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FIG. 7. Radioactivity profiles for the fractionation of cellular DNA and RNA on CsSO₄ gradients. Cultures of CEM (A) and Molt-4 (B) cells were set up as described under "Experimental Procedures." Cellular nucleic acids from cells grown in the presence of [³H]dCF for 24, 48, and 72 h were isolated and fractionated on CsSO₄ gradients. 0.15-ml fractions were collected and processed for trichloroacetic acid-precipitable counts.

FIG. 8. HPLC analysis of the nucleosides derived from the degradation of cellular nucleic acids from CEM (A) and Molt-4 (B) cells incubated with [³H]dCF. Digestion of total cellular nucleic acids using DNase I, RNase, snake venom phosphodiesterase I, and bacterial alkaline phosphatase was carried out, and HPLC analyses of the nucleosides was performed with the use of a C₁₈-Bondapak column under the conditions as described under "Experimental Procedures." Fractions of 0.5 ml were collected for liquid scintillation counting.

DNA and RNA at the rate of 30 pmol/10⁶ cells. In contrast, araC was incorporated into DNA alone (15 pmol/10⁶ cells). These results are apparently comparable to the rate of incorporation of other natural nucleosides into DNA if similar conditions are used (14). Thus, the level of incorporation of dCF into nucleic acids was considerably lower, 1–2 pmol/10⁶ cells, than that observed for other nucleosides. However, when the intracellular concentration of dCF achieved in cells is considered, 4 μM versus the intracellular concentration achieved for araA or dAdo, 400–500 μM, the incorporation of dCF into nucleic acids was relatively efficient.

DISCUSSION

Deoxycoformycin is a transition-state enzyme inhibitor which has attracted clinical interest because it is a selective immunosuppressant agent. When administered to patients, dCF precipitates a series of biochemical events that result in selective lymphocyte lysis (at high drug concentration) or impairment of immune function without lysis (at low drug concentration). Hereofore, the action of dCF was thought to result solely from its inhibition of the catalytic activity of adenosine deaminase.

In this study, we have shown for the first time that dCF entered the major nucleoside anabolic pathways in lymphoblastoid cells. We attributed the inability of others to demonstrate significant metabolism of dCF to two factors: 1) low specific activity radiolabeled dCF, and 2) instability of the phosphorylated derivatives of dCF to low pH extraction conditions. The level of low specific activity was addressed satisfactorily when we obtained a uniformly labeled dCF from Moravek Biochemicals which was 5 times the specific activity of previous preparations of dCF. All nucleotide extractions were carried out in 60% methanol, accompanied by rapid sample processing to solve the experimental problem of nucleotide instability. The substantial variations we observed among experiments in recovery of dCF nucleotides was directly related to the length of time between sample extraction and analysis. Thus, with modified procedures, dCF was shown to be converted to mono-, di-, and triphosphorylated derivatives and ultimately incorporated into DNA and, to a far lesser extent, into RNA. The first step in the process, conversion to the monophosphate nucleoside, did not occur via the enzymes Ado kinase and dCyd kinase. Thus, dCF did not compete in kinase reactions with nucleosides adenosine and deoxyadenosine that accumulate when ADA is inhibited. When dCF was present as the triphosphorylated derivative, it functioned as a fairly efficient substrate for an as yet unidentified nucleotide-polymerizing activity and was incorporated into DNA as demonstrated by recovery of intact dCF following hydrolysis of nucleic acids.

When total nucleic acids were hydrolyzed, we were able to isolate not only radiolabeled dCF and its ring-opened structure but also radiolabeled dAdo and Ado. These natural nucleosides appeared to be incorporated into DNA and RNA.

TABLE V

| Nucleoside analogue | Time of incubation | Amount incorporated into cellular nucleic acids (pmol/10⁶ cells) |
|---------------------|--------------------|---------------------------------------------------------------|
|                     |                    | CEM                | Molt-4               |
| [³H]dCF             | 24                 | 1.0               | 0.5                  |
|                     | 48                 | 1.8               | 1.2                  |
|                     | 72                 | 2.1               | 1.5                  |
| [³H]araA            | 24                 | 30                | ND                   |
| [³H]araC            | 24                 | 15                | ND                   |

* The deamination of araA was inhibited in these cells.
* ND, not determined.
since they were not isolated in control experiments in which labeled dCF was incubated with DNA and carried through the isolation and fractionation procedures. The conversion of radiolabel from dCF to Ado and dAdo did not appear to occur metabolically, as in no instance were radiolabeled nucleotides of Ado or dAdo isolated from cells incubated with dCF. Isotope exchange from dCF to Ado and dAdo specifically (but not other nucleotides) did not appear to be a feasible mechanism, but we cannot completely rule out this possibility. However, when radioactive dCF was incubated with cells deficient in both adenosine kinase and deoxyadenosine kinase and nucleosides were generated from cellular nucleic acids, we observed a similar pattern with radioactivity associated with Ado and dAdo. This experiment seems to indicate that isotope exchange is not occurring at the nucleoside level. Other potential mechanisms for exchange of radiolabel involve the DNA itself by close positioning of dCF and dAdo monomers, or by a DNA repair enzyme which may be capable of converting the dCF ring structure to dAdo.

The existence of phosphorylated derivatives of dCF and incorporation of these derivatives into cellular DNA raises questions about the mechanism of action of the drug. Since dCF has been shown here to be converted to nucleotide forms, it is possible that these compounds inhibit multiple enzymes. As an example, dCF monophosphate, enzymatically synthesized from dCF using a preparation of 5'-nucleoside phosphotransferase from Serratia marcescens (15), has been shown by Frieden et al. (16) to be a competitive inhibitor of AMP deaminase with a Ki of $10^{-8}$ M. Concentrations of dCF monophosphate in excess of this $Ki$ (3 x $10^{-10}$ to 1.8 x $10^{-8}$ M), are easily achievable, as shown herein, in lymphoblasts at an extracellular dCF concentration of 10 $\mu$M.

Recently, two groups have observed abrupt phenotypic shifts in residual leukemic cells in certain patients treated with high levels of dCF. Murphy et al. (17) have reported that during the course of a 1-month treatment with dCF, a child with typical acute lymphoblastic leukemia quite suddenly experienced total marrow replacement of lymphoblasts showing T-cell characteristics with myeloblasts. Similarly, Hershfield et al. (18) have reported that in a single patient treated aggressively with dCF, there was a rapid conversion of leukemic cells from lymphoblast morphology to a typical promyeloblastic morphology. The potential role of dCF or any of its metabolites in precipitating phenotypic shifts in multipotent hematopoietic stem cells is unknown, but it is reasonable to consider that phosphorylated derivatives of dCF may have multiple effects on the anabolic pathways in cells which are exposed to the drug for prolonged intervals. These effects may include unusual organ toxicities which have been associated with the use of high levels of this drug (3) as well as profound alterations in cellular morphology that may be a reflection of changes in gene expression in hematopoietic cells.

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Table 1: Accumulation of dAMP in CEM and Molt-4 Cells.

|          | CEM (nmol) | Molt-4 (nmol) |
|----------|------------|---------------|
| 0 hours  | 8.5        | 10            |
| 5 hours  | 12.4       | 12.4          |
| 10 hours | 17.5       | 17.5          |
| 20 hours | 22.6       | 22.6          |

Methods

Deoxycoformycin and deoxycoformycin phosphodiestase II were purified as described previously. The purified deoxycoformycin phosphodiestase II was incubated with dAMP at 37°C for 5 hours. The resulting mixture was analyzed by HPLC, and the absorbance at 260 nm was measured.

Figure 1: Accumulation of dAMP in CEM and Molt-4 Cells.

Figure 2: Effects of deoxycoformycin in the growth of CEM (panel A) and Molt-4 (panel B) cells. Cells were incubated with deoxycoformycin at 10, 20, and 30 μM for 72 hours. The results are presented as mean ± SEM of at least three experiments.

Table 2: Accumulation of dAMP in CEM and Molt-4 Cells.

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| 20 hours | 22.6       | 22.6          |

Results

Deoxycoformycin was incubated with dAMP at 37°C for 5 hours. The resulting mixture was analyzed by HPLC, and the absorbance at 260 nm was measured.

Figure 1: Accumulation of dAMP in CEM and Molt-4 Cells.

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J. Biol. Chem. 1984, 259:9426-9433.

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