Regulation of Ribonucleic Acid Synthesis by Polyamines

REVERSAL BY SPERMINE OF INHIBITION BY METHYLGLYOXAL BIS(GUANYLHYDRAZONE) OF RIBONUCLEIC ACID SYNTHESIS AND HISTONE ACETYLATION IN RABBIT HEART

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The relationship between polyamines and RNA synthesis was studied by considering the action of spermine on histone acetylation in perfused heart. In addition, the effect of methylglyoxal bis(guanyldihydrazone), inhibitor of putrescine-activated *S*-adenosylmethionine decarboxylase activity, on RNA and polyamine specific radioactivity and on acetylation of histone fractions was also investigated in perfused heart. Different concentrations of spermine and/or methylglyoxal bis(guanyldihydrazone) were injected into the heart, 15 min after beginning the perfusion. The results demonstrate that spermine stimulates the specific radioactivity of RNA of subcellular fractions. Acetylation of the arginine-rich histone fractions, involved in the regulation of RNA transcription, is enhanced by spermine. The perfusion with methylglyoxal bis(guanyldihydrazone) causes a decrease in the specific radioactivity of polyamines and RNA, and in acetylation of histone fractions. However, spermine is able to reverse the methylglyoxal bis(guanyldihydrazone) inhibition when injected simultaneously. From these results we may assume a possible role for spermine in the regulation of RNA transcription.

During the last 10 years, numerous studies have been carried out to define the biochemical significance of the polyamines spermine and spermidine. It is of particular note that in animal tissues several lines of evidence have indicated a relationship between polyamines and rapid tissue growth (see reviews by Williams-Ashman *et al.*, 1969; Cohen, 1971; Tabor & Tabor, 1972; Bachrach, 1973). An increase in spermine and spermidine content, found in regenerating liver after partial hepatectomy, may be a cause of the elevated rate of nucleic acid synthesis (Raina *et al.*, 1970; Snyder *et al.*, 1970). Similar results were obtained more recently, during experimental myocardial hypertrophy. In fact, the early event, in response to an increased work load of the heart, is a rapid induction of polyamine synthesis (Caldarera *et al.*, 1971; Russell *et al.*, 1971; Feldman & Russell, 1972; Caldarera *et al.*, 1974).

Several studies have been carried out in recent years on methylglyoxal bis(guanyldihydrazone) {1,1'-[(methylethanediyldiene)-dinitrilo]diguanidine}, a specific inhibitor of putrescine-activated *S*-adenosylmethionine decarboxylase activity, which is an enzyme responsible for spermidine synthesis. The

action of this drug has been studied by Williams-Ashman & Schenone (1972) in yeast and rat prostate, and provides a useful means of investigating more deeply the biochemical role of polyamines. Pegg (1973) showed an inhibitory effect on putrescine-activated *S*-adenosylmethionine decarboxylase activity when methylglyoxal bis(guanyldihydrazone) was added *in vitro* to the enzyme obtained from a soluble kidney extract from untreated rats. When the rats were injected with the drug and the incorporation of [¹⁴C]putrescine into polyamines was studied 8 h later, the specific radioactivity of polyamines was considerably decreased. However, by 20 h after administration of the inhibitor, spermidine synthesis was resumed. Human lymphocytes, activated by phytohaemagglutinin, also showed inhibition of the same enzyme activity when incubated with methylglyoxal bis(guanyldihydrazone) (Kay & Pegg, 1973). A paradoxical enhancement was observed by Pegg *et al.* (1973) in dialysed extracts of kidney, ventral prostate and testis of rats previously injected with the drug. It has also been observed that sublethal doses of methylglyoxal bis(guanyldihydrazone) injected into rats induced, after a few days, an evident increase in the activity of putrescine-dependent *S*-adenosylmethionine decarboxylase of normal and regenerating liver and thymus (Hölttä *et al.*,

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1973). The degree of inhibition of putrescine-dependent *S*-adenosylmethionine decarboxylase activity by methylglyoxal bis(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 (Corti *et al.*, 1974).

The effect of the drug on the synthesis of nucleic acids has been studied by several authors. In sarcoma-180 ascites cells, decreases of 43% and about 30% in the rates of DNA and RNA synthesis respectively were found to be caused by methylglyoxal bis(guanylhydrazone) (Sartorelli *et al.*, 1965). Also, when it is added with phytohaemagglutinin to human lymphocytes, it inhibits the incorporation of [^3H]uridine into RNA and [^{14}C]phenylalanine into protein (Kay & Pegg, 1973).

In the work described in the present paper we have carried out experiments to study the relationship between the polyamines, spermine and spermidine, and the modulation of RNA synthesis. In particular we have considered the action of spermine on histone acetylation in perfused and methylglyoxal bis(guanylhydrazone)-treated heart.

Experimental

Materials

Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Spermine tetrahydrochloride was supplied by Fluka A.G., Buchs, Switzerland; acrylamide, *NN'*-bisacrylamide and *NNN'*-tetramethylethylenediamine were purchased from Bio-Rad Laboratories, Richmond, Calif., U.S.A. [$5\text{-}^3\text{H}$]Ribose (2.9 Ci/mmol), [$1,4\text{-}^{14}\text{C}$]putrescine (54 mCi/mmol) and sodium [$1\text{-}^{14}\text{C}$]acetate (59 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Perfusion of hearts

Rabbits (3 months old, weighing about 2 kg) were used in all experiments. The perfusion of the hearts was performed without recirculation, (drip-through) retrogradely through the aorta, in a Langendorff (1895) apparatus. The composition of the perfusion medium was: 154 mM-NaCl, 5.6 mM-KCl, 2.2 mM- CaCl_2 , 5.9 mM- NaHCO_3 , 5.5 mM-glucose. The flow rate of the perfusion medium was 10 ml/min. The radioactive precursors were added to the medium at the beginning of perfusion; 15 min later various concentrations of spermine or methylglyoxal bis(guanylhydrazone), dissolved in the perfusion medium, were injected by a pump into the heart at a rate of 1 ml/min. The left ventricle was

separated at the end of the experiment and used for several determinations.

Isolation of nuclei

The isolation of nuclei was based on the method of Hnilica *et al.* (1966). The rabbit hearts were homogenized in 0.25 M-sucrose containing 2 mM- CaCl_2 and 2 mM- MgCl_2 in an Ultra-Turrax homogenizer. The homogenate was strained through six layers of cheesecloth, underlaid with an equal volume of 0.34 M-sucrose containing 2 mM- MgCl_2 and centrifuged at 1000g for 20 min. The pellet was dissolved in 0.25 M-sucrose containing 1 mM- CaCl_2 and 1 mM- MgCl_2 , underlaid with 0.34 M-sucrose alone and centrifuged at 1000g for 15 min. The nuclei were purified by resuspending the pellet in 2.2 M-sucrose and centrifuging at 105 000g for 60 min.

Separation of subcellular fractions

Subcellular fractions were separated as described by Jones & Jones (1969). The left ventricles were homogenized in 8 vol. of medium containing (final concentrations) 0.25 M-sucrose, 4 mM-Tris-HCl, pH 7.2, and 1 mM-EDTA. After sedimentation of the nuclear fraction by centrifugation at 800g for 10 min, the supernatant was centrifuged at 17 000g for 4 min and the mitochondrial pellet was washed twice in the same buffer. The combined supernatants were centrifuged at 15 000g for 15 min. The ribosomes were sedimented by centrifugation at 105 000g for 45 min. The post-ribosomal fraction was precipitated with 1.2 M- HClO_4 and centrifuged at 10 000g for 10 min.

Analytical methods

[$5\text{-}^3\text{H}$]Ribose (50 $\mu\text{Ci}/100\text{ ml}$ of perfusion medium) was used as precursor of RNA and of the acid-soluble fraction. The RNA of subcellular fractions was determined by the method of Fleck & Munro (1966). The hydrolysed RNA was neutralized, estimated spectrophotometrically by measuring the E_{260} and then dried. Finally, 10 ml of a scintillation 'cocktail' (Insta-Gel; Packard Instrument Co., La Grange, Ill., U.S.A.) was added and the radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer. For the total acid-soluble fraction, the left ventricles were homogenized with 0.6 M- HClO_4 and the homogenate was centrifuged at 5000g for 10 min; the pellet was washed with 0.2 M- HClO_4 . The extracts were collected, neutralized with 4 M-KOH and the KClO_4 was removed by centrifugation. The acid-soluble fraction was adsorbed on an ion-exchange resin column (1 cm $\times$ 12 cm) of Dowex 1 (X8; formate form; 200-400

mesh). This was followed by 50ml of water to displace non-exchangeable material. Elution of total free nucleotides with 10M-formic acid was then carried out until no material absorbing at E_{260} was found in the eluate (Moruzzi *et al.*, 1968). Samples (10ml) of the eluate were dried completely in a vacuum oven at 70°C and redissolved at 20°C in 1 ml of water. Finally, 10ml of scintillation 'cocktail' was added and the radioactivity measured.

The polyamines were determined by the method of Raina & Cohen (1966). [1,4- 14 C]Putrescine (5 μ Ci/100ml) was added to the perfusion medium at the beginning of perfusion. The left ventricles were homogenized in a glass Potter homogenizer in 3% (w/v) HClO_4 . The extracts were neutralized with 4M-KOH and the KClO_4 was removed by centrifugation. The spermine and spermidine were extracted with butanol; the extracts were evaporated to dryness and then dissolved on 0.1 M-HCl. The polyamines were separated by electrophoresis at 9V/cm of Whatman no. 1 paper for 3h in 0.1M-sodium citrate buffer, pH3.5. The spermine and spermidine, stained with ninhydrin, were eluted with water-acetic acid-ethanol (1:5:4, by vol.) containing 2% (w/v) cadmium acetate, and then determined by spectrophotometry at 505nm. For radioactivity measurements, the coloured bands were cut out and placed in counting vials with 10ml of scintillation 'cocktail' (Insta-Gel).

Sodium [1- 14 C]acetate (25 μ Ci/100ml of perfusion medium) was used for acetylation of histone fractions in perfused heart. The histones were extracted from purified nuclei as follows (Hnilica *et al.*, 1966). The nuclear pellet was suspended in 0.14M-NaCl containing 0.01M-trisodium citrate and centrifuged at 1600g for 20min. The sediment was washed in 0.1M-Tris-HCl (pH7.6) and centrifuged at 1600g for 15min. The resulting sediment was extracted with 95% (v/v) ethanol, centrifuged and the histones were extracted with 0.2M-HCl. The histone fractions were precipitated by adding 8vol. of acetone; they were washed twice and dried under vacuum. All the operations were performed at 0-4°C. Analytical gel electrophoresis was performed, as described by Panyim & Chalkley (1969), with 0.9M-acetic acid at 2mA/7.5cm gel at 80-120V. Histones (1mg/ml) were dissolved in 0.9M-acetic acid in 15% (w/v) sucrose. The amount of solution applied to the gels was 50 μ l. The gels were scanned at 280nm by an ISCO model UA-4 absorbance monitor with an ISCO model 658 gel-scanner transport attachment. The histone content was calculated by integration of peak areas; calf thymus histone fraction (20 μ g), extracted and purified by the method of Johns (1964), was used as protein standard. For radioactivity measurements, the gels were stained with 0.1% (w/v) Amido Black in ethanol-acetic acid (20:7, v/v) and de-stained with 0.9M-acetic acid.

The coloured bands were cut out, dissolved in 2M-NaOH and added to 10ml of scintillation 'cocktail' (Insta-Gel).

Results

In the perfusion experiments we have observed that spermine is able to stimulate RNA synthesis. The changes in specific radioactivity of RNA after perfusion with various concentrations of spermine are reported in Fig. 1. All concentrations of this amine tried stimulate [^3H]ribose incorporation into RNA. In particular substantial increases are evident for nuclear and mitochondrial RNA, whose values after treatment with 2mM-spermine are 4-5-fold higher than the control at all times studied. Changes are observed for ribosomal RNA 20min after the injection of spermine; the post-ribosomal RNA fraction is enhanced after treatment with 1.0mM-spermine, but a dramatic fall occurs with 2mM-spermine. In all subcellular fractions, RNA synthesis is enhanced after 5min by the addition of spermine, and continues to increase during the time considered, the only exception being the post-ribosomal fraction in the presence of 2mM-spermine.

Fig. 2 shows the electrophoretic patterns of all histone fractions obtained from rabbit heart in comparison with those from calf thymus. The relative amounts and mobility of each of the five main histone fractions are quite similar. The only detectable difference between histone fractions concerns the appearance of considerable microheterogeneity of histone F1 fraction in the rabbit heart compared with calf thymus.

Table 1 reports the action of spermine on the acetylation of histone fractions. The data show that addition of spermine to perfused heart causes a change in the rate of incorporation of sodium [1- 14 C]acetate into these fractions, particularly into the arginine-rich histones. In fact, 5min after spermine addition, an increase in specific radioactivity is observed of about 200% for histone F2a1, 60% for histone F2a2 and 80% for histone F2b; 10min after spermine addition a considerable increase in the histone F2a1 (+180%) and F2b (+42%) fractions is found, but after 20min only histone F2a1 showed an increase (+41%). Decreased acetylation is observed 20min after spermine perfusion of the heart for the F2a1 and F2b fractions. Acetylation of histone F1 fraction was inhibited by 1mM-spermine throughout.

The changes in polyamine biosynthesis after treatment with methylglyoxal bis(guanyldrazone) are shown in Fig. 3. A progressive decrease in incorporation of [14 C]putrescine into spermidine is noted after the injection of various concentrations of the drug; the maximal inhibition (45%) of spermidine synthesis is noted with a concentration of 2 μ M.

Fig. 1. Effect of spermine on RNA specific radioactivity of subcellular fractions in perfused rabbit heart

The perfusion rate was 10 ml/min. The heart was perfused for 20, 25 and 30 min with perfusion medium containing [^3H]-ribose. At 15 min after the beginning of the perfusion, spermine solutions, at a rate of 1 ml/min, were added for (a) 5, (b) 10 or (c) 20 min. The details for separation of subcellular fractions and for RNA hydrolysis are reported in the Experimental section. Six animals were used for each point; the S.E.M. was less than 10%. ●, Nuclear RNA; □, mitochondrial RNA; ▲, ribosomal RNA; ■, post-ribosomal RNA.

Fig. 2. Electrophoretic patterns of histone fractions

Densitometric tracing of histone fractions from rabbit heart (—) and calf thymus (----). Electrophoresis was performed with 7 cm gels at 2 mA/gel for 3 h 30 min at room temperature.

A slight increase in spermine synthesis, however, is caused by 0.25–1.00 μM -methylglyoxal bis(guanylhydrazone), but with 2 μM the synthesis of this amine is also inhibited by 45%. Fig. 4 shows the incorporation of [^3H]ribose into RNA and total free nucleotides after addition of methylglyoxal bis(guanylhydrazone) to the perfused rabbit heart. The maximum decrease (50%) in the incorporation of labelled precursor into both RNA and free nucleotides is observed with 2.0 μM drug. Slight increases are obtained with low doses of methylglyoxal bis(guanylhydrazone).

Table 2 reports the incorporation of [^3H]-ribose into RNA of subcellular particles under the action of 2.0 μM -methylglyoxal bis(guanylhydrazone) alone or with 1 mM-spermine. The specific radioactivity of nuclear RNA is decreased by 50% under the action of the inhibitor. Addition of spermine together with the drug completely prevents the fall in precursor incorporation and causes the RNA specific radioactivity to rise above the control values. Similar behaviour is noted for the post-ribosomal RNA fraction. Mitochondrial RNA specific radioactivity increases when methylglyoxal bis(guanylhydrazone) is injected together with

Table 1. *Effect of spermine on acetylation of histone fractions of perfused rabbit heart*

The heart was perfused with perfusion medium containing sodium [1-¹⁴C]acetate; 15 min after the start of the perfusion, spermine (1 mM) at a rate of 1 ml/min was added for 5, 10 or 20 min. The data are expressed as d.p.m./μg of protein. For technical details see the Experimental section. The results are means ± S.E.M. with numbers of determinations in parentheses.

Histone fraction	Experimental conditions	Time after injection of 1 mM-spermine (min)	5		10		20	
			(d.p.m./μg)	Change (%)	(d.p.m./μg)	Change (%)	(d.p.m./μg)	Change (%)
F1	Control+Spermine		115 ± 8 (8)		129 ± 8 (6)		150 ± 14 (5)	
			34 ± 6 (8)	-73	85 ± 8 (6)	-34	96 ± 11 (5)	-36
F3	Control+Spermine		160 ± 10 (8)		174 ± 14 (6)		214 ± 19 (5)	
			162 ± 12 (8)	0	191 ± 12 (6)	+10	201 ± 18 (5)	-6
F2b	Control+Spermine		88 ± 6 (8)		114 ± 9 (6)		152 ± 16	
			158 ± 13 (8)	+80	161 ± 13 (6)	+41	168 ± 14 (5)	+10
F2a2	Control+Spermine		129 ± 9 (8)		154 ± 9 (6)		218 ± 14	
			200 ± 10 (8)	+55	178 ± 10 (6)	+16	216 ± 14 (5)	0
F2a1	Control+Spermine		203 ± 11 (8)		233 ± 15 (6)		293 ± 18 (5)	
			594 ± 18 (8)	+192	654 ± 19 (6)	+180	413 ± 21 (5)	+41

Fig. 3. *Effect of methylglyoxal bis(guanyldrazone) on polyamine synthesis in perfused rabbit heart*

The specific radioactivity was expressed as d.p.m./μmol of polyamine. The perfusion rate, without recirculation, was 10 ml/min. The heart was perfused with perfusion medium containing [1,4-¹⁴C]putrescine throughout the period of the experiment (25 min). At 15 min after the beginning of the perfusion, the indicated concentrations of methylglyoxal bis(guanyldrazone), at a rate of 1 ml/min, were injected for 10 min. Six animals were used for each point; the S.E.M. was less than 10%. ●, Spermine; ▲, spermidine.

Fig. 4. *Effect of methylglyoxal bis(guanyldrazone) on [5-³H]ribose incorporation into RNA and total free nucleotides of perfused rabbit heart*

The perfusion rate was 10 ml/min. The heart was perfused with perfusion medium containing [5-³H]ribose throughout the period of the experiment (25 min). At 15 min after the beginning of perfusion, the indicated concentrations of methylglyoxal bis(guanyldrazone), at a rate of 1 ml/min, were injected for 10 min. The RNA hydrolysis and free nucleotide separation are reported in the Experimental section. Six animals were used for each point; the S.E.M. was less than 10%. ●, RNA; ▲, total free nucleotides.

spermine (+100%). Under the same experimental conditions no changes are observed for ribosomal RNA specific radioactivity. The effect of methylglyoxal bis(guanyldrazone), added alone or

together with spermine, on histone acetylation is shown in Table 3. Acetylation of histone F2b and F2a2 fractions is decreased after methylglyoxal bis(guanyldrazone) perfusion, and this inhibition

Table 2. *Effect of methylglyoxal bis(guanyldihydrazone) or methylglyoxal bis(guanyldihydrazone) plus spermine on RNA specific radioactivity of subcellular particles of perfused rabbit heart*

The perfusion rate was 10 ml/min. The heart was perfused with perfusion medium containing [5-³H]ribose for study of RNA specific radioactivity. At 15 min after the beginning of perfusion, methylglyoxal bis(guanyldihydrazone) (2 μ M) alone or together with spermine (1 mM), at a rate of 1 ml/min, was injected for 10 min. The results are means $\pm$ S.E.M. of six determinations in all cases.

Subcellular particles	RNA specific radioactivity (d.p.m./ μ g of RNA)		
	Control	Methylglyoxal bis(guanyldihydrazone)	Methylglyoxal bis(guanyldihydrazone)+spermine
Nuclei	16 $\pm$ 0.7	9 $\pm$ 0.9	20 $\pm$ 0.7
Mitochondria	21 $\pm$ 1.3	20 $\pm$ 1.1	32 $\pm$ 2.0
Ribosomes	45 $\pm$ 2.5	42 $\pm$ 2.8	50 $\pm$ 2.8
Post-ribosomal	58 $\pm$ 3.4	32 $\pm$ 2.0	56 $\pm$ 3.0

Table 3. *Effect of methylglyoxal bis(guanyldihydrazone) or methylglyoxal bis(guanyldihydrazone) plus spermine on acetylation of histone fractions of perfused rabbit heart*

The heart was perfused with perfusion medium containing sodium [1-¹⁴C]acetate, at a rate of 10 ml/min, throughout the period of the experiments (25 min). At 15 min after the beginning of perfusion, methylglyoxal bis(guanyldihydrazone) (2 μ M) alone or together with spermine (1 mM), at a rate of 1 ml/min, was injected for 10 min. The results are means $\pm$ S.E.M. of six determinations in all cases.

Histone fractions	Histone acetylation (d.p.m./ μ g of protein)		
	Control	Methylglyoxal bis(guanyldihydrazone)	Methylglyoxal bis(guanyldihydrazone)+spermine
F1	129 $\pm$ 6	102 $\pm$ 5	88 $\pm$ 4
F3	174 $\pm$ 9	153 $\pm$ 9	391 $\pm$ 15
F2b	114 $\pm$ 6	55 $\pm$ 3	159 $\pm$ 10
F2a2	154 $\pm$ 9	43 $\pm$ 2	192 $\pm$ 10
F2a1	233 $\pm$ 10	188 $\pm$ 6	537 $\pm$ 15

is prevented by the addition of spermine. Further, the acetylation of histone F3 and F2a1 fractions, which is unchanged under the action of methylglyoxal bis(guanyldihydrazone) alone, is enhanced when spermine is also added.

The action of spermidine on perfused and methylglyoxal bis(guanyldihydrazone)-treated heart was not substantially different from that of spermine (C. M. Caldarera, A. Casti, C. Guarnieri, G. Moruzzi, unpublished work).

Discussion

An increase in biosynthesis and accumulation of the polyamines spermine and spermidine is almost always associated with a more rapid incorporation of labelled precursors into nucleic acids (Raina & Jänne, 1970). This phenomenon is particularly evident in regenerating liver (Snyder *et al.*, 1970), in chick-embryo development (Caldarera *et al.*, 1965; Moruzzi *et al.*, 1968; Caldarera & Moruzzi, 1970), in growing cells (Goldstein, 1965) and in

other experimental models (Kostyo, 1966; Caldarera *et al.*, 1969). The development of cardiac hypertrophy is known to be followed by various alterations in myocardial metabolism. In fact, it has been suggested that mechanical or chemical stimulation of myocardial tissue results in an increase in the size of the existing cells (hypertrophy), by stimulation of protein synthesis, and not an increase in cell number (hyperplasia) (Rabinowitz & Zak, 1972). In particular, the formation of RNA and protein is considerably enhanced in the hypertrophying heart (Florini & Dankberg, 1971). We have observed a close relationship between polyamine biosynthesis and the increase in specific radioactivity of RNA of subcellular fractions during the early stage of compensatory hyperfunction of the heart by overload (Caldarera *et al.*, 1974; Moruzzi *et al.*, 1974). These observations may indicate a role for polyamines in the mechanism of activation of protein synthesis. On the other hand, the gene-regulation mechanism involves the histone fractions. In fact, various experiments have shown that the inhibitory effect of histones on RNA synthesis can be removed by

acetylation of arginine-rich fractions (Pogo *et al.*, 1968). In molecular terms, the acetylation of lysine residues results in a decrease in the net positive charge on the basic protein. This would be expected to weaken the interaction between the histones and the negatively charged DNA, and would presumably lead to changes in the fine structure of the chromatin (Allfrey, 1970). Other chemical modifications of histones, such as phosphorylation and methylation, can occur, but phosphorylation is not affected by agents inhibiting RNA synthesis (Hnilica, 1967), and histone methylation occurs well after the general de-repression required by DNA replication (Shepherd *et al.*, 1971).

To study the relationship between polyamines and RNA synthesis, we have carried out experiments on the effect of spermine on [^3H]ribose incorporation into RNA of subcellular fractions and on [^{14}C]acetate incorporation into histone fractions. The increase in specific radioactivity of RNA in subcellular fractions from perfused rabbit heart under the action of different spermine concentrations demonstrates that this biogenic amine is able to stimulate RNA synthesis. To explain the effect of polyamines on stimulation of RNA synthesis, also observed by other authors under different experimental conditions, various hypotheses have been suggested. Abraham's (1968) results and those of other authors (Caldarera *et al.*, 1968; Moruzzi *et al.*, 1975) demonstrated that the polyamines are able to activate DNA-dependent RNA polymerase activities. Other evidence suggests that polyamines, by binding to RNA, prevent the action of ribonuclease (Schlenk & Dainko, 1966). A third hypothesis of polyamine action on RNA synthesis may be related to a modification of the cell-wall membrane in such a way as to facilitate the entry of nucleic acid precursors (Gibson & Harris, 1974; Amatruda & Lockwood, 1974). None of these hypotheses definitively clarifies the role of polyamines in regulation of RNA synthesis. So we have considered the effect of spermine on [^{14}C]acetate incorporation into histone fractions, because, as is well known, histone acetylation precedes the changes in the template activity of the chromatin during gene activation (Berlowitz & Pallotta, 1972).

The separation and characterization by gel electrophoresis of heart histone fractions and comparison with calf thymus histones was carried out since the latter are well characterized (Panyim *et al.*, 1971). Our results, obtained by densitometric tracing of myocardial and thymus histones, demonstrate similar electrophoretic behaviour of histones from the two tissues except that the histone F1 fraction obtained from the heart shows microheterogeneity. Recent studies have clearly shown that some of the microheterogeneity of lysine-rich histones (F1 fraction) which occurs in various organs and species

(Jergil *et al.*, 1970) can be ascribed to variation in the degree of phosphorylation. The results obtained on histone acetylation in the spermine-perfused heart show that this polyamine causes an increase (+200%) in [^{14}C]acetate incorporation into the arginine-rich histone fraction, F2a1, which is involved in the gene-regulation mechanism of RNA synthesis. This result, although difficult to explain, may be related to researches demonstrating that spermine may uncouple the histone-DNA complex and allow RNA transcription to proceed (Schwimmer, 1968; Agrell & Heby, 1971). Moreover, spermine may also act on histone acetyltransferase and histone deacetylase activities, which are responsible for acetate turnover in histone fractions.

The cause-and-effect relationship between spermine and the increase in histone acetylation and RNA synthesis of subcellular particles may be studied by the use of a specific inhibitor of polyamine synthesis, methylglyoxal bis(guanylhydrazone). Earlier reports (Williams-Ashman & Schenone, 1972; Hölttä *et al.*, 1973; Corti *et al.*, 1974) showed that this drug is an inhibitor of putrescine-activated *S*-adenosylmethionine decarboxylase activity, the key enzyme of polyamine synthesis. In accord with Pegg (1973), who observed an inhibitory effect on the enzyme and a decrease in specific radioactivity of the polyamine from rat liver and kidney which had been subjected to methylglyoxal bis(guanylhydrazone) treatment, we have observed a 50% decrease in polyamine synthesis in perfused rabbit heart. Similarly, methylglyoxal bis(guanylhydrazone) causes a decrease in acetylation of histone fractions and in specific radioactivity of nuclear and post-ribosomal RNA. Kay & Pegg (1973) also showed a decrease in RNA and protein synthesis in phytohaemagglutinin-activated and methylglyoxal bis(guanylhydrazone)-treated lymphocytes. The inhibition of the increase in protein synthesis is the earliest event observed after the addition of methylglyoxal bis(guanylhydrazone). Therefore Kay & Pegg (1973) affirm that it would be premature to conclude that polyamines are directly involved in the increase in protein synthesis. Nevertheless, our results demonstrate that inhibition by methylglyoxal bis(guanylhydrazone) of polyamine synthesis causes a decrease in [^{14}C]acetate incorporation into arginine-rich histone fractions, which are responsible for control of protein synthesis. Under these conditions, we have observed that the addition of spermine causes a higher incorporation of [^{14}C]acetate into histone F2a1 and F2a2 fractions. This may suggest that spermine is involved in gene de-repression and consequently in stimulation of protein synthesis. It is likely that some of the polyamine molecules present in the cells are bound to the cellular structures, and some play a metabolic function. Therefore the spermine added may play a metabolic role either by regulating the histone acetyl-

ase of deacetylase enzymes, or by making the histone fractions available to the enzyme, or by both mechanisms.

Another explanation of our results on histone acetylation and RNA specific radioactivity is the formation of a DNA-methylglyoxal bis(guanyldiazotization) complex, which would interfere with RNA synthesis. In fact, complex-formation between the deoxyribonucleosides and methylglyoxal bis(guanyldiazotization) indicates that ionic bonding to phosphate groups and hydrogen-bonding to a ring component in the intact nucleic acid structure may occur (Sartorelli *et al.*, 1965). On the basis of this molecular model, the added spermine may prevent the formation of the DNA-methylglyoxal bis(guanyldiazotization) complex by reversible coupling with DNA in such a way as to allow the RNA transcription to proceed.

In conclusion, our results on perfused heart suggest that spermine is able to stimulate strongly gene transcription by altering the fine structure of chromatin.

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