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Effect of Methylglyoxal Bis(guanylhydrazone) on Polyamine Metabolism in Normal and Regenerating Rat Liver and Rat Thymus

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1. Injections of sublethal doses of methylglyoxal bis(guanylhydrazone), a potent inhibitor of putrescine-activated *S*-adenosylmethionine decarboxylase *in vitro*, resulted after a few days in an immense increase in the activity of *S*-adenosylmethionine decarboxylase in normal and regenerating rat liver and in rat thymus. The increase in the activity of *S*-adenosylmethionine decarboxylase was at least partly due to a marked lengthening of the half-life of the enzyme. 2. In regenerating liver and thymus there was also a moderate stimulation of the activity of ornithine decarboxylase (EC 4.1.1.17) and a marked accumulation of tissue putrescine. 3. Injection of methylglyoxal bis(guanylhydrazone) into the rat also markedly decreased the activity of diamine oxidase (EC 1.4.3.6) in thymus. 4. In no cases where doses of methylglyoxal bis(guanylhydrazone) close to the LD_{50} dose for the rat were used was it possible to lower tissue spermidine content to any significant extent. 5. Methylglyoxal bis(guanylhydrazone) seemed to act as a competitive inhibitor for the substrate *S*-adenosylmethionine and as an uncompetitive inhibitor for the activator putrescine in the decarboxylation of *S*-adenosylmethionine *in vitro*. 6. In the diamine oxidase reaction, with putrescine as the substrate, methylglyoxal bis(guanylhydrazone) was a non-competitive inhibitor for putrescine.

Williams-Ashman & Schenone (1972), on the basis of findings by Mihich (1963), made the observation that methylglyoxal bis(guanylhydrazone) strongly and apparently quite specifically inhibited eukaryotic putrescine-stimulated *S*-adenosylmethionine decarboxylase. This opened a new avenue for attempts to solve the physiological function(s) of mammalian polyamines. The finding that methylglyoxal bis(guanylhydrazone) inhibited *S*-adenosylmethionine decarboxylase was especially noteworthy because it is likely that *S*-adenosylmethionine decarboxylase functions as the rate-limiting enzyme in the synthesis of spermidine (and spermine) in mammalian tissues (Hannonen *et al.*, 1972; Hölttä & Jänne, 1972).

Methylglyoxal bis(guanylhydrazone) has been used as an inhibitor of spermidine formation in cultured human lymphocytes activated by phytohaemagglutinin (Kay & Pegg, 1973). The administration of methylglyoxal bis(guanylhydrazone) during the lymphocyte activation resulted after 48h in a complete inhibition of the incorporation of labelled thymidine into DNA and of radioactive phenylalanine into protein. It is noteworthy that the inhibition could be reversed by spermidine, thus indicating that the effects observed after the administration of methylglyoxal bis(guanylhydrazone) most probably were due to the inhibition of spermidine synthesis. Methylglyoxal bis(guanylhydrazone) has been also used *in vivo* to inhibit spermidine synthesis in the rat (Pegg, 1973). The consequences after injections of large doses of the compound into the rat were a complete inhibition of spermidine synthesis from exogenous radioactive putrescine and an accumulation of putrescine in liver and kidney.

The specificity of the inhibitor for eukaryotic *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972) and the findings by Mihich (1963) of the anti-proliferative properties of the compound have evoked enthusiasm about the possibilities of solving the physiological functions and importance of polyamines in mammalian tissues.

In the present paper we have followed the effects of sublethal doses of methylglyoxal bis(guanylhydrazone) on polyamine metabolism in regenerating and normal rat liver and in rat thymus. It is remarkably difficult to prevent tissue spermidine from accumulating after partial hepatectomy even by using doses of the inhibitor close to the lethal dose. Methylglyoxal bis(guanylhydrazone) also increased the activity of *S*-adenosylmethionine decarboxylase and decreased the activity of diamine oxidase. Therefore the diversity of the effects of methylglyoxal bis(guanylhydrazone) on polyamine metabolism *in vivo* may make it difficult to interpret the meaning of the metabolic changes after the administration of the inhibitor.

Experimental

Materials

Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Labelled and unlabelled *S*-adenosylmethionine and decarboxylated *S*-adenosylmethionine (*S*-methyladenosylhomocysteamine) were prepared and purified as described previously (Pegg & Williams-Ashman, 1969; Jänne *et al.*, 1971*a*; Raina & Hannonen, 1971). [1,4-^{14}C]Putrescine (sp. radioactivity 17.5Ci/mol) was purchased from New England Nuclear Corp., Dreieichenhain, West Germany, and purified before use on a column (H^+ form; 1cm × 5cm) of Dowex 50 (Raina *et al.*, 1970). DL-[1-^{14}C]Ornithine (sp. radioactivity 37Ci/mol) and dihydroxyphenyl[1-^{14}C]alanine (sp. radioactivity 9Ci/mol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and treated before use as described previously (Raina & Jänne, 1968; Jänne & Williams-Ashman, 1971*a*). Actinomycin D was kindly supplied by Merck, Sharp and Dohme, Rahway, N.J., U.S.A.

Preparation of tissue extracts

Female rats of Wistar or Sprague–Dawley strain, weighing about 100g, were used in all experiments. The rats were killed by decapitation; tissues were removed immediately and homogenized with 2vol. of ice-cold 0.25M-sucrose–1mM-2-mercaptoethanol–0.3mM-EDTA. The homogenates were centrifuged at 100000$g_{max.}$ for 45min and the supernatant fractions were used for enzyme assays or for further purification of the enzyme activities.

Partial purification of S-adenosylmethionine decarboxylase from rat liver

The cytosol fraction prepared from about 200g of normal rat liver was subjected to chromatography on a column (3cm×30cm) of DEAE-cellulose (Whatman DE-52) previously equilibrated with 25mM-potassium phosphate buffer (pH7.2) containing 0.1mM-EDTA and 0.5mM-dithiothreitol. The column was washed with 100ml of the buffer used for the equilibration and connected to a linear gradient of 0.1–0.3M-NaCl in the same buffer (total gradient volume was 1000ml). *S*-Adenosylmethionine decarboxylase activity was eluted from the column at about 0.15M-NaCl. The most active fractions were pooled (49ml), concentrated in an Amicon ultrafiltration cell and applied to a column (2.6cm×70cm) of Sephadex G-150 previously equilibrated against the same buffer as that used in DEAE-cellulose chromatography. The most active fractions (38ml) were pooled and used for the characterization of the inhibition of *S*-adenosylmethionine decarboxylase by methylglyoxal bis(guanylhydrazone).

Partial purification of diamine oxidase from rat thymus

A thymus cytosol fraction was prepared from 10g of thymus and fractionated with solid $(NH_4)_2SO_4$ (Schwarz/Mann, Orangeburg, N.Y., U.S.A., special enzyme grade) at 0°C. The proteins precipitated between 30 and 50% saturation of $(NH_4)_2SO_4$ were dissolved in a small volume of 25mM-Tris–HCl buffer (pH7.2) containing 0.1mM-EDTA and dialysed overnight against 100vol. of the same buffer. The dialysed $(NH_4)_2SO_4$ fraction was used as the enzyme in the inhibition studies.

Assay of enzyme activities

The activity of ornithine decarboxylase was assayed in the presence of saturating concentrations of L-ornithine (2mM). The other ingredients of the incubation mixture are described by Jänne & Williams-Ashman (1971*a*). The activity of *S*-adenosylmethionine decarboxylase was assayed after appropriate dilution of the samples obtained from animals treated with methylglyoxal bis(guanylhydrazone) (3mg twice a day and killed 12h after the last injection).This ensured that no inhibition was present in the assay system (usually a dilution of 1:15 was sufficient) in the presence of 0.2mM-*S*-adenosylmethionine and 2.5mM-putrescine (Pegg & Williams-Ashman, 1969; Raina & Hannonen, 1971; Jänne & Williams-Ashman, 1971*b*). The activity of spermidine synthase was determined in the presence of 0.5mM-radioactive putrescine and 0.1mM-decarboxylated *S*-adenosylmethionine (Jänne *et al.*, 1971*b*; Raina & Hannonen, 1971). Diamine oxidase was assayed in the presence of 0.4mM-radioactive putrescine as the substrate by the method of Okuyama & Kobayashi (1961) as modified by Tryding & Willert (1968). It was also necessary to dilute thymus extracts from methylglyoxal bis(guanylhydrazone)-treated animals to avoid inhibition by the drug present in the enzyme preparation. The activity of tyrosine aminotransferase of rat liver was determined by the method described by Granner & Tomkins (1970) Aromatic L-amino acid decarboxylase from pig kidney was assayed with dihydroxyphenyl-[1-^{14}C]alanine as substrate (Lowenberg, 1971).

Analytical methods

Putrescine, spermidine and spermine were measured from trichloroacetic acid extracts by the method of Raina & Cohen (1966). Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

Effect of methylglyoxal bis(guanylhydrazone) on the activities of polyamine-synthesizing enzymes in normal and regenerating rat liver

The changes in ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities after treatment with methylglyoxal bis(guanylhydrazone) are shown in Fig. 1. Injections of methylglyoxal bis(guanylhydrazone) (3mg twice a day) caused a tremendous increase in *S*-adenosylmethionine decarboxylase activity in liver. At 48h the increase in the enzyme activity was more than 30-fold compared with control animals. At the same time ornithine decarboxylase activity tended to decrease in the methylglyoxal bis(guanylhydrazone)-treated animals. Injection of 6mg of methylglyoxal bis(guanylhydrazone)/100g of body wt. daily into intact rats increased the concentration of putrescine by 20–50%, whereas no significant changes were found in the concentrations of spermidine and spermine in the liver.

The effects of methylglyoxal bis(guanylhydrazone) *in vivo* on ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities in regenerating rat liver are shown in Fig. 2. Again a tremendous increase (about 100-fold) in *S*-adenosylmethionine decarboxylase activity was evident, but contrary to the situation in normal liver there was also an increase in ornithine decarboxylase activity in

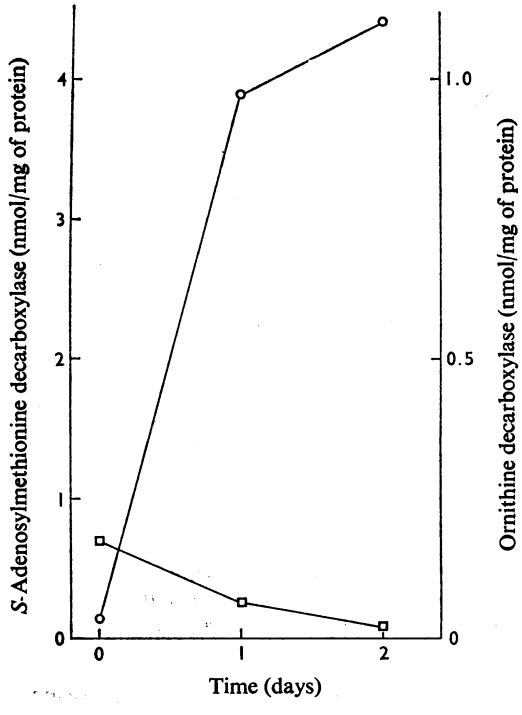

Fig. 1. *Effect of methylglyoxal bis(guanylhydrazone) in vivo on the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in normal rat liver*

The rats received intraperitoneal injections of methylglyoxal bis(guanylhydrazone), 3mg twice a day. The enzyme activities were measured as described in the text. The values are means obtained from three or four rats. □, Ornithine decarboxylase; ○, *S*-adenosyl-L-methionine decarboxylase.

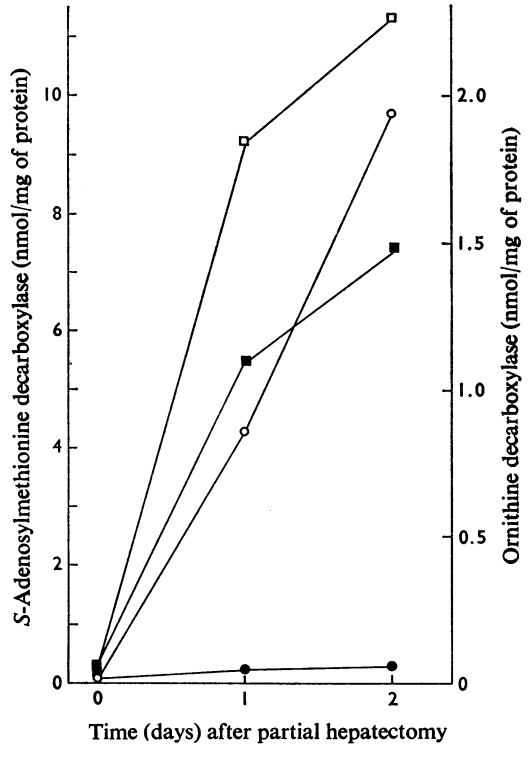

Fig. 2. *Effect of methylglyoxal bis(guanylhydrazone) in vivo on ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in regenerating rat liver*

The rats were partially hepatectomized and killed at the time-points indicated. The administration and dose of methylglyoxal bis(guanylhydrazone) were as described in Fig. 1. Three to four rats were in each group. Ornithine decarboxylase activity: ■, regenerating control; □, methylglyoxal bis(guanylhydrazone)-treated. *S*-Adenosyl-L-methionine decarboxylase activity: ●, regenerating control; ○, methylglyoxal bis(guanylhydrazone)-treated.

methylglyoxal bis(guanylhydrazone)-treated animals. There was a marked accumulation of putrescine in livers of methylglyoxal bis(guanylhydrazone)-treated animals. The concentrations of putrescine increased from 42nmol/g of liver in intact rats to 120nmol/g of liver during 48h liver regeneration, and methylglyoxal bis(guanylhydrazone) given to hepatectomized animals further increased the concentration of putrescine to 550nmol/g of liver. The concentration of spermidine and spermine showed practically no changes compared with the animals not receiving the drug. The daily dose used in this experiment (6mg) is close to the LD_{50} dose given by Mihich (1963) for the rat. An increase of the daily dose to 9mg/100g body wt. led to a prevention of the increased accumulation of spermidine 14h after partial hepatectomy, but this dose caused a serious toxic effect, killing the rats in less than 2 days. There was no change in spermidine synthase activity after treatment with methylglyoxal bis(guanylhydrazone).

In both normal and regenerating liver it appears that the compensatory mechanisms after the administration of methylglyoxal bis(guanylhydrazone) were sufficient to prevent any swift decrease in spermidine concentration of liver. The maintenance of the spermidine content after methylglyoxal bis(guanylhydrazone) injections was most likely due to the very large increase in *S*-adenosylmethionine decarboxylase activity in regenerating and normal liver and also partly due to the stimulation of ornithine decarboxylase activity in regenerating liver. A small increase in *S*-adenosylmethionine decarboxylase activity after a single injection of methylglyoxal bis(guanylhydrazone) occurred about 4h after the injection, and the activity tended to increase for at least 2 days. The slow increase in the enzyme activity, as well as the fact that injections of actinomycin D [0.2mg/100g body wt. twice a day given at the same times as methylglyoxal bis(guanylhydrazone)] failed to prevent the increase, suggested that the increase in *S*-adenosylmethionine decarboxylase activity might not be due to an increase in the rate of synthesis of new enzyme protein. In agreement with Pegg *et al.* (1973) we found that the increase in *S*-adenosylmethionine decarboxylase activity after the treatment with methylglyoxal bis(guanylhydrazone) at least partly was a result of a marked increase in half-life of the enzyme. In fact the half-life of *S*-adenosylmethionine decarboxylase in liver increased from about 45min to more than 7h after only a short (24h) treatment with methylglyoxal bis(guanylhydrazone) as judged by the decay of the enzyme activity after the injection of cycloheximide (0.8mg/100g of body wt.).

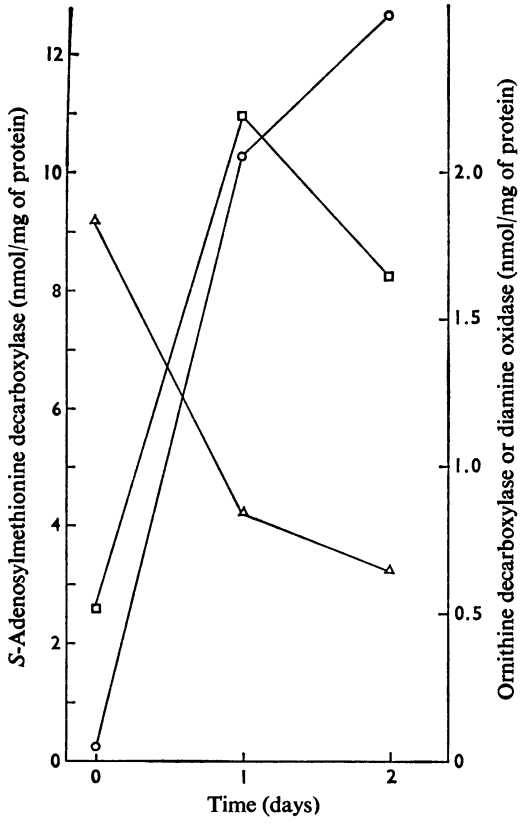

Fig. 3. *Ornithine decarboxylase, S-adenosyl-L-methionine decarboxylase and diamine oxidase activities in rat thymus after treatment with methylglyoxal bis(guanylhydrazone)*

The animals were treated with methylglyoxal bis(guanylhydrazone) as described in Fig. 1. Three to four rats were in each group. The enzyme activities were determined as described in the text. □, Ornithine decarboxylase; ○, *S*-adenosyl-L-methionine decarboxylase; △, diamine oxidase.

Effect of methylglyoxal bis(guanylhydrazone) on polyamine concentrations and the activities of some enzymes involved in the metabolism of the polyamines in rat thymus

The effect of methylglyoxal bis(guanylhydrazone) on ornithine decarboxylase, *S*-adenosylmethionine decarboxylase and diamine oxidase activities in rat thymus is shown in Fig. 3. As in liver, there was a very large increase in *S*-adenosylmethionine decarboxylase activity after treatment with methylglyoxal bis(guanylhydrazone). A moderate increase in ornithine decarboxylase activity also occurred and it is noteworthy that the treatment with methylglyoxal bis(guanylhydrazone) resulted in a decrease in diamine oxidase activity in thymus.

Table 1. *Effect of methylglyoxal bis(guanylhydrazone) on the concentration of polyamines in rat thymus*

The animals were treated with methylglyoxal bis(guanylhydrazone) as described in Fig. 1. Polyamines were determined from pooled samples of three rats in each group.

Time (days)	Polyamine (nmol/g wet wt. of tissue)		
	Putrescine	Spermidine	Spermine
0 (control)	201	1102	494
1	917	1292	445
2	1254	1390	442

As shown in Table 1, injections of methylglyoxal bis(guanylhydrazone) caused a remarkable accumulation of putrescine and there was also a slight increase in spermidine concentration in thymus. Thus the reactions in thymus were even more complex than in liver, since diamine oxidase activity was also affected by methylglyoxal bis(guanylhydrazone). It is possible that the rather marked accumulation of putrescine (6-fold) in thymus after the injections of methylglyoxal bis(guanylhydrazone) was partly due to the inhibition of spermidine synthesis, the increase in ornithine decarboxylase activity and decreased oxidation of putrescine by diamine oxidase. Apart from the effect on diamine oxidase activity (which is practically absent from the cytosol fraction of liver), the events occurring after treatment with methylglyoxal bis(guanylhydrazone) in polyamine metabolism were qualitatively comparable in liver and thymus.

Inhibition of S-adenosylmethionine decarboxylase by methylglyoxal bis(guanylhydrazone) in vitro

It has been suggested by Williams-Ashman & Schenone (1972) that methylglyoxal bis(guanylhydrazone) preferentially inhibits eukaryotic putrescine-activated *S*-adenosylmethionine decarboxylase by interacting with the putrescine site of the enzyme. It was therefore slightly surprising that at least low concentrations of methylglyoxal bis(guanylhydrazone) in the presence of saturating concentrations of putrescine showed competitive inhibition with respect to *S*-adenosylmethionine when partially purified *S*-adenosylmethionine decarboxylase from liver cytosol fraction was used as the source of enzyme (Fig. 4). [A similar finding has been reported by Corti *et al.* (1973).] In the absence of putrescine the inhibitor also increased the apparent K_m value for *S*-adenosyl methionine (which was markedly increased by omitting putrescine from the reaction mixture); however, at the same time there was a slight increase in the maximum reaction velocity. As shown in Fig. 4, methylglyoxal bis(guanylhydrazone) is

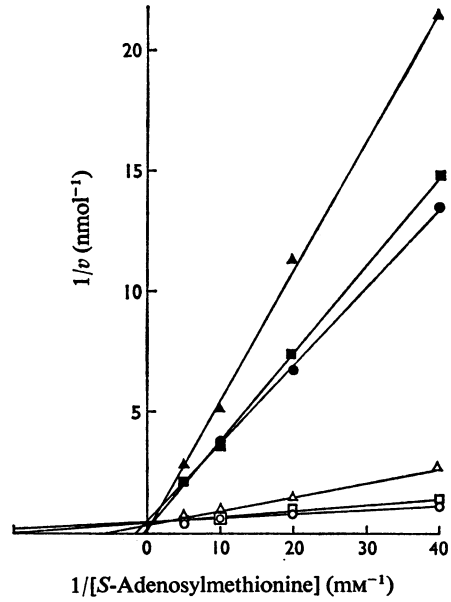

Fig. 4. *Lineweaver–Burk plot of the effect of substrate concentration on the S-adenosyl-L-methionine decarboxylase activity of rat liver in the absence or presence of methylglyoxal bis(guanylhydrazone)*

The enzyme activity was determined by using partially purified enzyme preparation (1.5mg of protein) in the absence or presence of saturating concentrations of putrescine and varying the concentrations of *S*-adenosyl-L-methionine with or without methylglyoxal bis(guanylhydrazone). The double reciprocals were plotted by the least-squares method. Assay in the presence of putrescine: ○, no additions; □, 0.33μM- and △, 0.67μM-methylglyoxal bis(guanylhydrazone). Assay in the absence of putrescine: ●, no additions; ■, 0.67μM- and ▲, 3.3μM-methylglyoxal bis(guanylhydrazone).

a potent inhibitor of *S*-adenosylmethionine decarboxylase. The apparent K_i for the compound was less than 1μM when the decarboxylation was assayed in the presence of saturating concentrations of putrescine. Much higher concentrations were needed to cause a 50% inhibition in *S*-adenosyl methionine decarboxylase activity in the absence of putrescine. The inhibition constant for liver *S*-adenosylmethionine decarboxylase is in good agreement with those reported by Williams-Ashman & Schenone (1972) and Pegg (1973) for prostate and kidney enzyme respectively.

A few more experiments were undertaken with partially purified liver *S*-adenosylmethionine decarboxylase preparations to evaluate further the interrelations between putrescine and methylglyoxal

bis(guanylhydrazone) in the decarboxylation of *S*-adenosylmethionine. The stimulation of *S*-adenosylmethionine decarboxylase activity by putrescine in the absence or presence of two concentrations of methylglyoxal bis(guanylhydrazone) is illustrated in Fig. 5. In this figure the reciprocal of putrescine concentration is plotted against the reciprocal of $v-v_0$, where v_0 is the reaction rate in the absence of putrescine and v is the rate in the presence of various concentrations of putrescine. This plot gives an apparent K_m for putrescine as the effector of the reaction. The 'effector K_m' for putrescine in the decarboxylation of *S*-adenosylmethionine was about 0.01 mM. The inclusion of 0.5 μM-methylglyoxal bis(guanylhydrazone) resulted in a decrease in this K_m value and a concomitant decrease in the maximum velocity of the reaction. An increase in the concentration of methylglyoxal bis(guanylhydrazone) caused further decreases in the effector K_m value and the maximum velocity. The three lines are approximately parallel (Fig. 5), indicating that methylglyoxal bis(guanylhydrazone) acts as an uncompetitive inhibitor for putrescine. Thus the inhibitor combines with an enzyme form that cannot then bind putrescine, for instance with an enzyme–putrescine complex. The net effect of methylglyoxal bis(guanylhydrazone) on the *S*-adenosylmethionine decarboxylase reaction appears to be a gradual removal of the stimulation of the enzyme activity by putrescine. Thus in the presence of the inhibitor the reaction appears to be saturated at lower concentrations of putrescine and finally the stimulation by putrescine completely disappears. This is in good agreement with the observations made by Williams-Ashman & Schenone (1972).

Inhibition of diamine oxidase by methylglyoxal bis(guanylhydrazone) in vitro

Intraperitoneal injections of methylglyoxal bis(guanylhydrazone) were shown to decrease diamine oxidase activity in thymus. Methylglyoxal bis(guanylhydrazone) was also found to be a potent inhibitor of thymus diamine oxidase *in vitro*. Fig. 6 illustrates the inhibition of diamine oxidase activity by minute concentrations of methylglyoxal bis(guanylhydrazone). The type of the inhibition clearly

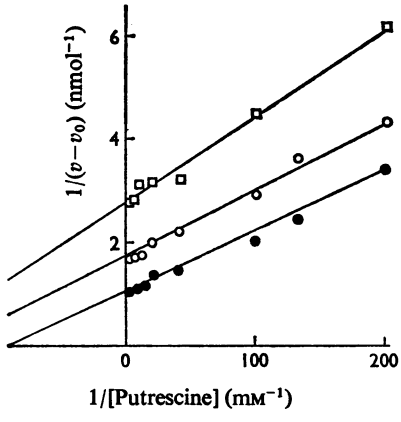

Fig. 5. *Lineweaver–Burk plot of the effect of putrescine on the S-adenosyl-L-methionine decarboxylase activity of rat liver in the absence or presence of methylglyoxal bis(guanylhydrazone)*

The enzyme activity was determined by using partially purified enzyme preparation (0.75 mg of protein) and varying the concentrations of putrescine (the effector of the reaction) with or without methylglyoxal bis(guanylhydrazone). v_0, reaction velocity in the absence of putrescine; v, reaction velocity in the presence of putrescine. The double reciprocals were plotted by the least-squares method. ●, No additions; ○, 0.5 μM-, and □, 1.0 μM-methylglyoxal bis(guanylhydrazone).

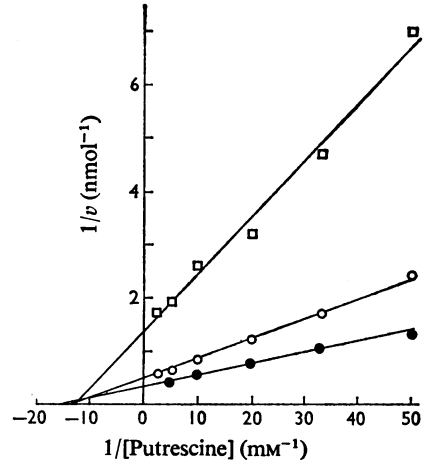

Fig. 6. *Lineweaver–Burk plot of the effect of substrate concentration on the diamine oxidase activity of rat thymus in the absence or presence of methylglyoxal bis(guanylhydrazone)*

The enzyme activity was determined by using dialysed $(NH_4)_2SO_4$ fraction from thymus cytosol as the source of enzyme (0.50 mg of protein) and varying the concentrations of putrescine in the absence or presence of the inhibitor, methylglyoxal bis(guanylhydrazone). The double reciprocals were plotted by the least-squares method. ●, No additions; ○, 0.5 μM-, and □, 2.5 μM-methylglyoxal bis(guanylhydrazone).

appears to be non-competitive with respect to putrescine, compared with the competitive inhibition with respect to *S*-adenosylmethionine and uncompetitive inhibition with respect to putrescine in the *S*-adenosylmethionine decarboxylase reaction. The inhibition constant for methylglyoxal bis(guanylhydrazone) in the diamine oxidase reaction was as low as 0.7 μM, i.e. it was of the same order of magnitude as in the *S*-adenosylmethionine decarboxylase reaction.

The inhibition of diamine oxidase by methylglyoxal bis(guanylhydrazone) is not very surprising since it is known that aminoguanide, an integral part of the methylglyoxal bis(guanylhydrazone) molecule, is a powerful inhibitor of diamine oxidase from various sources (for references see Kapeller-Adler, 1970). It is unlikely that methylglyoxal bis(guanylhydrazone) simply acts as an antagonist of putrescine (an effector for *S*-adenosylmethionine decarboxylase and substrate for diamine oxidase) since concentrations of methylglyoxal bis(guanylhydrazone) up to 1 mM did not have any effect on the activity of spermidine synthase (which uses putrescine as substrate) or of ornithine decarboxylase (which gives putrescine as the product). It is also somewhat unlikely that methylglyoxal bis(guanylhydrazone) acts solely as a carbonyl reagent. Methylglyoxal bis(guanylhydrazone) did not inhibit various pyridoxal phosphate enzymes such as ornithine decarboxylase, tyrosine aminotransferase of rat liver or aromatic L-amino acid decarboxylase from pig kidney. Nor did inclusion of pyridoxal phosphate reverse or prevent this inhibition by methylglyoxal bis(guanylhydrazone) in the diamine oxidase reaction. Further, methylglyoxal bis(guanylhydrazone) did not influence the absorption spectrum of an equimolar pyridoxal phosphate solution in the range 350–500 nm.

Discussion

The increase in biosynthesis and accumulation of the polyamines putrescine, spermidine and spermine is almost always associated with rapid growth [for references see Cohen (1971) and Williams-Ashman *et al.* (1972)]. There is an ever-increasing number of reports of indirect correlations between the synthesis and accumulation of polyamines and tissue or cell growth. However, the physiological functions of these compounds have not been solved. It appears that the only approach to solve the importance of polyamines for living animal cells is to find a specific inhibitor that blocks the synthesis of polyamines under conditions in which they normally accumulate.

Spermidine and spermine are synthesized in mammalian tissues by a pathway involving at least three different enzymes: a putrescine-activated *S*-adenosylmethionine decarboxylase, a spermidine synthase and a spermine synthase (Jänne & Williams-Ashman, 1971*b*; Raina & Hannonen, 1971; Jänne *et al.*, 1971*a*; Hannonen *et al.*, 1972). It appears likely that the first-mentioned enzyme, *S*-adenosylmethionine decarboxylase, is the rate-limiting enzyme in the synthesis of spermidine and spermine in mammalian tissues (Hannonen *et al.*, 1972: Hölttä & Jänne, 1972; Pegg, 1973). The discovery by Williams-Ashman & Schenone (1972) that methylglyoxal bis(guanylhydrazone) acts as a powerful and apparently specific inhibitor only for putrescine-activated *S*-adenosylmethionine decarboxylase gave a very useful tool to study and possibly to solve the function(s) of polyamines in mammalian tissues. In fact Pegg (1973) showed that spermidine synthesis in liver and kidney can be stopped by injections of methylglyoxal bis(guanylhydrazone) as judged by the cessation of the incorporation of [^{14}C]putrescine into spermidine. As shown in the present study, however, one should be very cautious in interpreting the effects of methylglyoxal bis(guanylhydrazone) on cellular metabolism. Intraperitoneal injections of methylglyoxal bis(guanylhydrazone) increase *S*-adenosylmethionine decarboxylase activity in all tissues studied and in some tissues ornithine decarboxylase activity is also stimulated. It is also difficult or impossible to achieve any real decrease in the content of tissue spermidine by using sublethal doses of methylglyoxal bis(guanylhydrazone), since the inhibition of spermidine formation due to the inhibition of *S*-adenosylmethionine decarboxylase is most probably reversed by a very large increase in the activity of the latter enzyme. The stimulation of ornithine decarboxylase also contributes to this. Methylglyoxal bis(guanylhydrazone) did not cause any drastic changes in RNA and DNA synthesis in regenerating rat liver (results not shown in this paper).

In agreement with the observation by Pegg *et al.* (1973) we found that the increase in the *S*-adenosylmethionine decarboxylase was apparently due to a marked prolongation of the half-life of *S*-adenosylmethionine decarboxylase. This might indicate that the inhibitor binds tightly to the enzyme and possibly changes its conformation to one that is more resistant toward the degradative systems.

The effect of methylglyoxal bis(guanylhydrazone) on polyamine metabolism becomes even more complicated because the drug also profoundly affects the degradation of putrescine by diamine oxidase, at least in some rat tissues. The role of diamine oxidase in the metabolism of cellular putrescine is not clear; however, it has been suggested that in tissue containing high diamine oxidase activity, e.g. rat thymus, this activity might be partly responsible for the regulation of tissue putrescine concentration (Beaven & Jong, 1973; Jänne & Hölttä, 1973). The same kind of conclusion can also be drawn from the present

experiments, where a several fold increase in putrescine concentration occurred in thymus after methylglyoxal bis(guanylhydrazone); this was not the case in normal liver not containing soluble diamine oxidase activity.

It seems likely that methylglyoxal bis(guanylhydrazone) is not the most ideal compound to be used as a specific inhibitor of polyamine synthesis in mammalian tissues. Repeated injections of the compound will result in a sequence of events leading to a partial compensation of the inhibitory action of the drug on spermidine synthesis. These events include at least a very large increase in *S*-adenosylmethionine decarboxylase due to a marked prolongation of the half-life of the enzyme and a marked accumulation of putrescine due to partly impaired synthesis of spermidine, increased ornithine decarboxylase activity and, in some tissues, inhibition of diamine oxidase activity both *in vivo* and *in vitro*. Further, nothing is known of the effect of the inhibitor on the conversion of spermine into spermidine (Siimes, 1967) and it is also unknown whether the drug could modify the concentration of *S*-adenosylmethionine.

However, methylglyoxal bis(guanylhydrazone) certainly is the first step to the 'rational pharmacology' of polyamines in mammalian tissues.

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