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Specific Inhibition of the Enzymic Decarboxylation of *S*-Adenosylmethionine by Methylglyoxal Bis(guanylhydrazone) and Related Substances

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Methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediylidene)-dinitrilo]diguanidine} is a very potent inhibitor of putrescine-activated *S*-adenosylmethionine decarboxylases from many different mammalian tissues, including sublines of mouse L1210 leukaemia that are resistant to the drug as well as sublines that are sensitive. The inhibition of purified rat ventral prostate *S*-adenosylmethionine decarboxylase is competitive with respect to the *S*-adenosylmethionine substrate, and is much greater in the presence than in the absence of the activator putrescine. Inhibition by the drug depends, among other things, on the nature of the aliphatic amines that can serve as stimulators of rat prostate *S*-adenosylmethionine decarboxylase. Effects of some congeners of methylglyoxal bis-(guanylhydrazone) on the enzyme are described.

The inhibition of putrescine-activated *S*-adenosylmethionine decarboxylases from rat ventral prostate and yeast by low concentrations of methylglyoxal bis(guanylhydrazone) {1,1'-[(methylethanediylidene)-dinitrilo]diguanidine} (Williams-Ashman & Schenone, 1972) has since been confirmed with enzyme preparations from rat liver and kidney (Pegg, 1973), plant lectin-stimulated lymphocytes (Fillingame & Morris, 1973*a*; Kay & Pegg, 1973) and mouse L1210 leukaemia cells (Corti *et al.*, 1973; Heby & Russell, 1973). The *S*-adenosylmethionine decarboxylase of *Physarum polycephalum*, which requires neither an amine nor a metal cofactor, was also shown by Mitchell & Rusch (1973) to be strongly inhibited by minute amounts of methylglyoxal bis-(guanylhydrazone). In contrast, the corresponding enzyme from *Escherichia coli*, which is activated by Mg^{2+} but not by putrescine or related aliphatic amines, is inhibited markedly only by concentrations of methylglyoxal bis(guanylhydrazone) that are two to three orders of magnitude higher than those that depress the activity of *S*-adenosylmethionine decarboxylase from the aforementioned eukaryotic cells (Williams-Ashman & Schenone, 1972). Treatment of rodents with methylglyoxal bis(guanylhydrazone) results in an expected depression of the incorporation of exogenous labelled putrescine into spermidine and spermine (Pegg, 1973) and a swift rise in the total putrescine concentration in various tissues (Heby & Russell, 1973; Pegg, 1973); similar observations were made after exposure of concanavalin A-stimulated lymphocytes to the drug (Fillingame & Morris, 1973*a*). The administration of methylglyoxal bis(guanylhydrazone) *in vivo* also elicits a very large and paradoxical rise in the putrescine-activated *S*-adenosylmethionine decarboxylase activity of a variety of animal cells, apparently as a consequence, at least in part, of a depression of the rate of intracellular degradation of this rapidly turning over enzyme (Fillingame & Morris, 1973*b*; Pegg *et al.*, 1973).

The present paper considers some novel features of the direct inhibition of animal tissue *S*-adenosylmethionine decarboxylases by methylglyoxal bis-(guanylhydrazone) and related substances. Some of the findings presented have been mentioned in a preliminary communication (Corti *et al.*, 1973). A number of the results in this paper are in agreement with the experiments of Hölttä *et al.* (1973), which were communicated to the authors in manuscript after the present investigations had been completed.

Materials and Methods

Male Sprague–Dawley rats (250–350 g) and female mice of the DBA/2J strain were provided with water and food *ad libitum*. Mouse L1210 leukaemia cells were obtained from DBA/2J females 4 days after the intraperitoneal inoculation of 2×10^6 cells. The mice were killed by cervical fracture, exsanguinated and the tumour cells were flushed out of the abdomen and suspended in a solution containing 100 mM-sodium phosphate buffer, pH 7.2, and 5 mM-dithiothreitol. After washing five times with this medium,

pellets of cells obtained from the final centrifugation were immediately frozen and stored for several days at −70°C. The tumour cells were then thawed and suspended in 10 mM-sodium phosphate buffer, pH 7.2, containing 0.1 mM-EDTA (disodium salt) and 2 mM-dithiothreitol. The suspensions were frozen and thawed five times in an ethanol–solid CO_2 bath and then centrifuged for 30 min at 35000*g* at 2°C. The supernatant fluids were used as a source of the tumour enzyme. Crude extracts of other tissues as shown in Table 1 were prepared by homogenization with 3 volumes of 10 mM-sodium phosphate buffer, pH 7.2, containing 2 mM-dithiothreitol and 0.1 mM-EDTA, followed by centrifugation at 35000*g* for 30 min at 2°C. Various Morris rat hepatomas (Williams-Ashman *et al.*, 1972*a*) were generously supplied by Dr. George Weber of the Department of Pharmacology, Indiana University Medical School, Indianapolis, Ind., U.S.A. Centrifuged extracts of these hepatomas were prepared after homogenization of non-necrotic tissue specimens with the aforementioned medium used for experiments with mouse leukaemia cells. Protein was determined by the method of Lowry *et al.* (1951) with serum albumin as standard.

Ornithine decarboxylase activities were determined by the method of Jänne & Williams-Ashman (1971*a*). Spermidine synthase activities were measured by the procedure of Jänne *et al.* (1971*a*). The enzymic decarboxylation of *S*-adenosyl-L-[*carboxy*-^{14}C]methionine was determined by the method of Pegg & Williams-Ashman (1969). The composition and temperatures of the reaction mixtures, the times of incubation and the sources of the enzyme preparations used for determination of *S*-adenosylmethionine decarboxylase activities are described in the text or the protocols. Unless otherwise stated, all reactions were initiated by the addition of the appropriate enzyme and suitable controls were invariably set up to correct for all non-enzymic reactions.

Hirudonine (diamidinospermidine) and arcaine (diamidinoputrescine) were generously given by Professor Seymour S. Cohen of the Department of Microbiology, University of Colorado School of Medicine, Denver, Col., U.S.A. Methylglyoxal bis(guanylhydrazone) in the form of its dihydrochloride monohydrate, and bis(guanylhydrazone) derivatives of related glyoxals (Freedlander & French, 1958; Podrebarac *et al.*, 1963; Podrebarac & Cheng, 1964) were obtained through the courtesy of Dr. Harry Wood of the Cancer Chemotherapy National Service Center of the National Cancer Institute, Bethesda, Md., U.S.A. All other substances were of commercial origin and of the highest grade of purity. Solutions of all drugs were prepared in water and were tested as enzyme inhibitors immediately. All pH measurements were made with a glass electrode and refer strictly to the stated temperatures.

Results and Discussion

Williams-Ashman & Schenone (1972) demonstrated that the *S*-adenosylmethionine decarboxylases of rat ventral prostate and baker's yeast were powerfully inhibited by micromolar concentrations of methylglyoxal bis(guanylhydrazone) when the activities were determined in the presence of saturating concentrations of the activator putrescine. At pH values near neutrality, the prostate enzyme exhibits small but definite decarboxylase activity in the absence of any amine activator; the extent of inhibition by the drug was markedly less when the prostate enzyme was determined without the addition of putrescine. Since baker's yeast *S*-adenosylmethionine decarboxylase is virtually inactive in the absence of putrescine (Coppoc *et al.*, 1971; Jänne *et al.*, 1971*b*), the effect of methylglyoxal bis(guanylhydrazone) on the yeast enzyme in the absence of putrescine could not be evaluated. Additional features of the inhibition of animal tissue *S*-adenosylmethionine decarboxylases by this drug are as follows.

Under standard conditions at 37°C (100 mM-sodium phosphate, pH 7.2; 0.2 mM-*S*-adenosylmethionine 2.5 mM-putrescine; 2.5 or 5 mM-dithiothreitol), the concentrations of methylglyoxal bis(guanylhydrazone) required for 50% inhibition of CO_2 release were in the range 0.5–2 μM when undialysed centrifuged extracts of rodent kidney, ventral prostate, heart, spleen or whole brain were used as the source of enzyme. The same range of concentrations of the drug was necessary for 50% inhibition of the activity of similar crude extracts of sublines of mouse L1210 leukaemia that were either sensitive or resistant to methylglyoxal bis(guanylhydrazone) (cf. Mihich, 1963, 1965). A typical experiment with mouse tissues is shown in Table 1. Other experiments (not shown) revealed that 50% inhibition of the putrescine-activated *S*-adenosylmethionine decarboxylase of crude extracts of rat Morris hepatomas 8999 and 3924A was achieved with approximately 1 μM-methylglyoxal bis(guanylhydrazone). Table 1 also illustrates the anomalous finding that 10 μM concentrations of the drug did not significantly affect the enzyme in fresh centrifuged extracts of mouse liver, as was previously reported by Heby & Russell (1973); even when the amount of mouse liver extract added to the reaction mixture was one-half of that shown in Table 1, raising the drug concentration to 40 μM evoked only 39% inhibition of CO_2 release. The latter result seemed to reflect the presence of substance(s) in crude and undialysed mouse liver extracts that prevent methylglyoxal bis(guanylhydrazone) from inhibiting the putrescine-activated decarboxylation of *S*-adenosylmethionine. This was suggested by experiments (not shown) indicating that after partial purification of the mouse liver decarboxylase by a single $(NH_4)_2SO_4$ fractionation followed by dialysis, the concentration

Table 1. *Inhibition of S-adenosylmethionine decarboxylase in crude extracts of various normal and malignant mouse tissues by methylglyoxal bis(guanylhydrazone)*

The preparation of the centrifuged tissue extracts is described in the text. The *S*-adenosylmethionine decarboxylase activities were determined in reaction mixtures containing 100mM-sodium phosphate buffer, pH7.2, 0.2mM-^{14}C-labelled *S*-adenosylmethionine (1.54c.p.m./pmol), 10mM-dithiothreitol, 2.5mM-putrescine and enzyme and drug in a final volume of 0.5ml. The tubes were incubated for 1h at 37°C.

Tissue	Amount of tissue extract added (mg of protein)	Amount of CO_2 released (pmol/h per mg of protein)	
		Control	+10μM-Methylglyoxal bis(guanylhydrazone)
Liver	2.4	2260	2520
Heart	0.74	437	0
Kidney	1.56	515	0
Spleen	1.44	543	0
Salivary gland	1.34	600	0
Thymus	0.48	238	0
Brain	0.66	1028	69
L1210 leukaemia (subline sensitive to the drug)	2.7	1392	101
L1210 leukaemia (subline resistant to the drug)	4.0	1421	93

of methylglyoxal bis(guanylhydrazone) required for 50% inhibition was lowered to roughly 1μM. In contrast with these results for mouse liver, the *S*-adenosylmethionine decarboxylase activity of crude centrifuged extracts of rat liver was inhibited more than 80% by 10μM-methylglyoxal bis(guanylhydrazone) under conditions identical with those involving mouse liver (see Table 1).

Inhibition of putrescine-activated rat ventral prostate *S*-adenosylmethionine decarboxylase by low concentrations of methylglyoxal bis(guanylhydrazone) (1μM or lower) was greater in experiments under the standard conditions with more purified enzyme preparations [e.g. stage 5 of the procedure of Jänne & Williams-Ashman (1971*b*) with 50μg of enzyme protein per assay system] than with less refined preparations [e.g. stage 2 of the method of Jänne & Williams-Ashman (1971*b*) with 2mg of enzyme protein per tube]. Higher concentrations of the drug (6μM) inhibited enzymes of different degrees of purity to practically the same extent (85%) The latter findings can probably be attributed to non-specific binding of methylglyoxal bis(guanylhydrazone) by contaminating proteins in the less refined enzyme preparations. Under conditions where the control rates of decarboxylation were constant with respect to time, the extent of inhibition by 1μM-methylglyoxal bis(guanylhydrazone) did not change significantly with time of incubation. In experiments where the final decarboxylase assays were performed at 37°C, prior incubation of the enzyme with 2μM concentrations of the drug for 10min at 25°C with or without 2.5mM-putrescine in the absence of the *S*-adenosylmethionine substrate hardly altered the degree of inhibition from that observed in reactions that were initiated by addition of the enzyme rather than of *S*-adenosylmethionine. The inhibitory effect of methylglyoxal bis(guanylhydrazone) on putrescine-stimulated decarboxylation of *S*-adenosylmethionine by the rat ventral prostate enzyme appeared to be reversible. This was indicated by previously reported experiments involving dialysis (Pegg *et al.*, 1973), and also by simple dilution experiments involving initial exposure of high concentrations of the enzyme to the drug in the presence of 2.5mM-putrescine, followed by 10-fold dilution of the enzyme into the standard putrescine-containing test medium.

At pH7.2 and 37°C, the decarboxylation of *S*-adenosylmethionine by the rat ventral prostate enzyme in the presence of a saturating concentration of putrescine (2.5mM) is considerably greater with 100mM concentrations of P_i than with Tris buffers (Pegg & Williams-Ashman, 1969). In a typical experiment, in which the control rate of CO_2 release in Tris buffer was 57% of that observed in phosphate buffer, the inhibition by 1μM-methylglyoxal bis(guanylhydrazone) in Tris buffer (55%) was very similar to that seen with phosphate buffer (61%). The difference in rate of decarboxylation in the absence of the inhibitor between Tris and phosphate buffers could not be accounted for by variations in the ionic strength of the reaction mixture, since addition of NaCl (200mM) did not significantly alter the rates of reaction in either system (cf. Williams-Ashman *et al.*, 1972*b*).

The degree of inhibition by methylglyoxal bis-

Table 2. *Effect of pH on the dependence of prostate S-adenosylmethionine decarboxylase on the concentration of S-adenosylmethionine and effect of this concentration on inhibition by methylglyoxal bis(guanylhydrazone)*

The enzyme (3.5 μg of protein in each tube) was obtained from step 6 (Bio-Gel P-200) of the procedure of Jänne & Williams-Ashman (1971*b*). The assay system contained, in a total volume of 0.25 ml, 100 mM-sodium phosphate buffer, pH 7.2, 2.5 mM-dithiothreitol, 2.5 mM-putrescine and the indicated concentrations of *S*-adenosylmethionine and inhibitor. The tubes were incubated at 37°C for 30 min.

		Decarboxylase activity (pmol of CO_2/30 min)					
		Controls		+ Methylglyoxal bis(guanylhydrazone) (1 μM)			
pH	*S*-Adenosylmethionine concn. (mM)	Without putrescine	With putrescine	Without putrescine	% inhibition	With putrescine	% inhibition
6.1	0.04	25	422	—	—	—	—
6.1	0.10	52	697	—	—	—	—
6.1	0.20	72	822	—	—	—	—
6.1	0.60	81	658	—	—	—	—
6.1	1.0	184	263	—	—	—	—
7.2	0.04	102	673	81	20	168	75
7.2	0.10	212	1172	183	14	416	64
7.2	0.20	296	1508	259	12	704	53
7.2	0.60	456	2473	407	11	1425	42
7.2	1.0	412	2067	392	4	1731	16
8.0	0.04	70	324	—	—	—	—
8.0	0.10	152	639	—	—	—	—
8.0	0.20	267	918	—	—	—	—
8.0	0.60	487	1893	—	—	—	—
8.0	1.0	445	2200	—	—	—	—

(guanylhydrazone) depends critically on the concentration of the *S*-adenosylmethionine substrate and also on the nature of the amine used as activator for the decarboxylases of mammalian tissue origin. The representative experiments summarized in Table 2 show that at pH 7.2 the extent of inhibition was much less in the absence than in the presence of a saturating concentration of putrescine, and that in both cases increasing the concentration of *S*-adenosylmethionine decreased inhibition by methylglyoxal bis(guanylhydrazone). Jänne & Williams-Ashman (1971*b*) have shown that with 0.2 mM-*S*-adenosylmethionine as substrate, the percentage enhancement of decarboxylase activity by putrescine increased markedly as the pH of the reaction mixture was progressively lowered from values of 8.7 to 5.8. These findings are corroborated in Table 2, which illustrates variations in the rate of decarboxylation of various initial concentrations of *S*-adenosylmethionine in the absence and presence of putrescine at three different pH values. At pH 6.1 and 7.2, but not at pH 8.0, there is inhibition of the reaction at the highest concentration of *S*-adenosylmethionine tested (1 mM) when excess of putrescine is added as activator; this accords with the findings at pH 7.2 reported for brain *S*-adenosylmethionine decarboxylase by Schmidt & Cantoni (1973). It is also evident from Table 2 that the degree of activation by putrescine at the three pH values examined was markedly dependent on the concentration of *S*-adenosylmethionine.

The dependence of the inhibition by methylglyoxal bis(guanylhydrazone) on the concentration of *S*-adenosylmethionine may be one factor that accounts for the differences in the amounts of the drug reported to be necessary for 50% inhibition of decarboxylase activity with enzymes from different mammalian tissues (Williams-Ashman & Schenone, 1972; Fillingame & Morris, 1973*a*; Kay & Pegg, 1973). Double-reciprocal plots of the putrescine-activated decarboxylase activities measured at pH 7.2 (see Table 2) versus *S*-adenosylmethionine concentrations in the absence and presence of methylglyoxal bis(guanylhydrazone) were approximately linear, nearly intersected on the ordinate and strongly suggested that inhibition by the drug was competitive with respect to *S*-adenosylmethionine (K_i approx. 1 μM). This agrees well with the findings of Hölttä *et al.* (1973). However, an exhaustive kinetic analysis of the inhibition by methylglyoxal bis(guanylhydrazone) at various pH values in the presence of different concentrations of *S*-adenosylmethionine was not undertaken. In the standard test system in 100 mM-phosphate buffer, the inhibition by 1 μM concentrations of the drug in the presence of 2.5 mM-putrescine did not differ very much at pH values of 6.5, 7.0 and 8.6.

Williams-Ashman & Schenone (1972) observed that high concentrations of methylglyoxal bis(guanylhydrazone) (10 μM) inhibited rat prostate *S*-adenosylmethionine decarboxylase by more than

Table 3. *Inhibition of rat ventral prostate S-adenosylmethionine by methylglyoxal bis(guanylhydrazone) in the presence of putrescine or spermidine as activators*

The experimental conditions are as in Table 2 except that 40 μg of rat ventral prostate enzyme of a lesser degree of purity (step 5 of the procedure of Jänne & Williams-Ashman, 1971*b*) was used.

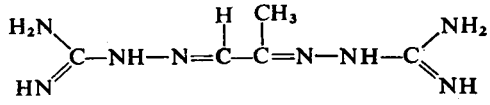
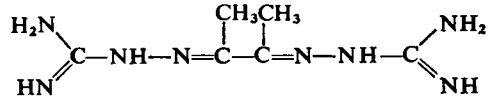
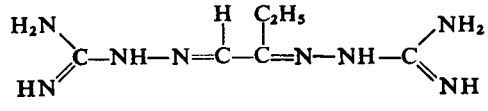
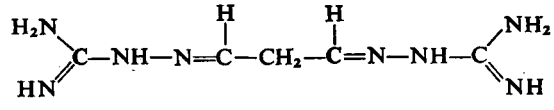
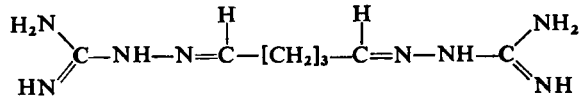
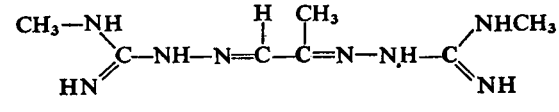
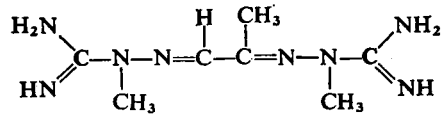
S-Adenosylmethionine concn. (mM)	Amine added as activator	Amount of CO_2 released (pmol/30min)		Inhibition by drug (%)
		Control	+1 μM-Methylglyoxal bis(guanylhydrazone)	
0.05	—	15	—	—
0.05	2.5mM-Putrescine	188	55	71
0.05	5mM-Spermidine	39	30	23
0.20	—	51	48	5
0.20	2.5mM-Putrescine	453	203	55
0.20	5mM-Spermidine	122	98	20
0.60	—	87	—	—
0.60	2.5mM-Putrescine	526	373	29
0.60	5mM-Spermidine	186	180	3

85% when either 1,3-diaminopropane, putrescine (1,4-diaminobutane), or cadaverine (1,5-diaminopentane) were used as activators of the enzyme (in the absence of the drug saturating concentrations of the three aliphatic diamines each gave different degrees of activation of the enzyme). Further experiments disclosed that the inhibition by lower concentrations of methylglyoxal bis(guanylhydrazone) depends on the nature of the amine added as enzyme activator. Previous studies (Pegg & Williams-Ashman, 1969; Jänne & Williams-Ashman, 1971*b*; Williams-Ashman *et al.*, 1972*b*) have established that spermidine as well as putrescine, but not spermine, accelerated the decarboxylation of *S*-adenosylmethionine by the enzyme from various animal tissues; the activation by saturating concentrations of spermidine (5mM) at pH 7.2 was strikingly less than that found with putrescine and the apparent affinity of the decarboxylase for spermidine was also less than that for putrescine. Table 3 shows that the inhibition of rat ventral prostate *S*-adenosylmethionine decarboxylase by methylglyoxal bis(guanylhydrazone) was less when spermidine rather than putrescine was used in saturating concentrations as an activator at pH 7.2 and 37°C, and also that, as found with putrescine, the inhibition by the drug with spermidine as activator was diminished when the *S*-adenosylmethionine concentrations were raised over the range 0.05–0.6mM.

Table 4 shows that with putrescine as activator, the bis(guanylhydrazones) of ethylglyoxal and dimethylglyoxal inhibited rat prostate *S*-adenosylmethionine decarboxylase to approximately the same extent as methylglyoxal bis(guanylhydrazone). The dimethylglyoxal and ethylglyoxal bis(guanylhydrazones) are devoid of anti-leukaemic actions (Mihich, 1963, 1965). These findings therefore indicate that there is no simple relationship between inhibition of putrescine-activated *S*-adenosylmethionine decarboxylase *in vitro* and the anti-proliferative actions *in vivo* by the drugs of this category. Table 4 also indicates that the bis(guanylhydrazones) of propane dialdehyde and pentane dialdehyde, which do not exert anti-leukaemic actions (Mihich, 1963, 1965), are relatively feeble inhibitors of the prostate *S*-adenosylmethionine decarboxylase in comparison with methylglyoxal bis(guanylhydrazone). The anti-leukaemic drug 4,4′-diacetyldiphenylurea bis(guanylhydrazone), which is not cross-resistant with methylglyoxal bis(guanylhydrazone) with respect to mouse L1210 leukaemia sublines (Mihich *et al.*, 1969), also did not inhibit the prostate enzyme at concentrations of 0.1mM under the conditions mentioned in Table 4. Substitution of methyl groups on the primary amino moieties at both ends of the methylglyoxal bis(guanylhydrazone) molecule markedly diminished its inhibitory power, whereas addition of methyl groups to both internal N'' nitrogen atoms had much less effect. Other experiments conducted under the conditions in Table 4 revealed that arcaine (diaminidoputrescine) gave 50 and 80% inhibition at 0.1mM and 1mM respectively, whereas hirudonine (diaminidospermidine) did not inhibit the enzyme at 0.1mM and gave 45% inhibition at 1mM. Thus the latter substances are very much less potent inhibitors than methylglyoxal bis(guanylhydrazone). Atabrine, which can be regarded as a substituted derivative of putrescine (Cohen, 1971), gave 45 and 85% inhibition at 0.1 and 1mM respectively. Cohen (1971) has pointed out that ethidium bromide can be viewed as a congener of spermidine; the putrescine-activated prostate *S*-adenosylmethionine decarboxylase was not affected by 0.1mM-ethidium bromide. No effects were observed on addition of hydrazine or aminoguanidine at concentra-

Table 4. *Inhibition of rat S-adenosylmethionine decarboxylase by congeners of methylglyoxal bis(guanylhydrazone)*

Experimental conditions as in Table 1. Prostate enzyme (0.07 mg) from step 5 of the procedure of Jänne & Williams-Ashman (1971*b*) was used in all tests. n.d. signifies not determined. Each value represents the results of at least two experiments.

Inhibitor	Formula	% Inhibition of CO_2 released by inhibitor at final concentration of: 1 mM	10 μM	1 μM
Methylglyoxal bis(guanylhydrazone)		100	100	50–60
Dimethylglyoxal bis(guanylhydrazone)		n.d.	100	80
Ethylglyoxal bis(guanylhydrazone)		n.d.	100	90
Propane dialdehyde bis(guanylhydrazone)		35	n.d.	n.d.
Pentane dialdehyde bis(guanylhydrazone)		75	n.d.	n.d.
Di-*N*″-methylglyoxal bis(guanylhydrazone)		95	10	0
Di-*N*″-methylglyoxal bis(guanylhydrazone)		n.d.	100	80

tions of 1 mM, although higher amounts of both the latter substances were inhibitory (Williams-Ashman & Schenone, 1972; Williams-Ashman *et al.*, 1972*b*).

Methylglyoxal bis(guanylhydrazone) is a remarkably specific inhibitor of only one of the three reactions involved in spermidine biosynthesis in animal tissues. Concentrations of the drug as high as 5 mM did not influence rat ventral prostate ornithine decarboxylase activity as determined with partially purified enzyme preparations (Jänne & Williams-Ashman, 1971*a*). Spermidine synthase activity of rat ventral prostate preparations (Jänne *et al.*, 1971*a*) was not affected by 0.1 mM-methylglyoxal bis(guanylhydrazone) when the reactions were followed at pH 7.2 and 37°C by measuring the incorporation of ^{14}C-labelled putrescine (0.1 mM) into spermidine with 0.2 mM-unlabelled decarboxylated *S*-adenosylmethionine as substrate.

Low doses of methylglyoxal bis(guanylhydrazone) depress the incorporation of labelled putrescine into spermidine and spermine and also cause an accumulation of putrescine in living cells (Fillingame & Morris, 1973*a*; Kay & Pegg, 1973; Pegg, 1973). The drug rapidly increases the putrescine content and decreases the net accumulation of spermidine and spermine content of concanavalin A-stimulated

lymphocytes (Fillingame & Morris, 1973*a*) and exerts similar effects on sensitive (but not resistant) sublines of L1210 leukaemia cells *in vivo* without affecting polyamine amounts in normal mouse liver, spleen or salivary gland (Mihich *et al.*, 1974). Pegg (1973) reported that methylglyoxal bis(guanylhydrazone) did not affect spermidine and spermine concentrations in rat kidney in experiments of short duration. Any comprehensive consideration of the mechanism by which the anti-proliferative effects of this drug can be antagonized by spermidine *in vivo* (Mihich, 1963, 1965) must take into account: (*a*) the very slow rates of turnover of spermidine and spermine in many animal cells (Russell, *et al.*, 1970; Shaskan & Snyder, 1973); (*b*) increases in the activity of *S*-adenosylmethionine decarboxylase (Fillingame & Morris, 1973*a*,*b*; Hölttä *et al.*, 1973) and also ornithine decarboxylase (Hölttä *et al.*, 1973) in various tissues after treatment with the drug *in vivo*; (*c*) the existence in L1210 leukaemia cells of an active-transport system for uptake of methylglyoxal bis(guanylhydrazone) that is more effective in sensitive as compared with resistant sublines, and which is competitively inhibited by spermidine (Dave & Caballes, 1973; Mihich *et al.*, 1974).

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