Effects of Methylglyoxal-bis(guanylhydrazone)\(^1\) on Polyamine Metabolism in Spleens of Mice with Disseminated L1210 Lymphoid Leukemia\(^2\)

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SUMMARY

Polyamine metabolism was studied in spleens of L1210 leukemic mice after i.p. injection of methylglyoxal-bis(guanylhydrazone). When added directly to the enzyme preparations, methylglyoxal-bis(guanylhydrazone) strongly inhibited the S-adenosyl-L-methionine decarboxylase activity (spermidine and spermine formation) without affecting the L-ornithine decarboxylase activity (putrescine formation). Accordingly, daily i.p. injections of methylglyoxal-bis(guanylhydrazone) resulted in a marked increase in the concentration of putrescine and a decrease in the concentration of spermidine and spermine. However, the increase in putrescine concentration following the drug treatment was not due to inhibition of spermidine and spermine synthesis only, but also to a marked increase in L-ornithine decarboxylase activity. Paradoxically, the activity of S-adenosyl-L-methionine decarboxylase was markedly increased as well, when assayed 24 hr after the injection of methylglyoxal-bis(guanylhydrazone). However, when assayed at earlier times, it was found that the S-adenosyl-L-methionine decarboxylase activity was markedly depressed for at least 8 to 12 hr after drug treatment but returned to or exceeded control levels within 24 hr. This early inhibition of enzyme activity probably accounts for the decrease in spermidine and spermine concentrations after the administration of methylglyoxal-bis(guanylhydrazone).

INTRODUCTION

Several years ago Mihich (15) observed that the anti-proliferative effects of methylglyoxal-bis(guanylhydrazone) against L1210 leukemia were prevented by the concurrent administration of spermidine. A study by Williams-Ashman and Schenone (26) indicated that these results might be due to inhibition of spermidine and spermine formation, since methylglyoxal-bis(guanylhydrazone) was found to be a very potent inhibitor of putrescine-stimulated S-adenosyl-L-methionine decarboxylase in extracts of mammalian tissues. S-Adenosyl-L-methionine decarboxylase catalyzes a key step in the formation of spermidine as well as spermine.

When studying the concentrations of the polyamines in spleens of leukemic mice as affected by treatment with various antineoplastic agents, we observed that all the drugs tested, with the exception of methylglyoxal-bis(guanylhydrazone), caused decreases in the concentrations of putrescine, spermidine, and spermine (8). After 5 days of treatment with methylglyoxal-bis(guanylhydrazone), putrescine concentration was almost 3 times higher and spermidine and spermine concentrations were significantly lower in spleens of treated compared to untreated leukemic mice (8). Accordingly, Fillingame and Morris (I) found that the addition of methylglyoxal-bis(guanylhydrazone) to transforming (concanavalin A-stimulated) lymphocyte cultures caused an almost complete cessation of the accumulation of both spermidine and spermine, whereas the accumulation of putrescine continued, at an even higher rate. In spite of the fact that spermidine and spermine formation was stopped, the activity of S-adenosyl-L-methionine decarboxylase in dialyzed extracts of the lymphocytes was found to be elevated after drug treatment. The activity of L-ornithine decarboxylase (EC 4.1.1.17) in the dialyzed extracts was markedly elevated as well.

In an attempt to determine the cause of the increase in putrescine concentration after treatment with methylglyoxal-bis(guanylhydrazone), we studied the activities of the polyamine-biosynthetic enzymes in extracts of spleens, heavily infiltrated with leukemic cells, at various times after a single injection of the drug (10). The activity of L-ornithine decarboxylase increased markedly and remained elevated for 24 hr. The activities of putrescine-stimulated and spermidine-stimulated S-adenosyl-L-methionine decarboxylase were significantly depressed during the 1st 4 to 6 hr, but subsequently the enzyme activities increased markedly and were still elevated at 24 hr after treatment.

The present investigation was made to study in detail the effects of daily i.p. injections of methylglyoxal-bis(guanylhydrazone) on the activities of the polyamine-biosynthetic enzymes and on the concentrations of the polyamines at various stages of treatment, a treatment known markedly

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\(^{1}\) Guanidine, 1,1\(-\{(\text{methylene}t\text{hane}d\text{i}l\text{yd}\text{ene})\text{dinitri}l\text{oni}tdi}, \text{dihydrochl}\text{o}r\text{i}d\text{e}, \text{monohydrat}e; \text{N}_{2}\text{C}_{4}\text{H}_{17}\text{O}_{2}; \text{HCl} \cdot \text{H}_{2}\text{O}; \text{NSC} 32946.

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to increase the survival time of L1210 leukemic mice (3, 6, 8, 11, 13, 15, 25). Particular interest was given to the study of the endogenous levels of the polyamines, since in vitro assay conditions of the enzymes in the polyamine-biosynthetic pathway may obscure in vivo effects of the drug. The in vitro assay conditions represent a 5-fold dilution of the tissues; and since methylglyoxal-bis(guanlyhydrzone) has a direct effect on S-adenosyl-L-methionine decarboxylase, the dilution of remaining drug may cause an overestimation of the activity of this enzyme.

**MATERIALS AND METHODS**

**Experimental Animals.** DBA/2 and C57BL/6 × DBA/2 F₁ (hereafter called BD2F₁) male mice, 18 to 20 g, were obtained from Microbiological Associates, Inc., Bethesda, Md. The mice were given standard laboratory chow and tap water ad libitum.

**Tumor Inoculation.** Lymphoid leukemia L1210 (line LE-29, generation R-08, obtained from Microbiological Associates) was carried in DBA/2 mice by weekly i.p. passages as previously described (7). For this study, mice of the BD2F₁ strain were used. L1210 ascites cells for inoculation were obtained from DBA/2 donor mice on the 7th day of tumor growth, and 1 x 10⁶ cells in 0.1 ml were inoculated i.p. into each recipient BD2F₁ mouse. All mice were inoculated on the same day with aliquots derived from the same tumor source and then divided into 2 groups, 1 of which was subjected to drug treatment.

**Drug Regimen.** A daily i.p. injection (Days 1 through 7) of 0.1 ml methylglyoxal-bis(guanlyhydrzone) (50 µg/g of body weight) was given to leukemic BD2F₁ mice, inoculated on Day 0 with tumor cells as described above, and to nonleukemic BD2F₁ mice. Methylglyoxal-bis(guanlyhydrzone) was dissolved in 0.85% NaCl solution immediately prior to use. Leukemic and nonleukemic mice of the same strain, sex, age, and weight were subjected to i.p. injections of 0.1 ml of 0.85% NaCl solution on Days 1 through 7 to serve as controls.

In a single experiment a single i.p. injection of the drug (50 µg/g) was given to leukemic mice on the 6th day of tumor growth.

**Autopsy.** The mice were killed by cervical dislocation in groups of 10, leukemic mice at 2, 4, 6, and 8 days after tumor inoculation and nonleukemic mice on Days 0, 2, 4, 6, and 8. Five mice were used for enzyme assays, and 5 mice were used for determination of endogenous pools of polyamines.

In studying the effects of a single i.p. injection of methylglyoxal-bis(guanlyhydrzone) on the enzymes in the polyamine-biosynthetic pathway, groups of 5 leukemic mice were killed 1, 2, 4, 8, 12, and 24 hr after treatment.

**Preparation of Spleen Extracts for Enzymatic Assays.** The spleens were rapidly excised, chilled on ice, rinsed in 0.85% NaCl solution, blotted, weighed, and homogenized with a Duall tissue grinder (Kontes Glass Co., Vineland, N. J.) at 0–2°C in 5 volumes of 0.05 M sodium:potassium phosphate buffer, pH 7.2, containing 1.0 mm dithiothreitol. The supernatant fraction obtained after 20 min centrifugation at 20,000 × g at 2°C was used for the estimation of enzyme activities.

**Enzyme Assays.** L-Ornithine decarboxylase activity was determined by measuring the release of ¹⁴CO₂ from DL-ornithine-¹⁴C as previously described (7, 22). Reaction mixtures consisted of 0.1 to 0.4 ml of spleen extract; 20 µM pyridoxal-5-phosphate; 5 µCi of DL-ornithine-¹⁴C monohydrochloride (specific activity, 12.8 mCi/m mole); and 1.45 to 1.75 ml of 0.05 M sodium:potassium phosphate buffer, pH 7.2, containing 1.0 mm dithiothreitol, to make a total volume of 2.00 ml.

Putrescine-stimulated and spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity was determined by measuring the release of ¹⁴CO₂ from S-adenosyl-L-methionine-carboxyl-¹⁴C (7, 18) in the presence of putrescine and spermidine, respectively. Reaction mixtures consisted of 50 to 100 µl of spleen extract; 20 µM pyridoxal-5-phosphate; 2.5 mm putrescine dihydrochloride or 5.0 mm spermidine trihydrochloride; 0.2 µCi of S-adenosyl-L-methionine-carboxyl-¹⁴C (specific activity, 7.3 mCi/m mole); and 70 to 120 µl of 0.05 M sodium:potassium phosphate buffer, pH 7.2, containing 1.0 mm dithiothreitol, to make a final volume of 200 µl. Complete absorption of the ¹⁴CO₂ was achieved with 0.2 ml of a 2:1 (v/v) mixture of ethanolamine and 2-methoxyethanol contained in a polypropylene center well. After the reaction had been stopped and all ¹⁴CO₂ had been absorbed, the center well was placed in a scintillation vial containing 2 ml of ethanol and 10 ml of 0.4% Omnifluor in scintillation grade toluene.

The release of ¹⁴CO₂, i.e., enzyme activity, was linear for the duration of the incubation period (30 min at 37°C) for the enzymes assayed and proportional to the amount of spleen extract added.

Radioactivity was assayed with a Beckman LS-150 liquid scintillation system, at an efficiency of approximately 90%. All values were corrected against a “boiled enzyme” assay.

**Preparation of Spleen Extracts for Polyamine Determinations.** The spleens were rapidly excised, chilled on ice, rinsed in 0.85% NaCl solution, blotted, weighed, and homogenized with a Duall tissue grinder at 0–2°C in 20 volumes of ice-cold 0.2 M perchloric acid. The homogenates were stored at 0°C for 1 hr and were then centrifuged for 10 min at approximately 1000 × g at 2°C. The supernatant fractions obtained were used for the determination of endogenous concentrations of putrescine, spermidine, and spermine.

**Quantitative Polyamine Analysis.** The spleen extracts were dansylated according to the method of Seiler and Wiechmann (23, 24). The details of the method as applied have been described elsewhere by Heby et al. (9). Briefly, 200 µl of the spleen extracts were dansylated by the addition of 400 µl 1-dimethylamino-naphthalene-5-sulfonyl chloride (30 mg/ml acetone) and 100 µl of a saturated Na₂CO₃ solution. After 16 hr in the dark at room temperature, allowing quantitative dansylation of the polyamines, 100 µl proline (150 mg/ml) were added to eliminate the excess of the reagent by conversion to its proline derivative. The 1-dimethylamino-naphthalene-5-sulfonamides were extracted into 500 µl of benzene. The benzene layer was
evaporated to dryness, and the residue was dissolved in 50 µl benzene. Aliquots (5 to 20 µl) of the benzene extracts were applied to heat-activated thin-layer chromatography plates, and the sulfonamides were separated by unidimensional development in ethyl acetate:cyclohexane (2:3, v/v). Tissue samples and standard samples were chromatographed on the same plate. Fluorescence intensities of the dansylated polyamines were measured with an Aminco-Bowman spectrophotofluorometer (American Instruments Co., Inc., Silver Spring, Md.) equipped with an Aminco thin-layer chromatography scanner and recorder (excitation wavelength, 365 nm; emission wavelength, 500 nm).

Chemicals. Methylglyoxal-bis(guanylylhydrazone) dihydrochloride monohydrate was obtained from Microbiological Associates, through the courtesy of Dr. Harry B. Wood, Jr., National Cancer Institute, Bethesda, Md. DL-Onnitol was purchased from Calbiochem, San Diego, Calif. The polyamines were recrystallized 3 times before use. L-Dimethylamino-naphthalene-5-sulfonic acid (specific activity, 12.8 mCi/mmol), S-adenosyl-L-methionine-carboxyl-14C (specific activity, 7.3 mCi/mmol), and Omnifluor [PPO: PO-bis(o-methylstyryl)benzene, 98:2] were purchased from New England Nuclear,Boston, Mass. Pyridoxal 5-phosphate, dithiothreitol (Cleland's reagent), t-proline, putrescine dihydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride were purchased from Calbiochem, San Diego, Calif. The polyamines were recrystallized 3 times before use. 1-Dimethylamino-naphthalene-5-sulfonic acid (10% solution in acetone) was obtained from Pierce Chemical Co., Rockford, Ill. Baker Intra-Analyzed GC-spectrophotometric quality solvents (Baker Chemical Co., Phillipsburgh, N. J.) were used. All other chemicals were of reagent grade.

RESULTS

Survival Time. The mean survival time of BD2F1 mice given i.p. inoculations of 1 x 10^6 L1210 ascites cells was 8.2 days. Leukemic mice treated daily with i.p. injections of methylglyoxal-bis(guanylylhydrazone) (50 µg/g of body weight) on Days 1 through 7 had a mean survival time of 14.9 days.

Spleen Weight. A 2-fold increase in the wet spleen weight occurred between the time of tumor inoculation and leukemic death (Table 1). When methylglyoxal-bis(guanylylhydrazone) was administered daily from Days 1 through 7 to leukemic mice, the rate of increase of the spleen weight changed significantly. The spleen weight of the drug-treated leukemic mice was 23% lower than that of control leukemic mice by Day 8. In a group of mice that received the drug regimen but were not inoculated with the tumor, the spleen weight was slightly decreased during early treatment.

L-Ornithine Decarboxylase Activity. The L-ornithine decarboxylase activity in spleens of leukemic mice was significantly increased 2 days after tumor inoculation and was maximal on Day 4 of tumor growth (Table 1). Peak activity was approximately 4 times higher than the activity in spleens of nonleukemic mice. When methylglyoxal-bis(guanylylhydrazone) was injected daily from Days 1 through 7 into leukemic mice, the enzyme activity was found to be markedly elevated at all times assayed. It was 2 times higher in spleens of drug-treated leukemic mice than in leukemic control mice 6 to 8 days after tumor inoculation. When the drug was injected into nonleukemic mice, following the same drug regimen as for leukemic mice, only an initial increase, lasting from Days 2 through 4, was observed. During this time maximal enzyme activity was 2.2 times higher than in nonleukemic control mice. When methylglyoxal-bis(guanylylhydrazone) was added directly to the reaction mixture, it did not significantly affect the enzyme activity, even at a 2 mM concentration. After a single i.p. injection of methylglyoxal-bis(guanylylhydrazone) (50 µg/g), the activity of 6-day leukemic spleen L-ornithine decarboxylase measured in vitro increased to a value more than 2 times higher than that of control 6-day leukemic spleen extracts (Table 3). It remained at this level for at least 12 hr.

Putrescine-stimulated S-Adenosyl-L-methionine Decarboxylase Activity. The putrescine-stimulated S-adenosyl-L-methionine decarboxylase activity in spleens of leukemic mice was increased 2 days after tumor inoculation, and from Day 4 until leukemic death it was fairly constant at a level more than 2-fold above normal (Table 1). When methylglyoxal-bis(guanylylhydrazone) was administered daily from Days 1 through 7 to leukemic mice, the enzyme activity increased markedly. It was 2- to 3-fold higher than in spleens of leukemic control mice 2 to 8 days after tumor inoculation. When nonleukemic mice received the drug regimen, the enzyme activity was 3 to 5 times higher than in nonleukemic control mice. Methylglyoxal-bis(guanylylhydrazone) added directly to the reaction mixture strongly inhibited the enzyme activity in the spleen extract, even at a low concentration (5 µM) (Table 2). After a single i.p. injection of methylglyoxal-bis(guanylylhydrazone) (50 µg/g), the activity of 6-day leukemic spleen putrescine-stimulated S-adenosyl-L-methionine decarboxylase measured in vitro was less than 10% of that of control 6-day leukemic spleen extracts (Table 3). It increased gradually after a 4-hr depression, reaching 130% of the control value 24 hr after injection of the inhibitor.

Spermidine-stimulated S-Adenosyl-L-methionine Decarboxylase Activity. The spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity in spleens of leukemic and nonleukemic mice, drug-treated as well as 0.85% NaCl solution-treated, exhibited patterns of changes rather similar to those observed for the putrescine-stimulated S-adenosyl-L-methionine decarboxylase activity (Table 1). In the control groups (leukemic and nonleukemic mice subject to i.p. injections of 0.85% NaCl), the activity of the putrescine-stimulated S-adenosyl-L-methionine decarboxylase was twice that of the spermidine-stimulated enzyme at all times (Table 1). However, in leukemic and nonleukemic mice treated with methylglyoxal-bis(guanylylhydrazone), putrescine did not stimulate the activity of S-adenosyl-L-methionine decarboxylase to a greater extent than did spermidine; i.e., the activity of the spermidine-stimulated enzyme was as high as that of the putrescine-stimulated enzyme. Methylglyoxal-bis(guanylylhydrazone), when added directly to the reaction mixture, inhibited the activity of the
Drug Effects on Polyamine Metabolism

Table 1
Changes in mouse spleen after i.p. inoculation of 10^6 L1210 leukemia cells and treatment with methylglyoxal-bis(guanylhydrazone)

The spleen weights represent mean wet weights of 10 spleens. The spleens were homogenized in 5 volumes of 0.05 M sodium:potassium phosphate buffer, pH 7.2, containing 1.0 mM dithiothreitol. The 20,000 x g (20-mm) supernatant fraction was assayed for enzyme activities. L-Ornithine decarboxylase activity was determined by measuring the release of ^14CO_2 from DL-ornithine-l-^14C. Putrescine-stimulated and spermidine-stimulated S-adenosyl-L-methionine decarboxylase activity was determined by measuring the release of ^14CO_2 from S-adenosyl-l-methionine-carboxyl-l-^14C in the presence of putrescine and spermidine, respectively. The release of ^14CO_2, i.e., enzyme activity, was linear for the duration of the incubation period (30 min at 37°C) for the enzymes assayed and was proportional to the amount of spleen extract added. Each value represents the mean of 3 assays (variation < 10%) for each enzyme in an extract of 5 spleens. The entire experiment was repeated once, and the same general pattern of changes was observed. One representative experiment is presented.

<table>
<thead>
<tr>
<th>Days after tumor inoculation</th>
<th>Spleen weight (mg)</th>
<th>L-Ornithine decarboxylase activity (nmoles ^14CO_2/30 min/g, wet wt)</th>
<th>S-Adenosyl-L-methionine decarboxylase activity (nmoles ^14CO_2/30 min/g, wet wt)</th>
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<tr>
<td></td>
<td>T^a</td>
<td>TD</td>
<td>CD</td>
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<td>122</td>
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<tr>
<td>8</td>
<td>237</td>
<td>182</td>
<td>117</td>
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</table>

^a T, BD2F, male mice inoculated i.p. on Day 0 with 10^6 L1210 ascites tumor cells and given i.p. injections of 0.85% NaCl solution on Days 1 through 7; TD, BD2F, male mice i.p. inoculated on Day 0 with 10^6 L1210 ascites tumor cells and given i.p. injections of methylglyoxal-bis(guanylhydrazone) (50 µg/g of body weight) on Days 1 through 7; CD, control (nonleukemic) BD2F, male mice given i.p. injections of methylglyoxal-bis(guanylhydrazone) (50 µg/g of body weight) on Days 1 through 7; C, control (nonleukemic) BD2F, male mice given i.p. injections of 0.85% NaCl solution on Days 1 through 7.

Table 2
Inhibition of S-adenosyl-L-methionine decarboxylase activity in extracts of leukemia mouse spleens by methylglyoxal-bis(guanylhydrazone)

BD2F, male mice were inoculated i.p. on Day 0 with 10^6 L1210 ascites tumor cells; and on Day 3 and Day 7, groups of 5 mice were killed. The spleens were excised and homogenized in 5 volumes of 0.05 M sodium:potassium phosphate buffer, pH 7.2, containing 1.0 mM dithiothreitol. The 20,000 x g (20-mm) supernatant fraction was assayed for enzyme activities after the addition of methylglyoxal-bis(guanylhydrazone) to a final concentration of 0.05 to 50 µM. The enzyme activity was determined by measuring the release of ^14CO_2 from S-adenosyl-L-methionine-carboxyl-l-^14C in the presence of putrescine or spermidine as an activator of the enzyme. Each value represents the mean of 3 assays (variation < 10%) for enzyme activity in an extract of 5 spleens.

The activity of L-ornithine decarboxylase was not significantly affected by the drug concentrations used.

<table>
<thead>
<tr>
<th>Concentration of methylglyoxal-bis(guanylhydrazone) (µM)</th>
<th>Putrescine-stimulated</th>
<th>Spermidine-stimulated</th>
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<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 7</td>
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<tr>
<td></td>
<td>nmoles ^14CO_2/30 min/g, wet wt</td>
<td>%</td>
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<tr>
<td></td>
<td>0</td>
<td>50</td>
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<td>23</td>
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</table>

spermidine-stimulated enzyme to almost the same extent as that of the putrescine-stimulated enzyme (Table 2). After a single i.p. injection of methylglyoxal-bis(guanylhydrazone) (50 µg/g of body weight), the activity of 6-day leukemic spleen spermidine-stimulated S-adenosyl-L-methionine decarboxylase measured in vitro decreased to a value 30% that of control 6-day leukemic spleen extracts (Table 3). It increased gradually after a 4-hr depression, reaching a value more than 2-fold that of the control at 24 hr after the injection of the inhibitor.

Endogenous Levels of Putrescine. In spleens of leukemia mice, there was a decrease in the putrescine concentration 2 to 4 days after tumor inoculation, but on Days 6 to 8 its concentration was almost 2-fold above normal (Chart 1). Daily treatment with methylglyoxal-bis(guanylhydrazone) resulted in a continuous increase in putrescine concentration.
in spleens of leukemic mice. At 8 days after tumor inoculation, it was more than 3 times higher in drug-treated leukemic mice than in control leukemic mice. In spleens of nonleukemic mice the drug regimen caused an increase in putrescine concentration as well, but the increase was observed after the 1st treatment only. A concentration 2.5 times higher than in nonleukemic control mice was maintained from Day 2 and throughout the subsequent period of treatment.

Endogenous Levels of Spermidine. The spermidine concentration in the spleen increased progressively after tumor inoculation and was almost 2-fold above normal on Day 8 of tumor growth. When methylglyoxal-bis(guanylhydrazone) was administered to leukemic mice, a marked decrease in the spermidine concentration occurred; it was 25% lower than in spleens of leukemic controls on Day 2 of tumor growth. Subsequently, however, the spermidine concentration increased continuously, but it was 15 to 30% lower than in spleens of leukemic control mice at all times. A slight decrease in the spermidine concentration was observed in spleens of nonleukemic mice subjected to the drug regimen.

Endogenous Levels of Spermine. The spermine concentration in the spleen was rather constant throughout the experimental period in leukemic and nonleukemic control mice. In drug-treated leukemic and nonleukemic mice, the spermine concentration decreased markedly during treatment.

DISCUSSION

Considerable interest in methylglyoxal-bis(guanylhydrazone) as an anticancer agent was evoked several years ago. This drug was found to have clinical activity against acute myelocytic leukemia (4–6, 14, 21), some lymphomatous diseases, and tumors of the head and neck region (4, 20, 21). It was effective against several experimental tumors, e.g., Sarcoma 180 ascites tumor (11), L1210 lymphoid leukemia (3, 11, 15), and other leukemias (11). However, it was found to be inactive, at nontoxic doses, against leukemia L4946, Ehrlich ascites carcinoma, and several other experimental tumors (11). In studying the antitumor and toxicological effects of this compound, Mihich (15) found that its effects against L1210 leukemia were prevented by the concurrent
administration of spermidine by the same or different route. Because of the structural similarities between methylglyoxal-bis(guanylhydrazone) and spermidine, it was suggested that this drug might interfere with biological functions of this polyamine.

Not until recently were any studies performed to elucidate which effects methylglyoxal-bis(guanylhydrazone) might have on the polyamine-biosynthetic enzymes and the polyamine concentrations in vitro (1, 2, 7, 12, 16, 26) and in vivo (8, 10, 16, 17). Interestingly enough, Williams-Ashman and Schenone (26) observed that putrescine-stimulated S-adenosyl-L-methionine decarboxylases of rat ventral prostate and bakers' yeast were strongly inhibited by the drug upon its addition to the reaction mixture.

In an attempt to study the effects of methylglyoxal-bis(guanylhydrazone) on endogenous concentrations of the polyamines we used the L1210 leukemia, against which it had been shown active (8). Significant prolongation of survival time of L1210 leukemic mice was observed after 7 days of i.p. injections of the drug (50 μg/g/day). The ratio of survival time of drug-treated leukemic mice to leukemic control mice was 182%. The putrescine concentration increased continuously in spleens of drug-treated leukemic mice, and on the 8th day of tumor growth it was more than 3-fold that of leukemic control mice. Concomitantly, the spermidine and spermine concentrations decreased. In the nonleukemic mice, the putrescine concentration increased markedly after the 1st injection of the drug but remained at this level during subsequent injections. The continuous increase in putrescine concentration in spleens of leukemic mice during drug treatment is probably due to constant infiltration of tumor cells, as indicated by the increasing spleen weight. Also, the L-ornithine decarboxylase activity in spleens of drug-treated leukemic mice was increased during the entire experimental period, whereas the enzyme activity in drug-treated non-leukemic mice showed only an early increase, temporarily corresponding with the increase in putrescine concentration in this group of mice. The daily dose of the drug was not sufficient completely to suppress tumor cell multiplication and infiltration of the spleen. Higher doses of the drug exhibited extreme toxicity and could not be used.

The increase in putrescine concentration subsequent to treatment with methylglyoxal-bis(guanylhydrazone) seems to be due to both increase in L-ornithine decarboxylase activity (Table 1) and inhibition of spermidine and spermine synthesis (Chart 1; Table 3). The inhibition of spermidine synthesis in drug-treated mice seems contradictory to the results in Table 1, which show a marked elevation of S-adenosyl-L-methionine decarboxylase activity after drug treatment. However, the activities of these enzymes were greatly depressed for at least 8 to 12 hr after treatment (Table 3), but 1 day (as in Table 1) after treatment they were even higher than those of the controls. The early, transient inhibition of S-adenosyl-L-methionine decarboxylase by the drug probably causes the decrease in spermidine and spermine concentrations. Moreover, the inhibition exerted by the drug on this enzyme is probably even more pronounced in vivo, since the in vivo assay conditions represent a 5-fold dilution of the tissues. Pegg et al. (17) and Fillingame and Morris (2) were able completely to reverse the inhibition of the enzyme activities by extensive dialysis, i.e., by removal of any remaining drug from the tissue extracts. We were not able completely to reverse the early inhibition in extracts of leukemic spleens by extensive dialysis, but it was reversible in extracts of nonleukemic spleens (10).

The increased activity of S-adenosyl-L-methionine decarboxylase after treatment with methylglyoxal-bis(guanylhydrazone) seems to be due to inhibition of its degradation (2, 17). However, stimulation of its synthesis may also contribute to the increased activity. Daily treatment with the drug (50 μg/g/day) sufficed to suppress enzyme activity for several hr and thus to decrease the spermidine and spermine formation.

Whether the increased L-ornithine decarboxylase activity is due to stabilization towards breakdown or increased synthesis of enzyme molecules or both has yet to be determined. Fillingame and Morris (2) reported that the half-life of L-ornithine decarboxylase did not change during treatment with methylglyoxal-bis(guanylhydrazone).

Various effects of methylglyoxal-bis(guanylhydrazone) are abolished by the administration of spermidine, a fact that supports the idea that the in vivo effects of the drug may be due to interference with the synthesis and cellular function of the polyamines. For example, the antiproliferative effect of the drug (15) as well as its antimitochondrial action (19) are antagonized by spermidine.

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Effects of Methylglyoxal-bis(guanylhydrazone) on Polyamine Metabolism in Spleens of Mice with Disseminated L1210 Lymphoid Leukemia

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