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Cytotoxicity of imides-*N*-alkyl semicarbazones, thiosemicarbazones, acetylhydrazones and related derivatives

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The semicarbazones, thiosemicarbazones and acetyl-hydrazones of phthalimide, o-benzosulfimide, naphthalimide and diphenimide demonstrated potent cytotoxicity against murine and human leukemia cell growth and cultured cell growth from human solid tumors. The major site of inhibition in L1210 leukemia cells was DNA synthesis after 60 min incubated with the agents at 25, 50 and 100 μM. *De novo* synthesis of purines at the regulatory enzyme sites of PRPP amidotransferase and IMP dehydrogenase were the major targets of the agent. Thymidylate synthetase, dihydrofolate reductase and ribonucleoside reductase activities were inhibited by the agents in a manner which would contribute to the overall reduction of DNA synthesis and cell death. d(NTP) pools were significantly reduced and the evidence suggests that the agents interacted with DNA affording DNA strand scission which would interfere with both template utilization by the polymerases and also ultimately reduce nucleic acid synthesis.

Key words: Acetylhydrazones, cytotoxicity, semicarbazones, thiosemicarbazones.

Introduction

Previous studies have shown that cyclic imides, e.g. indandiones, 2,3-dihydrophthalazine-1,4-dione,[1] indazolones,[2] diphenimides and reduced diphenimides,[3] were potent cytotoxic/antineoplastic agents. These agents were effective against the growth of both leukemias as well as cells cultured from solid tumors of humans and mice. The mode of action of the cyclic imides was suppression of L1210 DNA and RNA synthesis by reducing *de novo* synthesis of purines. In addition, thymidylate synthetase and dihydrofolate reductase activities were inhibited, leading to reduced d(NTP) pool levels. Other cyclic imides similar to the above derivatives have previously demonstrated hypolipidemic activity. Compactin, an hypocholesterolemic agent, also suppresses cell proliferation and DNA synthesis of L929 cells.[4] Similar cross-over activity has been observed for sesquiterpene lactones[5] and amine-carboxyboranes.[6] Thus, at this time we initiated studies on a series of hypolipidemic semicarbazone cyclic imides and related derivatives to evaluate their cytotoxic activity.

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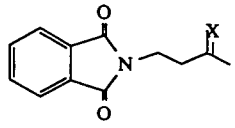
Materials and methods

Source of compounds

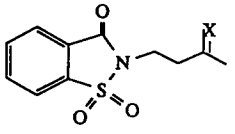
The imido-*N*-alkyl semicarbazones, thiosemicarbazones and acetylhydrazone derivatives were previously synthesized and characterized[9,10] (Figure 1). All radioisotopes were purchased from New England Nuclear (Boston, MA) unless otherwise indicated. Radioactivity was determined in Fisher Scintiverse scintillation fluid with correction for quenching. Substrates and cofactors were obtained from Sigma (St Louis, MO).

Pharmacological methods

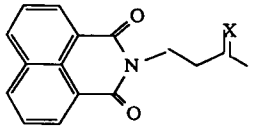
Compounds **Ia–VIIa** (Table 1) were tested for cytotoxic activity by homogenizing drugs in a 1 mM solution in 0.05% Tween 80/H_2O. These solutions were sterilized by passing them through an acrodisc (45 μM). The following cell lines were maintained by literature techniques:[8] murine L1210 lymphoid leukemia, human $Tmolt_3$ acute lymphoblastic T cell leukemia, colorectal adenocarcinoma SW480, lung bronchogenic MB-9812, osteosarcoma TE418, KB epidermoid nasopharynx, HeLa-S^3 suspended cervical carcinoma and glioma EH 118 MG. Geran *et al.*'s protocol[9] was used to assess the cytotoxicity of the compounds and standards in each cell line. Values for cytotoxicity were expressed as ED_{50}=



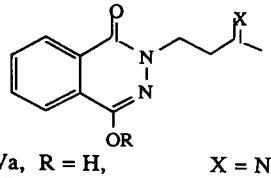
Ia, X = $NNHCONH_2$
Ib, X = $NNHCSNH_2$
Ic, X = $NNHCOCH_3$



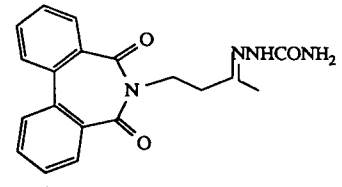
IIa, X = $NNHCONH_2$
IIb, X = $NNHCSNH_2$
IIc, X = $NNHCOCH_3$



IIIa, X = $NNHCONH_2$
IIIb, X = $NNHCSNH_2$
IIIc, X = $NNHCOCH_3$



IVa, R = H, X = $NNHCONH_2$
IVb, R = H, X = $NNHCSNH_2$
IVc, R = H, X = $NNHCOCH_3$
IVe, R = CH_3, X = $NNHCONH_2$
IVf, R = CH_3, X = $NNHCSNH_2$
IVg, R = CH_3, X = $NNHCOCH_3$
IVh, R = CH_2CH_3, X = O
IVi, R = CH_2CH_3, X = $NNHCONH_2$
IVj, R = CH_2CH_3, X = $NNHCSNH_2$
IVk, R = CH_2CH_3, X = $NNHCOCH_3$
IVl, R = $CH_2C_6H_5$, X = O
IVm, R = $CH_2C_6H_5$, X = $NNHCONH_2$
IVn, R = $CH_2C_6H_5$, X = $NNHCSNH_2$
IVo, R = $CH_2C_6H_5$, X = $NNHCOCH_3$



VIIa

Figure 1. Structures of hypolipidemic agents.

μg/ml, i.e. the concentration of the compound inhibiting 50% of cell growth. ED_{50} values were determined by the Trypan blue exclusion technique for L1210 and $Tmolt_3$ leukemias and HeLa-S^3 uterine carcinoma. A value of less than 4 μg/ml was required for significant activity of growth inhibition. Cytotoxicity was determined in the cell cultures derived from solid tumors by Liebovitz *et al.*'s method[10] utilizing crystal violet/MeOH and the 96-well plates were read at 580 nm (Molecular Devices, Menlo Park, CA).

Incorporation of labeled precursors into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein for 10^6 L1210 cells was obtained.[11] The concentration response at 10, 25, 50 and 100 μM required for inhibition of DNA, RNA and protein synthesis was determined after 60 min incubations. The incorporation of [^{14}C]glycine (53.0 mCi/mmol) into purines was obtained by the method of Cadman *et al.*[12] Incorporation of [^{14}C]formate (53.0 mCi/mmol) into pyrimidines was determined by the method of Chrisopherson *et al.*[13]

Enzyme assays

Inhibition of various enzyme activities was performed by first preparing the appropriate L1210 cell homogenates or subcellular fractions, then adding the drug to be tested during the enzyme assay. For the concentration response studies, inhibition of enzyme activity was determined at 10, 25, 50 and 100 μM of compounds **IIIa**, **IVc** and **IVn** after 60 min incubations. DNA polymerase α activity was determined in cytoplasmic extracts isolated by Eichler *et al.*'s method.[14] Nuclear DNA polymerase β was determined by isolating nuclei.[15] The polymerase assay for both α and β was described by Sawada *et al.*[16] with [^{3}H]TTP. Messenger-, ribosomal- and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate, individual RNA polymerase activities were determined using [^{3}H]UTP.[17,18] Ribonucleoside reductase activity was measured using [^{14}C]CDP with and without dithioerythritol.[19] The deoxyribonucleotides [^{14}C]dCDP were separated from the ribonucleotides by thin layer chromatography (TLC) on PEI plates. Thymidine, TMP and TDP kinase activities were determined using [^{3}H]thymidine (58.3 mCi/mmol) in the medium of Maley and Ochoa.[20] Carbamyl phosphate synthetase activity was determined by the method of Kalman *et al.*;[21] citrulline was determined colorimetrically.[22] Aspartate transcarbamylase activity was measured by the meth-

Table 1. Cytotoxicity of cyclic imide semicarbazone (ED_{50} values = μg/ml)

Compound	L1210 leukemia	$Tmolt_3$ leukemia	HeLa-S^3 uterine	Adenocarcinoma SW480	KB Naso	MB-9812 lung broncho	TE418 osteo sarcoma	EH118MG glioma
Ia	2.52	2.45	2.41	1.39	1.03	0.94	8.78	8.01
Ib	0.88	3.60	2.20	3.30	7.64	4.21	8.18	3.56
Ic	1.51	2.36	1.86	1.25	6.81	7.36	5.67	2.85
IIa	1.34	1.81	1.95	3.63	7.68	6.78	6.47	5.83
IIb	0.84	3.11	1.73	2.60	6.97	2.18	—	2.89
IIc	0.96	4.06	2.46	2.65	5.84	7.38	6.89	6.91
IIIa	0.80	0.51	5.60	6.19	3.21	4.54	7.95	8.41
IIIb	1.79	4.39	1.57	2.28	4.64	8.10	4.27	6.65
IIIc	0.65	3.97	2.17	1.36	4.77	6.26	7.28	2.65
IVa	2.96	2.10	2.12	4.59	3.48	4.52	4.33	7.88
IVb	1.45	3.59	2.96	1.09	5.53	6.55	7.57	6.85
IVc	0.57	2.97	2.17	1.88	3.39	8.55	5.84	3.37
IVe	0.90	1.74	3.96	7.15	5.45	5.91	6.67	8.37
IVf	1.06	4.12	1.52	1.76	4.64	5.52	6.09	5.54
IVh	1.57	1.56	3.36	1.45	3.34	6.58	5.29	5.86
IVl	3.27	3.35	2.07	3.21	5.27	8.85	7.54	2.51
IVj	1.26	1.67	4.55	1.33	2.81	7.39	7.57	5.06
IVk	0.74	1.65	1.98	0.96	5.78	6.55	4.81	4.96
IVl	2.78	2.54	4.21	0.97	3.77	7.57	5.23	8.70
IVm	1.84	3.87	3.18	1.68	3.84	7.59	6.42	6.74
Ivn	0.36	2.76	3.23	2.54	4.83	6.19	5.78	7.06
IVo	1.82	3.08	3.75	1.45	5.20	7.69	7.57	8.25
VIIa	2.91	5.63	1.91	1.96	1.91	1.41	7.86	—
5 FU	1.41	2.14	2.47	3.09	1.25	5.69	—	1.28
Ara C	2.76	2.67	2.13	3.42	2.84	4.69	—	1.88
Hydroxyurea	2.67	3.18	1.96	4.74	5.29	7.37	7.57	2.57

od of Kalman *et al.*;[21] carbamyl aspartate was determined colorimetrically.[23] OMP decarboxylase activity was determined using orotidine-5-monophosphate [carboxyl-^{14}C][34.9 mCi/mmol] by Appel's method.[24] Thymidylate synthetase activity was analyzed by Kampf *et al.*'s method.[25] The ^3H_2O measured was proportional to the amount of TMP formed from [^{3}H]dUMP. Dihydrofolate reductase activity was determined by the spectrophotometric method of Ho *et al.*[26] PRPP amidotransferase activity was determined by Spassova *et al.*'s method;[27] IMP dehydrogenase activity was analyzed with [8-^{14}C]IMP (54 mCi/mmol) (Amersham, Arlington Heights, IL) after separating XMP on PEI plates (Fisher Scientific, Pittsburg, PA) by TLC.[28] Protein content was determined for the enzymatic assays by the Lowry technique.[29]

After deoxyribonucleotide triphosphates were extracted,[30] levels were determined by the method of Hunting and Henderson[31] with calf thymus DNA, *Escherichia coli* DNA polymerase I, non-limiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 mCi of [^{3}H-methyl]dTTP or [5-^{3}H]dCTP.

The effects of compounds **IIIa**, **IVc** and **IVn** on DNA strand scission were determined by the methods of Suzuki *et al.*,[32] Pera *et al.*[33] and Woynarowski *et al.*[34] L1210 lymphoid leukemia cells were incubated with 10 μCi [methyl-^{3}H]thymidine, 84.0 Ci/mmol for 24 h at 37°C. L1210 cells (10^7) were harvested and then centrifuged at 600 *g* for 10 min in PBS. They were later washed and suspended in 1 ml of PBS. Lysis buffer (0.5 ml; 0.5 M NaOH, 0.02 M EDTA, 0.01% Triton X-100 and 2.5% sucrose) was layered onto a 5–20% alkaline–sucrose gradient (5 ml; 0.3 M NaOH, 0.7 KCl and 0.01 M EDTA); this was followed by 0.2 ml of the cell preparation. After the gradient was incubated for 2.5 h at room temperature, it was centrifuged at 12 000 r.p.m. at 20°C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the bottom of the gradient, neutralized with 0.2 ml of 0.3 N HCl and measured for radioactivity. Thermal calf thymus DNA denaturation studies and DNA viscosity studies were conducted after incubation of compounds **IIIa, IVc** and **IVn** at 100 μM at 37°C for 24 h.[35]

Statistics

The mean and standard deviation are designated by '$X \pm$ SD.' The probable level of significance (p) be-

tween test and control samples was determined by the Student's *t*-test with the raw data.

Results

All of the compounds demonstrated potent cytotoxicity against murine L1210 lymphoid leukemia cells. Compounds **Ib**, **IIb**, **IIc**, **IIIa**, **IIIc**, **IVc**, **IVe**, **IVk** and **IVn** demonstrated ED_{50} values less than 1 μg/ml. $Tmolt_3$ leukemia cell growth was significantly reduced by all of the derivatives except **IIc**, **IIIb**, **IVf** and **VIIa**. Compound **IIIa** resulted in an ED_{50} value of 0.51 μg/ml. Adenocarcinoma colon SW480 carcinoma growth was reduced by all of the derivatives except **IIIa**, **IVa** and **IVe**. Compounds **IVk**, **IVl** and **IVb** were the most effective agents with ED_{50} values of approximately 1 μg/ml in the colon SW480 screen. Hela-S^3 uterine carcinoma growth was reduced by **Ic**, **IIa**, **IIb**, **IIIb**, **IVf**, **IVk** and **VIIa** with ED_{50} values less than 2 μg/ml. KB nasopharynx growth was inhibited only by **Ia**, **IIIa**, **IVa**, **IVc**, **IVh**, **IVj**, **IVl**, **IVm** and **VIIa**. Lung bronchogenic MB-9812 cancer growth was significantly reduced by **Ia**, **IIb** and **VIIa**. Osteosarcoma bone TE418 growth was not reduced by any of the derivatives. Brain glioma EH 118MG growth was reduced by **Ib**, **Ic**, **IIb**, **IIIc**, **IVc** and **IVi**.

Three representative compounds of these chemical classes, compounds **IIIa**, **IVc** and **IVn**, which all demonstrated potent cytotoxic action, were selected for an in-depth metabolic study to determine the mode of action of these compounds for L1210 cell growth inhibition. Compounds **IIIa**, **IVc** and **IVn** all caused a concentration-dependent inhibition of DNA synthesis over 60 min with 28–47% reduction at 100 μM. Compound **IIIa** actually increased RNA synthesis, **IVc** caused a 35% reduction at 100 μM. **IVn** essentially had no effect on RNA synthesis over 60 min. Protein synthesis was not significantly affected by any of the three agents. DNA polymerase α activity was inhibited by compounds **IIIa** and **IVc** but not by **IVn**. m-RNA polymerase activity was inhibited by compounds **IIIa** and **IVn** but not compound **IVc**, which actually elevated the activity. r-RNA polymerase activity was inhibited only by compound **IVn**. t-RNA polymerase activity was inhibited significantly by all three derivatives from 36 to 65% at 100 μM. Purine *de novo* synthesis was inhibited significantly by all three agents. Both of the regulatory enzymes, i.e. PRPP amido transferase and IMP dehydrogenase, of the purine pathway were inhibited by the semicarbazone derivatives. Compounds **IIIa** and **IVc** inhibited marginally the activities of three regulatory enzymes in the pyrimidine pathway, e.g. carbamyl phosphate synthetase, aspartate transcarbamylase and OMP decarboxylase. Thymidylate synthetase activity was significantly inhibited by all of the derivatives greater than 30% at 100 μM. Thymidine kinase and TMP kinase activities were inhibited by compounds **IIIa** and **IVn**. Compound **IVa** only inhibited thymidine kinase activity. TDP kinase activity was inhibited only by compound **IIIa**. Ribonucleoside reductase activity was marginally inhibited by compounds **IIIa** and **IVn**, but compound **IVc** was more effective in its inhibition of this enzyme activity. Dihydrofolate reductase activity was significantly inhibited by all three agents at 100 μM. It should be noted that the semicarbazone derivatives caused inhibition of a number of enzymatic activities, but the effects of the agents were not always concentration dependent over the concentration range of 25–100 μM. d[NTP] pool levels were also affected by the semicarbazone derivatives with d[GTP] and d[TTP] pools being reduced more than d[ATP] and d[CTP] levels. *In vitro* studies with ct-DNA incubated with drugs for 24 h showed that all of the agents interfered with DNA in a manner to cause hyperchromic shifts at 260 nm, i.e. the maximum absorption of DNA complexed with drug shifted to a higher wave length. Thermal DNA denaturation studies showed that the control gave a T_m value of 74°C whereas compound **IIIa** afforded 59°C, **IVc** afforded 58°C and **IVn** afforded 62°C. ct-DNA viscosity studies showed that after 24 h incubation with drugs the control required 431 s to move through the reservoirs whereas compound **IIIa** required 441 s, **IVc** required 429 s and **IVn** required 434 s. After compounds **IIIa**, **IVc** and **IVn** at 100 μM were incubated with L1210 cells for 24 h smaller fragmented DNA was observed in the gradient (Figure 2).

Discussion

The *N*-butane-3-one semicarbazones, thiosemicarbazones, and acetylhydazones derivatives of phthalimide, *o*-benzosulfimide, 1,8-napthalimide, 2,3-dihydro-phthalazine-1,4-diones and diphenimides proved to be potent cytotoxic agents against the growth of L1210 lymphoid leukemia, $Tmolt_3$ T cell leukemia and HeLa-S^3 suspended uterine cancer cells. Selected agents were active against the growth of cultured human solid tumors, e.g. adenocarcinoma SW480 colon, KB nasopharynx and brain EH 118MG glioma. The agents were not active

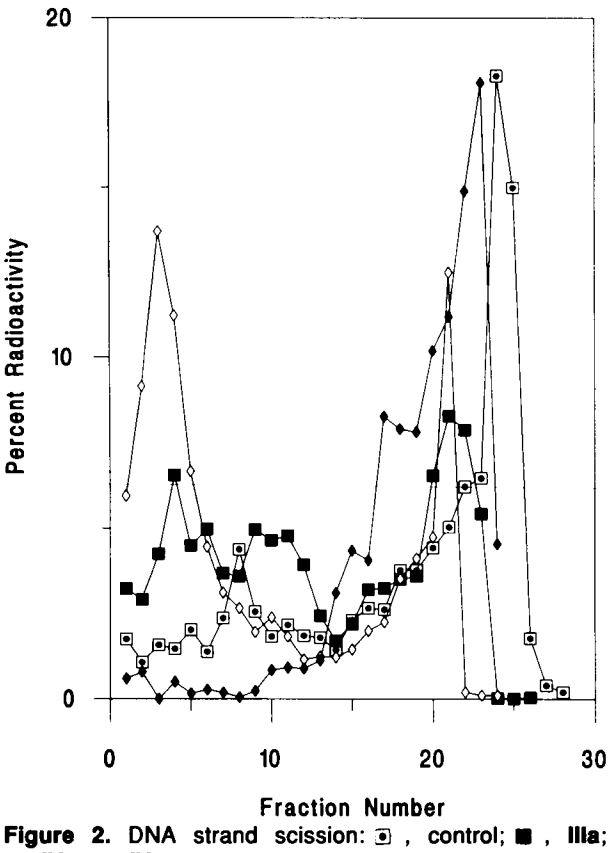


Figure 2. DNA strand scission: ⊡ , control; ■ , **IIIa**; ◇, **IVn**; ◆, **IVc**.

against the growth of bronchogenic MB-9812 lung and osteosarcoma TE418 bone cancer.

When the three typical compounds which afforded significant cytotoxic action were examined for their mode of action as antineoplastic agents in L1210 leukemia cells, all three compounds inhibited DNA synthesis over 60 min. The diphenimide acethylhydrazone **IVc** was the only compound of the three which significantly inhibited RNA synthesis. The inhibition of protein synthesis did not appear to be a target of this class of derivatives.

DNA template activity did appear to be affected by the derivatives since the naphthalimide semicarbazone **IIIa** and diphenimide acethylhydrazone **IVc** lowered the activity of DNA polymerase α and t-RNA polymerase. The thiosemicarbazone of diphenimide **IVn** reduced the activities of m-, r- and t-RNA polymerase. Further proof of the DNA template being affected by the agents were the ct-DNA studies, which suggested that the drugs interacted with the DNA strand but probably not by intercalation between bases, i.e. viscosity studies did not produce higher T_m values. The L1210 DNA strand scission denotes fragments of DNA which are consistent with altered T_m times in the ct-DNA viscosity studies, indicating smaller fragments of DNA, and with the reduced ability to utilize the DNA template by the polymerase in the L1210 studies after drug incubation.

L1210 purine *de novo* synthesis appeared to be the major site of inhibition of the agents. The two regulatory enzymes of the pathway, i.e. PRPP amido transferase and IMP dehydrogenase, were significantly inhibited by all three agents after 60 min incubation. The inhibition of this pathway by the agents was reflected in the reduced pool levels of d[ATP] and d[GTP] after 60 min incubation, which in turn should reduce both RNA and DNA synthesis causing ultimately cell death. Regulatory sites in the pyrimidine synthetic pathway were marginally inhibited in the presence of the naphthalimide semicarbazone **IIIa** and the diphenimide semicarbazone **IVa**. Thymidylate synthetase activity was significantly reduced by all three agents. The inhibition of this enzyme activity was reflected in the reduction of d[TTP] pools as observed after 60 min incubation with the agents. Dihydrofolate reductase activity was markedly reduced by all three agents. The inhibition of the activity of this rate limiting enzyme would affect *de novo* synthesis of purines because of the reduced capability of one-carbon transfer and may contribute to the overall reduction of nucleic acid synthesis. The inhibition of dihydrofolate reductase by the agents is consistent with the observed inhibition of thymidylate synthetase activity similar to the effects of methotrexate. The moderate reduction of nucleoside kinase and ribonucleoside reductase activities by all three agents is consistent with the observed reduction of d[NTP] pool levels. Thus, the metabolic effects of the semicarbazone derivatives on L1210 cells was not focused on a single event; however, the additive effects of the agents were sufficient to account for their cytotoxic action.

Other studies conducted on cyclic imides, e.g. 2.3-dihydrophthalazine-1,4-diones,[1] indazolones,[2] diphenimides and 6,7-dihydro-5H-dibenz[*c,e*] azepines,[3] cyclic imido alkyl ethers, thioethers, sulfoxides and sulfones,[36] and 3-imino-1-oxo-isoindolines[37] have demonstrated that the major target of cylic imides is the purine pathway with additional sites similar to those observed in this study. The imides and diphenimides have previously been shown to be free of any acute toxicity in mice.[38] Thus, these derivatives should offer a new chemical class of agents which may be available for development as antineoplastic agents.

Table 2. The effects of compounds **IIIa**, **IVc** and **IVn** on L1210 cell metabolism after 60 min incubation ($N = 6$)

Biochemical assay	Control	Percent of control								
		compound **IIIa**			compound **IVc**			compound **IVn**		
		25 μM	50 μM	100 μM	25 μM	50 μM	100 μM	25 μM	50 μM	100 μM
DNA synthesis	100 ± 6[a]	80 ± 6	77 ± 5*	72 ± 5*	71 ± 6*	65 ± 5*	53 ± 4*	71 ± 8*	66 ± 5*	64 ± 8*
RNA synthesis	100 ± 7[b]	125 ± 6	130 ± 6*	182 ± 6*	101 ± 6	84 ± 5	65 ± 5*	135 ± 7*	101 ± 6	90 ± 6
Protein synthesis	100 ± 7[c]	101 ± 6	89 ± 7	85 ± 5	107 ± 6	105 ± 6	103 ± 6	118 ± 7	86 ± 6	84 ± 3
DNA polymerase α	100 ± 6[d]	68 ± 5*	44 ± 4*	44 ± 5*	64 ± 4*	62 ± 4*	53 ± 4*	107 ± 6	102 ± 6	100 ± 4
m-RNA polymerase	100 ± 4[e]	104 ± 6	102 ± 5	63 ± 6*	125 ± 6	170 ± 7*	217 ± 8*	180 ± 7*	64 ± 3*	62 ± 5*
r-RNA polymerase	100 ± 7[f]	90 ± 5	102 ± 6	133 ± 7*	173 ± 6*	177 ± 5*	211 ± 6*	116 ± 7	102 ± 6	78 ± 5*
t-RNA polymerase	100 ± 6[g]	134 ± 7*	68 ± 5*	64 ± 4*	61 ± 6*	55 ± 4*	35 ± 7*	75 ± 6*	59 ± 6*	48 ± 6*
Purine synthesis	100 ± 6[h]	42 ± 3*	38 ± 4*	37 ± 5*	71 ± 5*	22 ± 3*	18 ± 4*	105 ± 6	47 ± 4*	12 ± 3*
PRPP amido transferase	100 ± 7[i]	95 ± 7	62 ± 5*	38 ± 4*	78 ± 6*	77 ± 5*	49 ± 4*	52 ± 5*	20 ± 3*	14 ± 3*
IMP dehydrogenase	100 ± 6[j]	65 ± 5*	57 ± 5*	50 ± 5*	44 ± 6*	42 ± 5*	40 ± 3*	46 ± 5*	38 ± 5*	27 ± 3*
Carbamyl phosphate synthase	100 ± 6[k]	82 ± 7	79 ± 6	77 ± 5*	117 ± 6	119 ± 5	121 ± 6	82 ± 8	81 ± 5	79 ± 5*
Asparatate transcarbamase	100 ± 7[l]	83 ± 7	81 ± 4	76 ± 5*	100 ± 7	96 ± 7	94 ± 5	74 ± 5*	73 ± 6*	73 ± 3*
OMP deacarboxylase	100 ± 6[m]	81 ± 6	78 ± 6*	74 ± 5*	92 ± 3	150 ± 6*	194 ± 5*	132 ± 6*	89 ± 6	81 ± 5*
Thymidylate synthetase	100 ± 5[n]	172 ± 8*	32 ± 4*	17 ± 3*	151 ± 5*	36 ± 4*	26 ± 6*	80 ± 5*	34 ± 4*	16 ± 3*
Thymidine kinase	100 ± 6[o]	62 ± 5*	62 ± 6*	31 ± 4*	76 ± 5*	77 ± 5*	103 ± 6	61 ± 4*	59 ± 5*	40 ± 3*
TMP kinase	100 ± 7[p]	58 ± 5*	55 ± 6*	34 ± 4*	64 ± 5*	94 ± 6	100 ± 6	102 ± 5	91 ± 6	34 ± 7*
TDP kinase	100 ± 6[q]	66 ± 4*	62 ± 5*	38 ± 3*	88 ± 7*	106 ± 6	105 ± 7	51 ± 5*	102 ± 6	100 ± 6
Riconucleoside reductase	100 ± 5[r]	83 ± 5	76 ± 5*	71 ± 6*	78 ± 6*	67 ± 5*	56 ± 5*	88 ± 6*	71 ± 6*	69 ± 5*
Dihydrofolate reductase	100 ± 5[s]	258 ± 7*	120 ± 6	34 ± 3*	218 ± 7*	97 ± 4	39 ± 4*	65 ± 6*	49 ± 5*	20 ± 3*
d[ATP]	100 ± 6[t]			80 ± 5*			8 ± 75			96 ± 6
d[GTP]	100 ± 5[u]			51 ± 4*			69 ± 5*			5 ± 2*
d[CTP]	100 ± 5[v]			79 ± 6*			78 ± 4*			83 ± 6
d[TTP]	100 ± 6[w]			40 ± 5*			57 ± 5*			34 ± 4*

Control values for 10^6 cells/h: [a] 29801 d.p.m.; [b] 29196 d.p.m.; [c] 6564 d.p.m.; [d] 5318 d.p.m.; [e] 1338 d.p.m.; [f] 5823 d.p.m.; [g] 3644 d.p.m.; [h] 92251 d.p.m.; [i] 0.055 OD units/mg protein; [j] 20875 d.p.m.; [k] 0.426 μmol citrulline; [l] 0.066 mol aspartate carbamylate; [m] 44743 d.p.m.; [n] 51560 d.p.m.; [o] 4562 d.p.m.; [p] 666 d.p.m.; [q] 275 d.p.m.; [r] 92975 d.p.m.; [s] 0.133 OD units/mg protein; [t] 32.39 pmol; [u] 23.79 pmol; [v] 86.29 pmol; [w] 22.04 pmol.

*$p = 0.001$ Student's t-test.

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