4. M. R. Hokin, G. B. Benfey and L. E. Hokin, *J. biol. Chem.* **233**, 814 (1958).
5. S. S. Jafferji and R. H. Michell, *Biochem. J.* **154**, 653 (1976).
6. K. Egawa, B. Sacktor and T. Takenawa, *Biochem. J.* **194**, 129 (1981).
7. J. Durrell and M. A. Sodd, *J. Neurochem.* **13**, 487 (1966).
8. M. R. Hokin and L. E. Hokin, *J. gen. Physiol.* **50**, 793 (1967).
9. L. M. Jones, S. Cockcroft and R. H. Michell, *Biochem. J.* **182**, 669 (1979).
10. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
11. J. Schacht, E. A. Neale and B. W. Agranoff, *J. Neurochem.* **23**, 211 (1974).
12. C. A. Harrington, D. C. Fenmore, and J. Eichberg, *Analyt. Biochem.* **106**, 307 (1980).
13. E. Santiago-Calvo, S. Mule, C. M. Redman, M. R. Hokin and L. E. Hokin, *Biochim. biophys. Acta* **84**, 550 (1964).
14. W. C. Bowman and M. J. Rand, *Textbook of Pharmacology* Chap. 22. Blackwell Scientific Publications, London (1980).
15. R. H. Michell and C. J. Kirk, *Biochem. J.* **198**, 247 (1981).
16. P. J. Marshall, D. E. Boatman and L. E. Hokin, *J. biol. Chem.* **256**, 844 (1981).

*Biochemical Pharmacology*, Vol. 31, No. 19, pp. 3133–3137, 1982.
Printed in Great Britain.

0006-2952/82/193133–05 $03.00/0


# Some biochemical characteristics of L1210 cell lines resistant to 6-mercaptopurine and 6-thioguanine and with increased sensitivity to methotrexate

(*Received* 15 *June* 1981; *accepted* 25 *March* 1982)

In experimental animal tumor systems, drug resistance has been demonstrated for all functional classes of anticancer agents [1]. In man, the recurrence or increased growth of leukemias and solid tumors with continuing, unchanged drug treatment suggests that overgrowth of drug-resistant tumor cells is also a therapeutic problem. With L1210 and P388 murine leukemias, we have shown that tumor cells resistant to a single drug or combination of drugs can be controlled in animal models by further treatment with anticancer agents of other classes [1]. Moreover, in some cases, drug-resistant tumor cells exhibit increased sensitivity to alternate drugs.

The 6-mercaptopurine (6MP)* and 6-thioguanine (6TG) resistant L1210 leukemia cells (L1210/6MP, L1210/6TG) used in this investigation are deficient in the purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase, EC 2.4.2.8 [2, 3]. Currently, their ability to convert hypoxanthine, guanine, 6MP and 6TG to 5′-ribonucleotide derivatives is 10%, or less, than that of the parent cell line (L1210/0). This suggests that the cells rely heavily on *de novo* purine synthesis.

By inhibiting dihydrofolate reductase (DHFR), EC 1.5.1.3, methotrexate (MTX) depletes the pool of one-carbon units carried by tetrahydrofolate derivatives [4–6] and, as a result, anabolic processes requiring one-carbon units, including the *de novo* synthesis of purines, are inhibited [7, 8]. The toxic effects of an MTX-induced purineless state might be amplified in cells that have a reduced capacity to salvage purine bases. In support of this prediction, Browman and Csullog [9] have reported that DNA synthesis in cell lines with impaired purine salvage was approximately 40% of that in parent drug-sensitive cells after MTX administration to tumor-bearing mice. Although cultured cells resistant to purine analogs did not exhibit collateral MTX sensitivity [9], such *in vitro* sensitivity has been noted in our laboratories [10]. In this investigation, we confirm the increased MTX sensitivity *in vivo* of an L1210 cell line resistant to 6MP, first observed by Law *et al.* [11], and report that a line of L1210 cells resistant to 6TG is similar in response. We have also investigated the biochemical bases for these increases in sensitivity.

The parent L1210 tumor cell line and the cell lines resistant to 6MP and 6TG were maintained by weekly serial passage in female DBA/2 mice as described previously [12]. To test sensitivity of the three tumor cell lines to MTX, 6-methylthiopurine ribonucleoside (6-MeMPR), 6MP or 6TG, cells were harvested from the peritoneal cavities of mice on day 6 or 7 post-implant, counted and inoculated intraperitoneally into healthy 6- to 10-week-old male or female BALB/c × DBA/2 ($CDF_1$) mice. In each experiment, the inoculum was titrated in 10-fold dilutions down to one cell to determine cell doubling time and to estimate the total body burden of tumor cells at the time of treatment. Tumor cell inoculum sizes, drug treatment schedules and the number of animals treated are given in Table 1. The approximate number of tumor cells alive after the last drug treatment was calculated from the life spans of treated mice (relative to those of control mice), duration of treatment, and cell doubling time [13]. Drug toxicity was based on the absence of gross evidence of leukemia (ascites or splenomegaly) at the death of treated mice.

To determine the intracellular MTX concentration in the three tumor cell lines following treatment of tumor-bearing mice, 3 mg/kg of [3′,5′,9(n)-$^3$H] MTX ([$^3$H]MTX) [9.4 μCi/mg, Amersham/Searle Corp., Arlington Heights, IL, radiochemical purity 95% by high pressure liquid chromatography (HPLC)], dissolved in 2% $NaHCO_3$, was administered subcutaneously to female $CDF_1$ mice that had received intraperitoneal tumor cell implants ($10^5$ to $10^6$ cells/mouse) 6 days earlier. At 1, 2, 4, 12 and 24 hr after dosing, mice were killed by cervical dislocation, and L1210 cells were harvested from the peritoneal cavity with heparinized syringes. The cells were washed with saline, and erythocytes were lysed with $NH_4Cl$ [14]. The L1210 cells were then resuspended in saline, and a portion was counted with a model Z Coulter Counter. After lysis of the remaining cell suspension, 15 ml of Liquifluor (New England Nuclear Corp., Boston, MA) containing Bio-Solv BBS-3 (Beckman Instruments, Fullerton, CA) (14:1, v/v) was added to 0.1-ml samples and radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer (model 3315) equipped with an external standard.

* Abbreviations: 6MP, 6-mercaptopurine; 6TG, 6-thioguanine; L1210/6MP, 6MP-resistant L1210 cell line; L1210/6TG, 6TG-resistant L1210 cell line; L1210/0, parent L1210 cell line; DHFR, dihydrofolate reductase; MTX, methotrexate; 6-MeMPR, 6-methylthipurine ribonucleoside; HPLC, high pressure liquid chromatography; and ILS, increase in life span.

To determine ribonucleotide levels in L1210 cells following MTX treatment of tumor-bearing mice, the same procedure was used, except that mice were dosed with unlabeled MTX (supplied by Dr. J. A. R. Mead, Developmental Therapeutics Program, DCT, NCI). After being counted, cells were extracted with cold 0.5 N perchloric acid [15]. Neutralized extracts were lyophilized, and the residues were dissolved in water and analyzed for ribonucleotides by HPLC as described previously [16]. Ribonucleoside mono-, di- and triphosphates, purchased from P-L Biochemicals (Milwaukee, WI), were used to verify retention times.

For measurement of DHFR, tumor cells were harvested from untreated tumor-bearing mice, processed as described above, and stored as pellets at −20°. Cultured L1210/0 and L1210/6MP cells were propagated with Dulbecco's modified Eagle's Minimal Essential Medium (Flow Laboratories, McLean, VA) supplemented with 10% horse serum (Flow Laboratories) and 5 μM 2-mercaptoethanol. Cells in the log phase of growth were harvested by centrifugation (1000 *g*), washed with saline, and stored at −20°. DHFR activity was determined in crude extracts of lysed cells and in pH 5.1 supernatant fractions obtained by precipitating the extracts with HCl [17]. DHFR was assayed according to the method of Bertino *et al.* [18] with dihydrofolate and NADPH (Sigma Chemical Co., St. Louis, MO). The change in absorbance at 340 nm and an extinction coefficient of 12,300 [19] were used to calculate enzyme activity. Protein was determined by the method of Lowry *et al.* [20] with bovine serum albumin (Sigma) as the standard. MTX was dissolved in 2% $NaHCO_3$ (1 mM) and serially diluted with reaction buffer (0.05 Tris buffer–0.1 M KCl, pH 7.5) to obtain the concentrations (1–10 nM) needed in these assays.

MTX treatment did not cause a net reduction in the tumor cell burden of mice bearing L1210/0 cells (Table 1). Depending on the dose level used, tumor cell populations had increased by 0.5–2.0 $\log_{10}$ at the end of treatment, even though a 75–93% increase in life span (ILS) was observed [13]. In contrast, treatment with MTX increased the median life span of mice bearing L1210/6MP cells by as much as 200%, and, at a dose of 2 mg/kg, three animals out of ten were "cured", i.e. were alive 51 days after the last drug treatment. This is consistent with our estimates that L1210/6MP cell populations were reduced by about 6 $\log_{10}$. Expressed in terms of the number of tumor cells alive at the end of treatment, this respresents a kill of all but 1 or a few L1210/6MP cells. The administration of MTX at a dose level of 1.5 mg/kg increased the median life span of mice bearing L1210/6TG cells by 122% and reduced the tumor cell population by about 3 $\log_{10}$. With the same MTX treatment, the median life span of mice bearing L1210/0 cells increased by about 85% and the tumor cell burden increased by about 1 $\log_{10}$. Repeat experiments confirmed these observations (not shown).

If the increased MTX sensitivity of the L1210/6MP and L1210/6TG cell lines is due to their inability to utilize endogenous purines supplied by the host, one would expect these cells also to be more sensitive to 6-MeMPR, an inhibitor of *de novo* purine synthesis. In two different experiments (Table 1), optimal, nontoxic, single-dose 6-MeMPR treatment (123 mg/kg) increased the median life span of mice bearing the parent cell line 37 and 62% and reduced the tumor cell population by 3 and 5 $\log_{10}$. In the first experiment, identical therapy in mice bearing L1210/6MP cells increased the median life span 20% and reduced the tumor burden by only 1.5 $\log_{10}$. In the second experiment, the median life span of mice bearing L1210/6TG cells increased 33% and their tumor cell burden decreased by about 3 $\log_{10}$. The apparent cross-resistance of L1210/6MP and L1210/6TG cells to 6-MeMPR indicates that the differential response of these cell lines and the parent cell line to MTX is not due to the use of host-supplied purines by the parent line. Further support is provided by the observation that L1210/6MP cells, cultured without purines added to the medium, exhibit enhanced sensitivity to MTX as compared to the parent line [10]. The L1210/6TG cell line has not been placed in culture. Unlike the L1210/6MP cells used in this report, the L1210 lines with impaired purine salvage developed by Browman and Csullog [9] did not exhibit collateral MTX sensitivity *in vitro*. Resolution of this inconsistency will require investigation of the biochemical characteristics of each cell line.

Impaired uptake and retention of MTX are established mechanisms of resistance [4, 21–26]. Therefore [$^3$H]MTX levels in L1210/0, L1210/6MP and L1210/6TG cells were compared during a 24-hr period following administration of drug to tumor-bearing mice (Fig. 1). Radioactivity can be used as a measure of MTX concentration since mice do not excrete detectable amounts of MTX metabolites [27]. MTX glutamation does occur intracellularly in mammalian tissues but does not reduce the effective concentration of drug in L1210 cells [28]. Throughout the experiment, the concentration of [$^3$H]MTX in L1210/6MP cells was less than that in L1210/0 cells. The greatest difference was observed at 24 hr after treatment, when MTX levels in L1210/6MP cells were one-half of those in L1210/0 cells. [$^3$H]MTX levels in L1210/6TG cells, on the other hand, were consistently greater than those in the parent line during the 24 hr following treatment. After 4 hr, the concentration of MTX in L1210/6TG cells was double that in L1210/0 cells. The higher levels of MTX in L1210/6TG cells may account, wholly or in part, for their increased sensitivity; the lower levels of MTX in L1210/6MP cells indicate that the mechanism(s) responsible for increased MTX sensitivity in this cell line is not related to processes determining intracellular MTX concentration.

Responsiveness to MTX has been associated with DHFR activity [29–33], although correlations between changes in this enzyme and MTX sensitivity have not been consistent [25, 34–37]. DHFR activity was measured in all three cell lines passaged *in vivo* and in cultured L1210/0 and L1210/6MP cells. Similar amounts of DHFR activity were

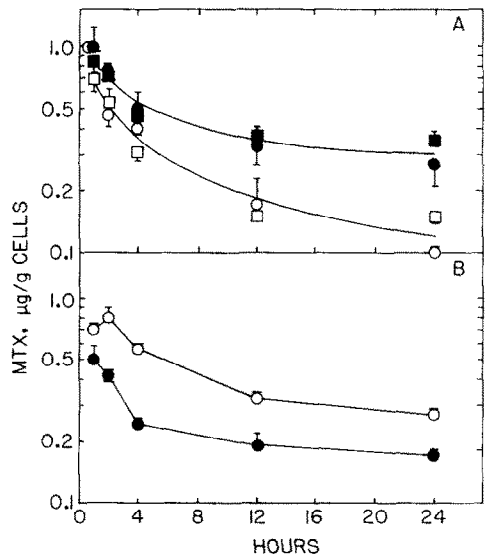


Fig. 1. [$^3$H]MTX concentration in L1210 leukemia cells following subcutaneous administration of 3 mg/kg of the drug to $CDF_1$ tumor-bearing mice. (A) L1210/0 and L1210/6MP; (B) L1210/0 and L1210/6TG. Symbols: for A, (●) L1210/0 (first experiment); (■) L1210/0 (second experiment); (○) L1210/6MP (first experiment); and (□) L1210/6MP (second experiment). For B, (●) L1210/0; and (○) L1210/6TG.

Table 1. Activity of methotrexate and 6-methylthiopurine ribonucleoside against L1210/0, L1210/6MP and L1210/6TG leukemias in $CDF_1$ mice

A. Comparison of L1210/0 and L1210/6MP Leukemias*

| | L1210/0 | | | L1210/6MP | | |
|---|---|---|---|---|---|---|
| | $10^5$ cells (Implant, i.p.†) $1.0 \times 10^6$ cells (Tumor Burden at Day 1) | | | $10^5$ cells (Implant, i.p.†) $6.5 \times 10^5$ cells (Tumor Burden at Day 1) | | |
| Treatment: MTX, i.p., q.d.‡ 1–9, Dose (mg/kg) | Day 60 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment§ | Day 60 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment |
| 2.5 | 0/10 | +75 | +2.0 | 1/10 | +183 | −5.8 (−5.4) |
| 2.0 | 0/10 | +93 | +0.5 | 3/10 | +200 | −5.8 (−5.7) |
| 1.5 | 0/10 | +87 | +1.0 | 1/10 | +175 | −5.8 (−5.4) |
| | $10^5$ cells (Implant i.p.) $1.0 \times 10^6$ cells (Tumor Burden at Day 1) | | | $10^5$ cells (Implant, i.p.) $5.5 \times 10^5$ cells (Tumor Burden at Day 1) | | |
| Treatment: 6-MeMPR, i.p., Day 1 only, Dose (mg/kg) | Day 30 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment | Day 30 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment |
| 185 | 0/10 | +62 | −5 | 0/10 | +20 | Toxic |
| 123 | 0/10 | +37 | −3 | 0/10 | +20 | −1.5 |
| 82 | 0/10 | +25 | −2 | 0/10 | +10 | −0.7 |

B. Comparison of L1210/0 and L1210/6TG Leukemias

| | L1210/0 | | | L1210/6TG | | |
|---|---|---|---|---|---|---|
| | $10^6$ cells (Implant, i.p.) $8.6 \times 10^6$ cells (Tumor burden at Day 1) | | | $10^6$ cells (Implant, i.p.) $3.7 \times 10^6$ cells (Tumor burden at Day 1) | | |
| Treatment: MTX, i.p., q.d. 1–9, Dose (mg/kg) | Day 45 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment | Day 45 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment |
| 1.5 | 0/10 | +85 | +1.2 | 0/10 | +122 | −3.1 |
| 1.25 | 0/10 | +78 | +1.3 | 0/10 | +109 | −2.3 |
| 1.0 | 0/10 | +71 | +1.4 | 0/10 | +86 | −0.9 |
| | $10^5$ cells (Implant, i.p.) $9.6 \times 10^5$ cells (Tumor Burden at Day 1) | | | $10^5$ cells (Implant, i.p.) $4.6 \times 10^5$ cells (Tumor Burden at Day 1) | | |
| Treatment: 6-MeMPR, i.p., Day 1 only, Dose (mg/kg) | Day 30 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment | Day 30 Survivors/ Total | Median Percent ILS | $Log_{10}$ Change in Cell No. at End of Treatment |
| 185 | 0/10 | +62 | −4.9 | 0/10 | +33 | −2.7 |
| 123 | 0/10 | +62 | −4.9 | 0/10 | +33 | −2.7 |
| 82 | 0/10 | +50 | −3.9 | 0/10 | +33 | −2.7 |

* Continued resistance of the L1210/6MP and L1210/6TG cell lines to 6MP and 6TG was confirmed in each experiment (not shown).

† Intraperitoneal.

‡ Every day.

§ $Log_{10}$ change = net log change in viable tumor cell population at the end of treatment as compared with the start of treatment, e.g. a −5.8 log change means there was a 99.9996% reduction and a +2 log change means there was a 100-fold increase in tumor burden at the end of treatment. Values are based on the median day of death (dying mice only) except those values in parentheses which are based on percent survivors [1, 13]. The log changes in tumor cell populations are reproducible to about one order of magnitude.

observed in crude extracts (2 nmoles per min per mg protein) and in pH 5.1 supernatant fractions (about 10 nmoles per min per mg protein) of L1210/0 and L1210/6TG cells propagated *in vivo*. Enzyme activity in the 6MP-resistant cell line was, however, only 30% of that in the parent cell line, with 0.6 nmole per min per mg protein being recovered in crude cell extracts and 3 nmoles per min per mg protein in pH 5.1 supernatant fractions. Cultured L1210/6MP cells also contained less DHFR activity (0.8 and 2 nmoles per min per mg protein for cell extracts and pH 5.1 supernatant fractions respectively) than the *in vitro* passaged parent line (4 and 14 nmoles per min per mg protein), which indicates that the decreased activity in L1210/6MP cells is not due to host influences. Supernatant fractions (pH 5.1) of *in vivo* passaged L1210/6MP and L1210/6TG cells were compared with those of parent L1210 cells for possible alterations in enzyme sensitivity to MTX, and no difference was found in the degree of DHFR inhibition by MTX when various concentrations of the drug were included in the assay (not shown). This demonstrates that the reduction in DHFR activity in L1210/6MP cells is due to decreased amounts of enzyme rather than to a change in enzyme

structure. While no difference in DHFR activity or sensitivity to MTX was detected between the parent and L1210/6TG cell lines, the reduced DHFR activity in L1210/6MP cells may account, in part, for their increased sensitivity to MTX.

Several investigators have studied the effect of MTX on ribonucleotide and deoxyribonucleotide pool levels in both normal and tumor cells [7, 8, 37–39]. Generally, these pools are depleted, although a variety of factors, including the cell line being studied, its growth state, and the dose of MTX, can alter the specific perturbations that are observed. Since the 6MP- and 6TG-resistant cell lines differ from each other in both DHFR activity and their capacity to accumulate MTX, ribonucleotide pools were examined to determine whether these differences would be reflected in pool sizes.

In all three cell lines, purine di- and triphosphate and pyrimidine triphosphate pools decreased from 2 to 6 hr after the administration of MTX to tumor-bearing mice. The data in Table 2 show nucleotide pool levels 4 hr after MTX treatment. At this time, pool sizes were at a minimum, and levels of GTP + GDP and CTP were significantly lower in L1210/6TG cells than in L1210/0 cells. This lower nucleotide level may be due to the high intracellular concentration of MTX that is maintained in L1210/6TG cells (Fig. 1) and may be related to increased MTX sensitivity. Although UTP levels appeared to be slightly higher in control L1210/6MP cells than in L1210/0 cells, pool sizes in these two cell lines were not significantly different following MTX treatment. No consistent differences in any pool levels were detected at 2, 6, 12, or 24 hr after MTX treatment. There appeared to be some recovery in nucleotide pools of all three cell lines by 12 hr (not shown); however, few cells were present at 12 and 24 hr after *in*

Table 2. Comparison of nucleotide pools in L1210/0, L1210/6MP and L1210/6TG leukemia cells 4 hr after *in vivo* exposure to methotrexate

| | | L1210/0 | | L1210/6MP | | | L1210/6TG | | |
|---|---|---|---|---|---|---|---|---|---|
| | Expt. No. | | | | | Student's t test* | | | Student's t test |
| ATP + ADP† | | | | | | | | | |
| Control | 1 | 20.6 ± 2.9 | (4)‡ | 24.1 ± 1.8 | (3)‡ | NS§ | | | |
| | 2 | 18.5 | (2)‡ | 25.1 | (2)‡ | $P < 0.02$ | | | |
| | 3 | 30.1 | (2) | | | | 44.7 ± 1.8 | (3) | $P < 0.005$ |
| | 4 | 19.5 ± 3.0 | (3) | | | | 25.2 | (2) | NS |
| 4 Hr | 1 | 45.0 ± 8 | (3) | 33.0 ± 5 | (4) | $P < 0.05$ | | | |
| (percent of | 2 | 43 | (2) | 44 | (2) | NS | | | |
| control) | 3 | 33 | (2) | | | | 24.0 ± 3 | (3) | $P < 0.05$ |
| | 4 | 51.0 ± 7 | (3) | | | | 44.0 ± 7 | (3) | NS |
| GTP + GDP | | | | | | | | | |
| Control | 1 | 3.7 ± 0.6 | (4) | 4.6 ± 0.3 | (3) | NS | | | |
| | 2 | 4.7 | (2) | 4.6 | (2) | NS | | | |
| | 3 | 6.8 | (2) | | | | 9.7 ± 0.4 | (3) | $P < 0.01$ |
| | 4 | 4.3 ± 0.8 | (3) | | | | 5.0 | (2) | NS |
| 4 Hr | 1 | 46.0 ± 3 | (3) | 39.0 ± 4 | (4) | NS | | | |
| (percent of | 2 | 43 | (2) | 45.0 ± 6 | (2) | NS | | | |
| control) | 3 | 40 | (2) | | | | 17.0 ± 2 | (3) | $P < 0.001$ |
| | 4 | 56.0 ± 10 | (3) | | | | 28.0 ± 5 | (3) | $P < 0.02$ |
| UTP | | | | | | | | | |
| Control | 1 | 2.2 ± 0.3 | (4) | 3.0 ± 0.3 | (3) | $P < 0.05$ | | | |
| | 2 | 2.5 | (2) | 3.8 | (2) | $P < 0.05$ | | | |
| | 3 | 3.9 | (2) | | | | 5.7 ± 0.9 | (3) | NS |
| | 4 | 2.4 ± 0.3 | (3) | | | | 3.4 | (2) | NS |
| 4 Hr | 1 | 36.0 ± 5 | (3) | 24.0 ± 8 | (4) | NS | | | |
| (percent of | 2 | 63 | (2) | 49 | (2) | NS | | | |
| control) | 3 | 48 | (2) | | | | 39.0 ± 5 | (3) | NS |
| | 4 | 93.0 ± 14 | (3) | | | | 88.0 ± 15 | (3) | NS |
| CTP | | | | | | | | | |
| Control | 1 | 0.3 ± 0.1 | (4) | 0.4 ± 0.0 | (3) | NS | | | |
| | 2 | 0.3 | (2) | 0.4 | (2) | $P < 0.05$ | | | |
| | 3 | 0.5 | (2) | | | | 0.7 ± 0.1 | (3) | NS |
| | 4 | 0.3 ± 0.0 | (3) | | | | 0.4 | (2) | $P < 0.01$ |
| 4 Hr | 1 | 31.0 ± 7 | (3) | | | | | | |
| (percent of | 2 | 46 | (2) | 42 | (2) | NS | | | |
| control) | 3 | 36 | (2) | | | | 21.0 ± 4 | (3) | $P < 0.02$ |
| | 4 | 100.0 ± 21 | (3) | | | | 41.0 ± 11 | (3) | $P < 0.02$ |

* Values obtained for the 6MP- and 6TG-resistant cell lines were compared with those obtained for the parent cell line.

† Purine monophosphates and pyrimidine mono- and diphosphates could not be unequivocally identified since nucleotide sugars and nucleoside phosphoglycerides have similar retention times.

‡ Control values are peak areas ($\times 10^{-6}$) ± S.D. obtained by HPLC analysis of neutralized perchloric acid extracts from $5 \pm 10^7$ L1210 cells. In experiments 1 and 2, 1 hr values were used as controls.

§ NS = not significant.

*vivo* exposure to MTX, making the determination of pool sizes difficult. On the basis of their increased sensitivity to MTX, one might expect that nucleotide pool levels in both the L1210/6MP and L1210/6TG cell lines would be greatly reduced compared to the parent cell line. That such was not the case might be due to the complicated regulation of nucleic acid metabolism [8, 40, 41].

In summary, L1210 cells resistant to 6MP and 6TG exhibit increased sensitivity to MTX compared to the parent line. The differential response of parent and purine analog-resistant cell lines to MTX is not due to host influences, for both L1210/6MP and L1210/6TG cell lines are cross-resistant to 6-MeMPR, an inhibitor of *de novo* synthesis, and cultured L1210/6MP cells are more sensitive to MTX than the parent cell line. Following treatment of tumor-bearing mice with MTX, the drug concentration in L1210/6TG cells was about 50% greater than in L1210/0 cells for 24 hr and may account, wholly or in part, for the increased sensitivity of the L1210/6TG cell line to MTX. L1210/6MP cells, however, accumulated less MTX than L1210/0 cells, indicating that an equivalent mechanism is not operative in these cells. DHFR activity in L1210/6TG cells was the same as that in L1210/0 cells, but activity in L1210/6MP cells was lower by 60%. Cultured L1210/6MP cells also exhibited a deficiency in DHFR activity as compared to the parent cell line. The sensitivity of the enzyme to MTX was the same for all three cell lines propagated *in vivo*. Therefore, the increased sensitivity of the L1210/6MP cell line to MTX may be due, in part, to decreased DHFR activity. Significantly lower levels of GTP + GDP and CTP in 6TG-resistant cells than in parent cells 4 hr after the administration of MTX to tumor-bearing mice may be related to the increased MTX sensitivity of these cells. Our results indicate that the observed alterations in drug sensitivity are associated with more than one biochemical change and that these changes are different in the two purine analog-resistant cell lines.

*Acknowledgements*—The authors thank Mr. Max Witt for assistance in the *in vivo* MTX sensitivity studies, Ms. Sue Shaddix for the assay of hypoxanthine-guanine phosphoribosyltransferase activity, Dr. Lee J. Wilkoff for supplying the cultured L1210 cell lines, and Mr. Glenn Duncan for performing the HPLC analyses. This work was supported by Contract NO1-CM-87162, NCI, NIH, DHHS.

*Kettering Meyer Laboratory*
*Southern Research Institute*
*Birmingham, AL35255, U.S.A.*

JANNA D. STROBEL-STEVENS*
SALAH M. EL DAREER
MARY W. TRADER
DONALD L. HILL

REFERENCES

1. F. M. Schabel, Jr., H. E. Skipper, M. W. Trader, W. R. Laster, Jr., T. H. Corbett and D. P. Griswold, Jr., in *Breast Cancer. Experimental and Clinical Aspects* (Eds. H. T. Mouridsen and T. Palshof), p. 199. Pergamon Press, Oxford (1980).
2. R. W. Brockman, *Cancer Res.* **20**, 643 (1960).
3. P. Stutts and R. W. Brockman, *Biochem. Pharmac.* **12**, 97 (1963).
4. R. W. Brockman, in *Antineoplastic and Immunosuppressive Agents* (Eds. A. C. Sartorelli and D. G. Johns), p. 352 Springer, Berlin (1974).
5. D. G. Johns and J. S. Bertino, in *Cancer Medicine* (Eds. J. F. Holland and E. Frei, III), p. 739. Lea & Febiger, Philadelphia (1973).
6. R. G. Moran, W. C. Werkheiser and S. F. Zakrzewski, *J. biol. Chem.* **251**, 3569 (1976).
7. W. M. Hryniuk, L. W. Brox, J. F. Henderson and T. Tamaoki, *Cancer Res.* **35**, 1427 (1975).
8. J. J. Kinahan, M. Otten and G. B. Grindey, *Cancer Res.* **39**, 3531 (1979).
9. G. P. Browman and G. Csullog, *Biochem. Pharmac.* **30**, 869 (1981).
10. L. J. Wilkoff and E. A. Dulmadge, *Proc. Soc. exp. Biol. Med.* **170**, 78 (1982).
11. L. W. Law, V. Taormina and P. J. Boyle, *Ann. N.Y. Aca. Sci.* **60**, 244 (1954).
12. H. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, *Cancer Chemother. Rep.* **35**, 1 (1964).
13. F. M. Schabel, Jr., D. P. Griswold, Jr., W. R. Laster, Jr., T. H. Corbett and H. H. Lloyd, *Pharmac. Ther.* (A) **1**, 411 (1977).
14. W. Boyle, *Transplantation* **6**, 761 (1968).
15. E. M. Scholar, P. R. Brown and R. E. Parks, Jr., *Cancer Res.* **32**, 259 (1972).
16. L. M. Rose and R. W. Brockman, *J. Chromat.* **133**, 335 (1977).
17. J. P. Perkins, B. L. Hillcoat and J. R. Bertino, *J. biol. Chem.* **242**, 4771 (1967).
18. J. R. Bertino, J. P. Perkins and D. G. Johns, *Biochemistry* **4**, 839 (1965).
19. B. L. Hillcoat, P. F. Nixon and R. L. Blakley, *Analyt. Biochem.* **21**, 178 (1967).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. L. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. G. A. Fischer, *Biochem. Pharmac.* **11**, 1233 (1962).
22. D. Kessel, T. C. Hall, D. Roberts and I. Wodinsky, *Science* **150**, 752 (1965).
23. B. M. Braganca, A. Y. Divekar and N. R. Vaidya, *Biochim. biophys. Acta* **135**, 937 (1967).
24. D. Kessel, T. C. Hall and D. Roberts, *Cancer Res.* **28**, 564 (1968).
25. K. R. Harrap, B. T. Hill, M. E. Furness and L. I. Hart, *Ann. N.Y. Acad. Sci.* **186**, 317 (1971).
26. F. M. Sirotnak and R. C. Donsbach, *Cancer Res.* **36**, 1151 (1976).
27. E. S. Henderson, R. H. Adamson, C. Denham and V. T. Oliverio, *Cancer Res.* **25**, 1008 (1965).
28. R. G. Poser, F. M. Sirotnak and P. L. Chello, *Biochem. Pharmac.* **29**, 2701 (1980).
29. G. A. Fischer, *Biochem. Pharmac.* **7**, 75 (1961).
30. M. T. Hakala, S. F. Zakryewski and C. A. Nichol, *J. biol. Chem.* **236**, 952 (1961).
31. M. E. Friedken, E. Crawford, S. R. Humphreys and A. Goldin, *Cancer Res.* **22**, 600 (1962).
32. W. F. Flintoff and K. Essani, *Biochemistry* **19**, 4321 (1980).
33. P. W. Melera, J. A. Lewis, J. L. Biedler and C. Hession, *J. biol. Chem.* **255**, 7024 (1980).
34. D. Roberts, I. Wodinsky and T. C. Hall, *Cancer Res.* **25**, 1899 (1965).
35. T. C. Hall, D. Roberts and D. H. Kessel, *Cancer, N.Y.* **2**, 135 (1966).
36. D. Roberts and I. Wodinsky, *Cancer Res.* **28**, 1955 (1968).
37. M. H. N. Tattersall, R. C. Jackson, S. T. M. Jackson and K. R. Harrap, *Eur. J. Cancer* **10**, 819 (1974).
38. A. Fridland, *Cancer Res.* **34**, 1883 (1974).
39. L. Skoog, B. Nordenskjold, S. Humla and T. Hagerstrom, *Eur. J. Cancer* **12**, 839 (1976).
40. W. C. Werkheiser, G. B. Grindey, R. G. Moran and C. A. Nichol, *Molec. Pharmac.* **9**, 320 (1973).
41. R. C. Jackson and K. R. Harrap, *Pharmac. Ther.* **4**, 245 (1979).

* Author to whom correspondence should be addressed.