The similarity of the structures and the cross-resistance to 6-thioguanine (6-TG) of some cell lines resistant to 6-mercaptopurine (6-MP) have led some investigators to assume that 6-TG and 6-MP have similar or identical metabolic effects. This cross-resistance can probably be explained on the basis of the resistance mechanism, which has been so thoroughly investigated by Brockman and his associates (2). Since it now appears that both 6-MP and 6-TG are active in the nucleotide form and that both reach this form by way of the guanine pyrophosphorylase, loss of the latter enzyme results in resistance to both agents. However, a 6-TG-resistant line of Ehrlich carcinoma cells was found to be sensitive to 6-MP (6), and a sensitive line of Ehrlich cells responded better to treatment with combinations of 6-MP and 6-TG than to any dose of either drug alone (4).

As will be reported, our investigations on 6-TG all support the concept that the incorporation of 6-TG into deoxyribonucleic acid (DNA) is closely linked to the toxicity produced in normal or neoplastic cells. Such was not the case when 6-MP was studied by Bieber et al. (1); incorporation of 6-MP into DNA was greater in a 6-MP-resistant line.

The data presented in Table 1 illustrate the relationship between incorporation to 6-TG into nucleic acids and response to therapy with the drug in a series of ascites cell mouse tumors and in growing tissues of the mouse. Therapy was routinely by intraperitoneal injection twice daily for 6 days at 0.5 mg/kg. Incorporation was measured 2 hours after injection with a single dose of 10 mg/kg. Further support for the concept of a direct relationship between the incorporation of 6-TG into cellular DNA and toxicity to the cells has been reported by LePage and Jones (7). It was established that: (a) susceptible tumors showed maximum response to treatment initiated at any time during the rapid-growth phase; (b) incorporation of 6-TG into tumor-cell nucleic acids was much greater in young, rapidly growing cell populations than in older populations; (c) maximum incorporation of 6-TG into the tumor-cell nucleic acids of susceptible tumors was reached with three treatments; multiple treatments of a resistant tumor did not increase the incorporation; (d) three treatments with 6-TG were sufficient to produce an essentially maximum tumor inhibition; (e) in susceptible tumors, a large part of the incorporation was in DNA; in two resistant tumors, the small incorporation observed was largely in RNA; (f) in tumor cells labeled with thioguanine-C¹⁴ and transferred to unlabeled hosts the radioactivity in DNA was completely retained over the

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**Basic Biochemical Effects and Mechanism of Action of 6-Thioguanine**

G. A. LePage

*(Life Sciences Research, Stanford Research Institute, Menlo Park, California)*

**SUMMARY**

The correlation between the carcinostasis produced by thioguanine (TG) and incorporation of the drug into cellular DNA reported earlier for ascites tumors in mice was extended to studies in early transplant generations of C3H mammary tumors and to spontaneous mammary tumors in C3H females. The incorporation appeared to be "geared" to DNA synthesis. Use was made of this finding to reduce toxicity to the host bone marrow and enhance tumor response by suitable spacing of treatments. The transplanted and spontaneous tumors were equally responsive. Complete regressions were noted in some of the latter.

Of four TG-resistant lines of mouse tumor cells, one achieved resistance because of loss of the activating (nucleotide-forming) enzyme. The other three lines do not lack this enzyme, and must achieve resistance through some other (as yet unexplained) mechanism, but respond to therapy with azaserine and TG.
time studied (in excess of twice the intermitotic time); retention was also almost complete in RNA. Such cells appear to remain viable but be unable to grow.

Extensions of these studies were based on the operating premise that incorporation of 6-TG into DNA was the biochemical event producing toxicity to cells. Attention was then directed to use of this premise to: (a) accomplish maximum effects on experimental tumors; (b) minimize toxicity to the bone marrow; (c) circumvent the resistance mechanism by which tumor cells avoid this damaging biochemical event. We have found it convenient to continue the use of ascites tumors for the development and study of resistance, but have progressed to the use of established solid tumor implants in compatible mice and spontaneous tumors of C3H mice.

Spontaneous mammary tumors in C3H mice of the Bittner strain were reported to be unresponsive to chemotherapy with antimetabolites (10, 11), whereas transplants of these tumors did show some response. From the Roscoe B. Jackson Laboratory we obtained C3H females of the Heston strain bearing spontaneous mammary tumors. Several of these tumors were transplanted in C3H females and studied in early (two to four) transplant generations. One experiment is described in Table 2. The second transplant generation of such a mammary tumor was into a large group of female C3H mice 4 months of age. When the tumors had become established (100 per cent “takes”), groups of six mice were selected, which had the same average tumor size as determined by caliper measurements. Thioguanine was given 3 times at either 12-hour or 24-hour intervals. Both groups were inhibited by the treatment, but inhibition of growth and incorporation of 6-TG into nucleic

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Survival*</th>
<th>DNA†</th>
<th>RNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180</td>
<td>160</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Ehrlich</td>
<td>205</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Ca-755</td>
<td>195</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td>L1210</td>
<td>200</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>TAS</td>
<td>185</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>6C3HED</td>
<td>97</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>Mecca lymphoarcoma</td>
<td>95</td>
<td>0.03</td>
<td>0.27</td>
</tr>
<tr>
<td>Ehrlich TGR I</td>
<td>90</td>
<td>0.41</td>
<td>0.55</td>
</tr>
<tr>
<td>Ehrlich TGR II</td>
<td>98</td>
<td>0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>S-180 TGR</td>
<td>97</td>
<td>0.23</td>
<td>0.47</td>
</tr>
<tr>
<td>Ca-755 TGR</td>
<td>88</td>
<td>0.17</td>
<td>0.41</td>
</tr>
<tr>
<td>L1210 TGR</td>
<td>99</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Toxic</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Intestinal mucosa</td>
<td>Nontoxic</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Per cent of control when treated twice daily for 6 days at 0.5 mg/kg.
† Micrograms of 6-TG per milliliter of cells.

<table>
<thead>
<tr>
<th>Incorporation of Thioguanine-8-C14 into Nucleic Acids and Inhibition of Growth in Transplanted Mammary Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Controls, sacrificed initially</td>
</tr>
<tr>
<td>Controls, sacrificed after 5 days</td>
</tr>
<tr>
<td>Tumors treated 3X at 12-hr. intervals</td>
</tr>
<tr>
<td>Tumors treated 3X at 24-hr. intervals</td>
</tr>
</tbody>
</table>

Groups were matched initially on the basis of tumor size, as measured in two dimensions with calipers. Thioguanine treatments were at 8.0 mg/kg. Those receiving thioguanine-8-C14 were sacrificed 2 hours after the final dose. There were six mice per group. The average tumor weight (or thioguanine content) is given, with the range in parentheses. Six groups of mice were used—i.e., the groups for which thioguanine incorporations are given were necessarily not the same groups as were used to obtain tumor weights after 5 days.
acids was greater with therapy given at 24-hour intervals. This is compatible with the much longer intermitotic time observed for these tumors, as compared with the rapidly growing ascites tumors. A similar experiment was conducted with C3H females bearing spontaneous mammary tumors. The results, presented in Table 3, are essentially the same as obtained with transplanted mammary tumors, in that both response to 6-TG therapy and incorporation of 6-TG into nucleic acids were obtained, with the better results obtained when dosages of 6-TG can be saved by the injection of suspensions of isologous bone marrow cells. In general, the more doses of 6-TG given, the lower is the total dose required to produce lethal effects. The tests here were conducted with healthy, female C3H mice 3–6 months of age. As indicated in Table 4, 6-TG given at 24-hour intervals was much less toxic than when given at 12-hour intervals. The result is that a considerable increase in the “therapeutic index” is possible if the dosage

### Table 3

INCORPORATION OF THIOGUANINE-8-C¹⁴ INTO NUCLEIC ACIDS AND INHIBITION OF GROWTH IN Spontaneous MAMMARY TUMORS OF C3H MICE

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor weights (mg.)</th>
<th>Thioguanine-C¹⁴ in nucleic acids (µg/gm cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, sacrificed initially</td>
<td>860 (830–970)</td>
<td>1.52 (0.93–1.92)</td>
</tr>
<tr>
<td>Controls, sacrificed after 7 days</td>
<td>1640 (1090–1820)</td>
<td>2.24 (1.63–2.92)</td>
</tr>
<tr>
<td>Treated 5X at 12-hr. intervals</td>
<td>1132 (940–1370)</td>
<td></td>
</tr>
<tr>
<td>Treated 5X at 24-hr. intervals</td>
<td>870 (642–996)</td>
<td></td>
</tr>
</tbody>
</table>

Groups were matched initially on the basis of tumor size, as measured in two dimensions with calipers. Thioguanine treatments were at 8.0 mg/kg. Those receiving thioguanine-8-C¹⁴ were sacrificed 2 hours after the final dose. There were eight mice per group. The average tumor weight (or thioguanine content) is given, with the range in parentheses.

### Table 4

TOXICITY TESTS WITH 6-THIOGUANINE IN C3H MICE

<table>
<thead>
<tr>
<th>No. mice</th>
<th>Dose (mg/kg)</th>
<th>Interval (hr.)</th>
<th>No. doses</th>
<th>Total dose (mg/kg)</th>
<th>No. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>4</td>
<td>12</td>
<td>10</td>
<td>40</td>
<td>15</td>
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<tr>
<td>15</td>
<td>8</td>
<td>24</td>
<td>5</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>24</td>
<td>5</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>24</td>
<td>5</td>
<td>80</td>
<td>2</td>
</tr>
</tbody>
</table>

Tests were conducted with C3H females, 3–6 months of age. Thioguanine was given intraperitoneally in isotonic saline at pH 8 in 0.25–0.75 ml. Mice were fed ad libitum and supplied with water containing 50 mg streptomycin/liter. The streptomycin was shown to have no influence on tumor growth and aided in avoiding deaths due to infections.
schedule used is adjusted to permit some bone marrow regeneration. Further, it was found that sublethal doses of 6-TG could be given repeatedly when a sufficient interval was allowed (7—9 days) to permit the mice to regain body weight. An experiment in which C3H mice bearing spontaneous mammary tumors were given two courses of treatments with 6-TG plus azaserine is presented in Table 5. Some complete regressions (eight of seventy-three) were obtained, and the treated tumors showed a good initial response. Although more treatments could be tolerated, the therapy was not continued beyond two courses in this experiment. A more comprehensive report of this study on host toxicity and the response of mammary tumors to 6-TG will appear (5).

If we are to have any appreciable success in the use of chemotherapy for the treatment of cancer, it is evident that we must find the mechanisms by which cancer cells achieve resistance to our drugs and find means for circumvention of resistance. This can most probably be accomplished through the use of drug combinations.

Brockman and his associates have clearly documented one mechanism by which cells achieve resistance to purine antimetabolites, including 6-TG. Sartorelli et al. (9) described an example of another, where ability to degrade the drug was involved. A solution to the latter mode of resistance was achieved with a combination of azaserine and 6-TG, since this combination produced a high percentage of complete remissions. We developed resistant sublines of ascites tumor lines (S180 TGR, Ehrlich TGR, CA 755 TGR, and L1210 TGR) by serial passage in mice treated with 6-TG at 0.6 mg/kg. Cell-free extracts of these resistant lines and their 6-TG-sensitive counterparts were assayed for capacity to form thioguananic acid (6-TGMP). Chart 1 shows the results of these assays. Loss of the phosphoribosylpyrophosphorylase was evidently involved in only one of the four, the L1210 TGR line. A more detailed report of the investigation of these resistant lines will appear (5).

We do not yet know the means by which the S-180, Ca-755, and Ehrlich lines achieve resistance to 6-TG. However, it is not by either ability to degrade the drug or loss of phosphoribosylpyrophosphorylase. However all three of these

![Chart 1](image)

**Chart 1.**—Enzymatic synthesis of thioguaninic acid by cell-free extracts from 6-TG sensitive and resistant ascites tumor cells.

### TABLE 5

<table>
<thead>
<tr>
<th>Day*</th>
<th>Controls</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor wt. (mg.)</td>
<td>Host wt. (gm.)</td>
</tr>
<tr>
<td>1</td>
<td>890</td>
<td>32.2</td>
</tr>
<tr>
<td>3</td>
<td>1210</td>
<td>32.5</td>
</tr>
<tr>
<td>5</td>
<td>1430</td>
<td>32.0</td>
</tr>
<tr>
<td>7</td>
<td>1630</td>
<td>32.7</td>
</tr>
<tr>
<td>9</td>
<td>1925</td>
<td>32.9</td>
</tr>
<tr>
<td>11</td>
<td>2210</td>
<td>32.6</td>
</tr>
<tr>
<td>13</td>
<td>2850</td>
<td>31.8</td>
</tr>
<tr>
<td>15</td>
<td>3100</td>
<td>31.5</td>
</tr>
<tr>
<td>17</td>
<td>3480</td>
<td>31.9</td>
</tr>
<tr>
<td>19</td>
<td>4300</td>
<td>31.6</td>
</tr>
</tbody>
</table>

* Day 1 was the first day of treatment. Treatments were with azaserine (1.0 mg/kg) and thioguanine (8.0 mg/kg) on days 1—5 and 10—14.

Tumor weights are given as the average weight of tumor per mouse, computed from 2-dimensional caliper measurements. A few of the mice had more than one tumor. Host weights are the averages, including tumors. There were 75 mice in each group. Two mice of each group died during the above period of observation, apparently as a result of tumor ulceration and infection. Eight mice in the treated group appeared to be tumor-free at 50 days, when the experiment was terminated. By this time, 69 control mice and fourteen treated mice had died.
lines respond to a combined treatment with aza-
serine plus 6-TG.

A second line of 6-TG-resistant Ehrlich cells
was derived by passage of Ehrlich cells in mice
 treated with 10 mg/kg doses of 6-TG, on the basis
that loss of phosphoribosylpyrophosphorylase con-
fers high resistance and would be favored by such
a high selection pressure. The resistant line which
emerged (Ehrlich TGR-II) was indeed found to
lack phosphoribosylpyrophosphorylase and to form
negligible amounts of 6-TGMP. It does not re-

In an effort to find a way to get TGMF forma-
tion in the tumor lines lacking phosphoribosylpy-
rophosphorylase (L1210 TGR, Ehrlich TGR II)
we tried the riboside, thioguanosine, with the hope
that the kinase necessary for conversion of this to
6-TGMP might be present. This effort was frus-
trated by the presence of a high level of cleavage
activity in these cells, by which thioguanosine
was rapidly cleaved to free base. We have at-
ttempted to find inhibitors of this cleavage reaction
and found that 9-methyl thioguanine had some in-
fluence. In L1210 TGR and Ehrlich TGR II the
minute amount of 6-TGMP formation obtained
with 6-TG was increased about two-fold by the use
of thioguanosine, and the 6-TGMP formation
with 2-methyl thioguanine and thioguanosine in
combination was increased about fifteen-fold and
five-fold, respectively. This led to relatively good
incorporation of 6-TG into RNA but had no in-
fluence on the negligible incorporation in DNA.
Survival experiments showed that this combined
treatment had little influence. This served to
emphasize the importance of achieving incorpora-
tion in DNA and perhaps indicates another type of
resistance arising out of poor capacity to convert
ribotides to deoxyribotides. We hope to circum-
vent this situation by the use of 2'-deoxythioguanos-
ine and a suitable inhibitor of cleavage.

Note added in proof: Experiments recently car-
ried out with the thioguanine-sensitive and -resis-
tant lines of Ca-755 have shown that the Km for
TG in the phosphoribosylpyrophosphorylase reac-
tion is the same with enzyme preparations from
the two lines. This eliminates one explanation sug-
gested for the resistance of the latter to TG.

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