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Stimulation of Eukaryotic Transcription by Glycerol and Polyhydroxylic Compounds†

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ABSTRACT: In the presence of glycerol, ethylene glycol, glucose, or sucrose there is a marked increase in the transcriptive activity of crude or partially purified rat thymic RNA polymerase II on calf thymus DNA. Transcription stimulation is concentration dependent and is pronounced at concentrations as low as 1–2% (v/v). Glycerol-mediated transcription stimulation is temperature dependent. On a molar basis, transcription stimulation appears to be correlated with the number of hydroxyl substituents (sucrose ≫ glucose > glycerol). From evidence obtained using glycerol and the antibiotic AF/013, we propose that glycerol and similar polyhydroxylic compounds (1) stabilize the association of the RNA polymerase and the DNA template and (2) permit RNA polymerase to transcribe through template stop signals or permit reinitiation to occur. These effects may be due to the capacity of polyhydroxylic compounds to weaken hydrophobic bonding and thereby alter the structure of the DNA template and RNA polymerase during interaction at promoter regions and during transcription. When glycerol is added to thymic nuclear homogenates, endogenous transcription is inhibited. Dimethyl sulfoxide stimulates transcription in reconstituted eukaryotic systems at concentrations up to 10% and then progressively inhibits transcription. These data suggest that polyhydroxylic compounds may be involved in the regulation of eukaryotic transcription. The uniformity of glycerol stimulation in reconstituted eukaryotic and prokaryotic transcriptive systems suggests that the mechanisms regulating transcription are similar. Virtually all eukaryotic RNA polymerase isolation and purification procedures utilize glycerol and/or sucrose, and assays for RNA polymerase contain glycerol as an ingredient of the assay medium or through the addition of RNA polymerase stored in high concentrations of glycerol. These observations suggest, therefore, that studies of eukaryotic transcription inadvertently contain altered transcription kinetics and overestimate RNA polymerase activity.

Glycerol is a component of cellular triacylglycerols and phosphoglycerides and enters the glycolytic pathway after conversion to dihydroxyacetone phosphate (Stryer, 1975). Glycerol is added to cell and enzyme preparations to prevent protein denaturation during freezing and thawing (Ashwood-Smith & Warby, 1972; Derrick et al., 1972; McKee & McCarty, 1973; Fansler & Loeb, 1974; Valeri, 1975). Glycerol is added to muscle preparations to alter surface membranes and permit the direct access of added components to muscle fibers (Yamaguchi & Fujino, 1972; Marston, 1973; Nakajima et al., 1973) and has been injected intravenously following cerebral infarction to reduce cerebral edema (Meyer et al., 1975). Glycerol has been used as a reaction medium, e.g., to facilitate acetylation and carboxymethylation (Bradbury & Jakoby, 1972; Siegel & Awad, 1973; Grove & Levy, 1975). While glycerol has been used extensively in protein isolation procedures and to provide a protective medium during ultracentrifugation, this use has been predicated upon the assumption that glycerol acts as an enzyme stabilizer. We demonstrate in the following paper that glycerol and other polyhydroxylic compounds may act to stimulate RNA polymerase activity in reconstituted eukaryotic transcriptive systems.

Materials and Methods

Materials

Unlabeled ribonucleoside triphosphates, calf thymus DNA, and crystalline bovine serum albumin were obtained from P-L Biochemicals. Anhydrous glycerol was Baker Analyzed Reagent grade. Sucrose, dithiothreitol, and other chemicals were of the highest grade available from Sigma Chemical Co. DEAE[1]-Sephadex A-25 was obtained from Pharmacia Fine Chemicals, Inc., and α-amanitin from Boehringer Mannheim Biochemicals. [5-^{3}H]Uridine 5′-triphosphate (24 Ci/mmol) was ordered from Amersham/Searle Corp. Scintanalyzed toluene was obtained from Fisher Scientific Co., and fluors and tissue solubilizer were from Packard Instrument Co. AF/013 was the generous gift of Dr. Renato Cricchio, Research Laboratories, Gruppo Lepitit, Milan, Italy.

Methods

Isolation of Crude RNA Polymerase from Rat Thymus Glands. Sprague-Dawley rats (breeding stock obtained from Simonsen Laboratories, Gilroy, Calif.) were decapitated and their thymus glands excised, blotted, and minced in cold buffer. Ten minced glands were homogenized in 12 mL of 2.0 M sucrose containing 0.5 M Tris-HCl, 0.025 M KCl, and 0.005 M $MgCl_2$ (2.0 M sucrose–TKM) with five and ten strokes, re-

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[1] Abbreviations used are: BSA, bovine serum albumin; RNAP (in figures), RNA polymerase; DEAE, diethylaminoethyl; DTT, dithiothreitol; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

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TABLE I: Effects of Adding Glycerol at 0 and 187 h on RNAP Activity at 187 h.[a]

glycerol (29%, v/v)	time of glycerol addition (h)	RNAP act. (dpm ± SEM)
−	−	8 226 ± 260
+	0	38 670 ± 1413
+	187	40 536 ± 2360

[a] Male Sprague-Dawley rats (60–120 g) were used to prepare a crude nuclear extract containing a high level of RNA polymerase II (RNAP) activity. Limiting RNA polymerase (150 μg total protein) was then used to transcribe calf thymus DNA (50 μg). The assay medium contained 10 μmol of Tris (pH 7.9); 0.5 μmol of 2-mercaptoethanol; 1.0 μmol of $MgCl_2$; 0.5 μmol of $MnCl_2$; glycerol [29% (v/v), when added]; 0.1 μmol each of ATP, CTP, and GTP; and 0.01 μmol of UTP including 5 μCi of [^{3}H]UTP (16 Ci/mmol). Final volume was 0.25 mL/assay. Following a 15-min incubation at 37 °C, acid-insoluble material was washed, dissolved, and counted as described under Methods. Each value gives the mean ± SEM of six replicates. Counting efficiency was 30%.

spectively, of the B and A pestles of a Dounce homogenizer. Nuclei were isolated according to a modified Blobel & Potter technique (1966). Cell homogenates were diluted to 23 mL with 2.0 M sucrose–TKM, and 21-mL aliquots were layered over 10 mL of 2.3 M sucrose–TKM. Gradients were centrifuged for 60 min at 70 000*g* in a Beckman SW 25.1 rotor. Purified nuclei were then lysed with 15 strokes of a Teflon pestle in a Potter-Elvehjem homogenizer utilizing a 0.05 M Tris buffer containing 0.005 M dithiothreitol, 0.025 M KCl, and 0.1 M NH_4Cl (DTT–Tris), adjusted to pH 8.0 with concentrated HCl. For studies on glycerol effects on endogenous RNA polymerase activity, aliquots of the nuclear homogenate (150 μg of protein) were assayed as described under "RNA Polymerase Assays".

The nuclear homogenate was incubated for 10 min at 25 °C, a procedure which has been found to result in the solubilization of 70% of the total nuclear RNA polymerase activity (Gabourel & Fox, 1971). The nuclear lysate was then centrifuged for 30 min at 280 000*g*. The resultant supernatant contained a high level of RNA polymerase activity and was referred to as soluble crude thymic RNA polymerase.

Purification of Rat Thymic RNA Polymerase II. Crude nuclear extract containing RNA polymerase activity was filtered through an Amicon PM 30 ultrafiltration membrane to reduce the extract volume and to reduce the quantity of small molecular weight proteins. The concentrated material (70–100 mg of protein) was then washed onto a 1.5 × 30 cm DEAE-Sephadex A-25 column with 120 mL of 0.05 M $(NH_4)_2SO_4$ in DTT–Tris buffer. The gradient was then developed with 40 mL of 0.1 M $(NH_4)_2SO_4$ in DTT–Tris, followed by a 320-mL linear gradient of 0.1–0.5 M $(NH_4)_2SO_4$ in DTT–Tris. Glycerol was not used in the purification procedure. A total of 120 4-mL fractions were collected at a flow rate of 1.5 mL/min. Protein was measured at 280 nm, and RNA polymerase activity was determined in each fraction as described under "RNA Polymerase Assays". The major RNA polymerase peaks were identified as RNA polymerases I and II on the basis of sensitivity to 2 μg/mL α-amanitin (e.g., see Chambon, 1975). Peaks at fractions 12 and 35 were 2 and 0% inhibited, respectively, by α-amanitin (RNA polymerase I), while peaks at fractions 41, 51, 71–78, and 87 were 95, 89, 96, and 74% inhibited by α-amanitin (RNA polymerase II). Pooled gradient fractions (71–78) of RNA polymerase II were used to examine glycerol effects on the transcription of calf thymus DNA.

RNA Polymerase Assays. Assays for RNA polymerase activity were performed according to the method of Chamberlin & Berg (1962), as modified by Roeder & Rutter (1969, 1970). Assay mixtures contained 10 μmol of Tris (pH 7.8); 0.5 μmol of 2-mercaptoethanol; 1.0 μmol of $MgCl_2$; 0.5 μmol of $MnCl_2$; 0.1 μmol each of ATP, CTP, and GTP; and 0.01 μmol of UTP. Tenfold increases or decreases in UTP concentration at constant specific activity indicated that the UTP concentration was not limiting. At the time of assay, calf thymus DNA or homologous chromatin (50 μg), variable amounts of glycerol, ethylene glycol, glucose, sucrose, Me_2SO, or AF/013 and crude nuclear RNA polymerase (150 μg of protein; Lowry et al., 1951) or purified RNA polymerase II (15 μg of protein; Popav et al., 1975) were added to give a total volume of 0.25 mL. Reaction rates were then determined by adding 5 μCi of [^{3}H]UTP followed by incubation at 37 °C. Time curves were obtained by removing aliquots at various times from flasks containing scaled-up assay volumes.

Incorporations were terminated by the addition of cold $Na_4P_2O_7$ (100 μg/mL) containing 1 mg/mL BSA, followed by 12% Cl_3AcOH. Precipitated material was collected by centrifugation at 12 000*g* for 10 min. Pellets were washed twice by dissolving them in 0.1 N NaOH followed by reprecipitation by Cl_3AcOH. RNA polymerase activity was then measured by dissolving the washed precipitates in 0.5 mL of Soluene followed by transfer to 5 mL of a scintillation mixture (4 g of diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]-benzene/L of toluene). All values were corrected by subtracting zero time counts (background counts due to nonspecific label binding) obtained by adding [^{3}H]UTP to samples maintained in the cold. Zero time values ranged from 300 to 400 dpm. Tritium was counted at approximately 30% efficiency in a Packard Tricarb scintillation spectrometer, and corrections were made for quenching when necessary with an automatic external standard. Corrections for color quenching by AF/013 were performed using a quenching curve calculated by adding known amounts of [^{3}H]UTP to samples incubated in the presence of variable concentrations of AF/013.

Results

In our original experiments, we isolated a crude thymic RNA polymerase fraction in the absence of glycerol and then stored the enzyme in the presence or absence of 29% (v/v) glycerol at −20 °C in an attempt to obtain data on the stability of RNA polymerase. When we assayed the fractions for RNA polymerase activity at 187 h after isolation, we found a marked reduction in the activity of the RNA polymerase fraction which had been stored without glycerol in comparison to the activity of the RNA polymerase fraction that had been stored in the presence of glycerol (Table I). To our surprise, however, when we added glycerol as a control to fractions which had been stored without glycerol, RNA polymerase activity increased to the level of the fractions that had been stored in glycerol. Increases in activity were similarly observed in an RNA polymerase sample that had been stored for 11 weeks at −20 °C in the absence of glycerol. When 15 μg of purified RNA polymerase II (see Methods) was examined in the incorporation assay outlined in the footnote to Table I, we obtained [^{3}H]UMP incorporation values (± SEM) of 1442 ± 142 dpm in the absence and 4394 ± 142 dpm in the presence of 25% (v/v) glycerol. This represents a 305% stimulation of [^{3}H]-UMP incorporation by 25% glycerol and is comparable to the degree of stimulation observed utilizing crude nuclear RNA polymerase. Subsequent experiments on glycerol effects were performed utilizing crude nuclear extract RNA polymerase.

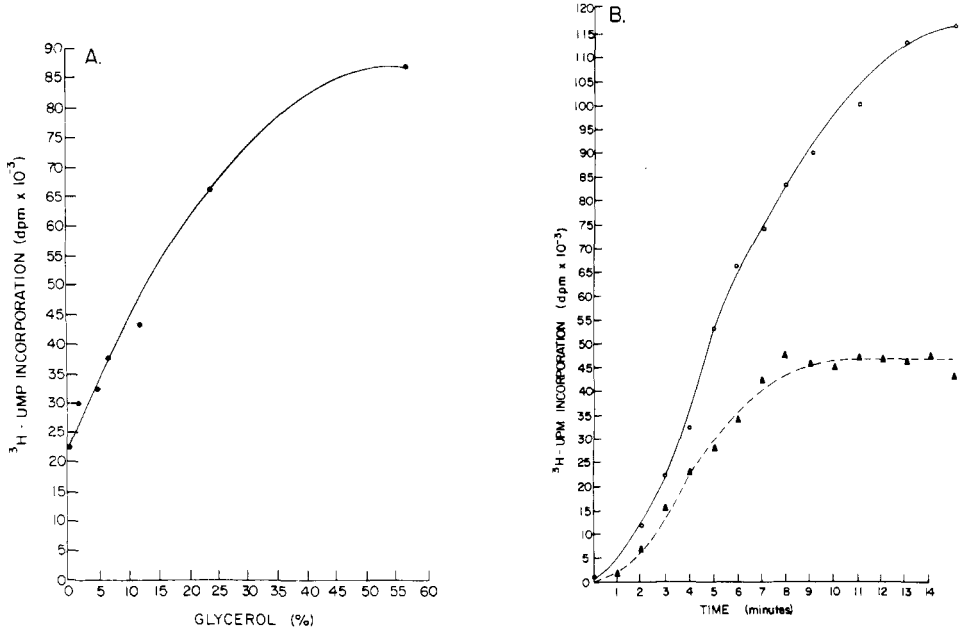

FIGURE 1: (A) Glycerol-mediated stimulation of transcription of calf thymus DNA by rat thymic RNA polymerase. RNA polymerase assays were performed as described in Table I. Glycerol in the concentrations shown was added to assays at 0 °C and prior to the addition of [^{3}H]UTP for a 15-min incorporation assay at 37 °C. Each point is the average of four values. (B) Time course of transcription in the presence (O–O) and absence (▲- - -▲) of glycerol. Glycerol (25%, v/v) or buffer was added to two flasks at 0 °C containing scaled-up reaction volumes. Following addition of [^{3}H]UTP and transfer to a shaking water bath at 37 °C, aliquots (0.25 mL) were removed at the times indicated and counted as described under Methods.

Figure 1A demonstrates the concentration-dependent stimulation of transcription produced by glycerol when crude rat thymic RNA polymerase was used to transcribe the calf thymus DNA. Figure 1B illustrates the time course of [^{3}H]-UMP incorporation in the presence and absence of 25% (v/v) glycerol. While glycerol stimulated an increase in the initial rate of transcription, a more pronounced glycerol effect was to permit incorporation to continue at a high rate for at least 14 min, while incorporation in the absence of glycerol plateaued by 8–10 min. In Figure 2, we have demonstrated that the addition of 25% (v/v) glycerol stimulated transcription even when added after the [^{3}H]UMP incorporation plateau was reached. DNA added at the plateau had no effect (data not shown), as expected from its being present in excess. RNA polymerase added at the plateau, or after glycerol, stimulated incorporation as expected from its being limiting (Figure 2).

In subsequent experiments, we preincubated various components of the transcriptive system with 25% (v/v) glycerol at 0 °C in an attempt to determine if glycerol acted specifically on the RNA polymerase or DNA template or facilitated the cooperative interaction between the RNA polymerase and DNA template to form an active transcriptive complex. In Figure 3, preincubation of the RNA polymerase or DNA template with 25% glycerol and ribonucleoside triphosphates for 2 min at 0 °C was followed by the addition of the DNA template or RNA polymerase, respectively. Transfer to a shaking water bath at 37 °C 2 min later resulted in similar levels of [^{3}H]UMP incorporation at 37 °C during the first 1.75 min, after which the sample in which the DNA template had been preincubated with glycerol incorporated somewhat higher levels of [^{3}H]UMP. Preincubation of the RNA polymerase or DNA template with glycerol at 0 °C was less effective in stimulating later [^{3}H]UMP incorporation at 37 °C than preincubation of the RNA polymerase + DNA template + glycerol and more effective than preincubation of the RNA polymerase + DNA template + buffer.

In order to determine if glycerol stabilized the transcription

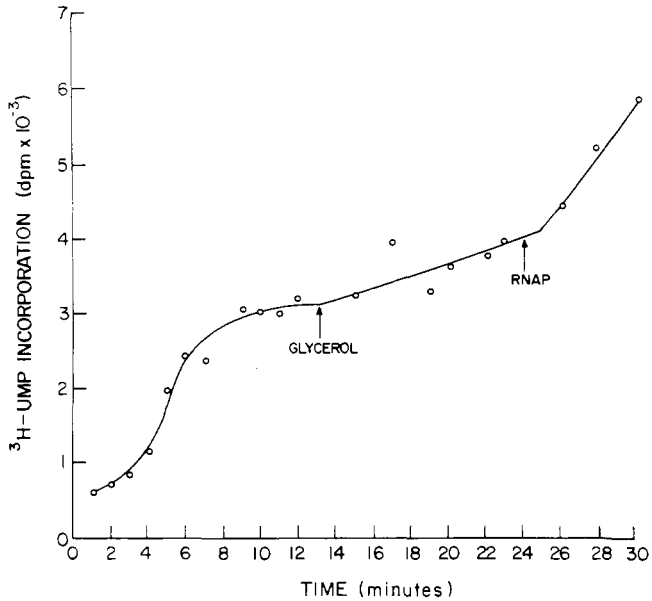

FIGURE 2: Stimulation of transcription by glycerol added at the incorporation plateau. RNA polymerase was assayed as described in Table I. Aliquots (0.25 mL) were removed for counting at the times indicated. A flask containing assay medium, RNA polymerase, and calf thymus DNA was permitted to reach the [^{3}H]UMP incorporation plateau at 37 °C in the absence of glycerol. Glycerol (25%, v/v) was then added at the time indicated and was found to increase the incorporation rate. A twofold increase in the concentration of RNA polymerase present in the assay resulted in a further rapid increase in the incorporation rate. Each point is the average of four to six values.

initiation complex, we made use of the rifamycin antibiotic AF/013. This antibiotic blocks formation of the transcription initiation complex in eukaryotic cells, as rifampicin has been demonstrated to do in prokaryotic cells (Wu & Goldthwait, 1969). We preincubated ribonucleoside triphosphates, RNA polymerase, and AF/013 (100 μg/mL in 3% Me_2SO) for 2

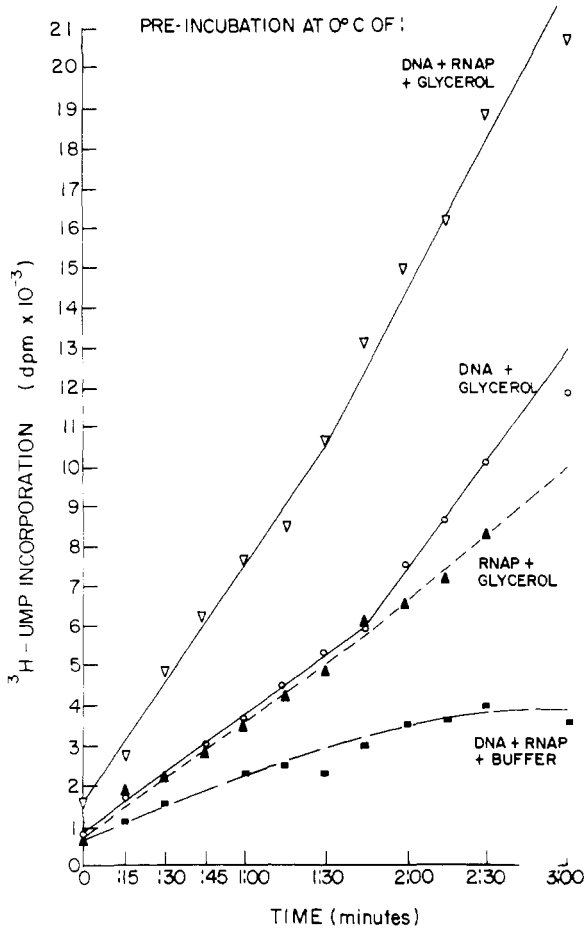

FIGURE 3: Effects of preincubating various components of the assay system with glycerol at 0 °C prior to the transcription assay during the first 3 min at 37 °C. RNA polymerase activity was assayed as described in Table I. Assay components indicated were preincubated together in flasks for 2 min at 0 °C and prior to the addition of RNA polymerase or DNA and [^{3}H]UTP. Two minutes later the flasks were transferred to 37 °C for incorporation assays. Aliquots (0.25 mL) were removed at the times indicated and counted.

min at 0 °C in the presence and absence of 25% (v/v) glycerol and prior to the addition of the DNA template. Two minutes after adding DNA, [^{3}H]UTP was added and the assay flasks were transferred to a shaking water bath at 37 °C. Aliquots were removed for counting at the times indicated. Preincubation of RNA polymerase with AF/013 results in an inactivation of RNA polymerase by AF/013 in the absence of protection of RNA polymerase by complex formation with the DNA template. In Figure 4A, it is apparent that 100 μg/mL AF/013 in 3% Me_2SO markedly reduced [^{3}H]UMP incorporation. In Figure 4B, data are presented from experiments in which RNA polymerase and DNA template are preincubated together with ribonucleoside triphosphates for 2 min at 0 °C in the presence and absence of 25% (v/v) glycerol. Two minutes following the addition of AF/013 (100 μg/mL in 3% Me_2SO), [^{3}H]UTP was added and the incubation flasks were transferred to a shaking water bath at 37 °C. This order of assay component addition allows RNA polymerase to attach to the DNA template which protects it from inactivation by AF/013. As RNA polymerase is limiting, AF/013 is expected to inactivate any RNA polymerase which dissociates from the DNA template or is released from the template following a round of transcription.

Compounds such as glucose, sucrose (Figure 5A), dimethyl sulfoxide (Figure 5B), and ethylene glycol (data not shown) were also effective in stimulating transcription in reconstituted eukaryotic systems. On a molar basis, sucrose was more effective in stimulating transcription than dimethyl sulfoxide, glucose, or glycerol. The stimulatory effects produced by sucrose, glucose, and glycerol appear to be related to the number of hydroxyl groups present. In contrast to the action of these polyhydroxylic compounds, dimethyl sulfoxide stimulated transcription in concentrations up to approximately 10% (v/v) (1.4 M) and then acted as a transcription inhibitor at higher concentrations. The 3% concentration of Me_2SO used to dissolve AF/013 should, therefore, produce a stimulation of transcription, and control curves are provided in those experiments which utilize Me_2SO.

Glycerol transcription stimulation was temperature dependent as shown in Table II. Transcription stimulation by 25% (v/v) glycerol was less pronounced at 23 °C than at 37 °C. Transcription stimulation was more pronounced at 46 than at 37 °C, in spite of decreases in absolute incorporation values. When we examined the effects of glycerol on endogenous RNA polymerase activity utilizing thymic nuclear homogenates (Table III), we found transcription inhibition rather than stimulation. The addition of exogenous calf thymus DNA partially reversed this effect, indicating that glycerol was stimulating transcription of exogenous calf thymus DNA while inhibiting transcription of endogenous chromatin.

Discussion

Glycerol produces a concentration-dependent stimulation of RNA polymerase activity rather than an enzyme stabilization (Table I). It is noteworthy that 2% (v/v) glycerol produces a 33, 5% glycerol a 53, and 25% glycerol a 300% stimulation of [^{3}H]UMP incorporation in a standard assay medium during a 15-min incubation at 37 °C (Figure 1A). Transcription stimulation appears to be maximal in the presence of approximately 60% (v/v) glycerol. This stimulation occurs when crude or purified RNA polymerase is used to transcribe calf thymus DNA. The observation that glycerol stimulates transcription utilizing purified RNA polymerase demonstrates that the stimulatory effects are not due to alterations in nuclease activity. Glycerol has been reported to be without effect on RNase activity up to 30% (v/v), and to inactivate RNase at higher concentrations (Elödi, 1961).

The presence of glycerol in the RNA polymerase assay system permits transcription to continue at a high rate for a longer period of time than that occurring in the absence of glycerol (Figure 1B). Addition of glycerol at the [^{3}H]UMP incorporation plateau reactivates incorporation when RNA polymerase is limiting (Figure 2). This effect suggests that RNA polymerase is completely attached to the DNA template and that incorporation is arrested at the plateau due to failure of RNA chain or RNA polymerase release. If glycerol facilitated the release of RNA polymerase and/or RNA from the DNA template, it could then permit glycerol to facilitate the reformation of an initiation complex. However, the fact that incorporation begins to plateau by 14 min in the presence of glycerol (Figure 1B) suggests that glycerol facilitates RNA chain elongation rather than reinitiation, as facilitated reinitiation should support continued incorporation.

In experiments in which the RNA polymerase or DNA template was preincubated with glycerol (Figure 3), the steeper slope of [^{3}H]UMP incorporation in the assay in which the DNA template was preincubated with glycerol provides some evidence of more pronounced effects on the DNA template than on the RNA polymerase. It seems evident from Figure 3, however, that, since preincubation of either the RNA polymerase or the DNA template with glycerol does not

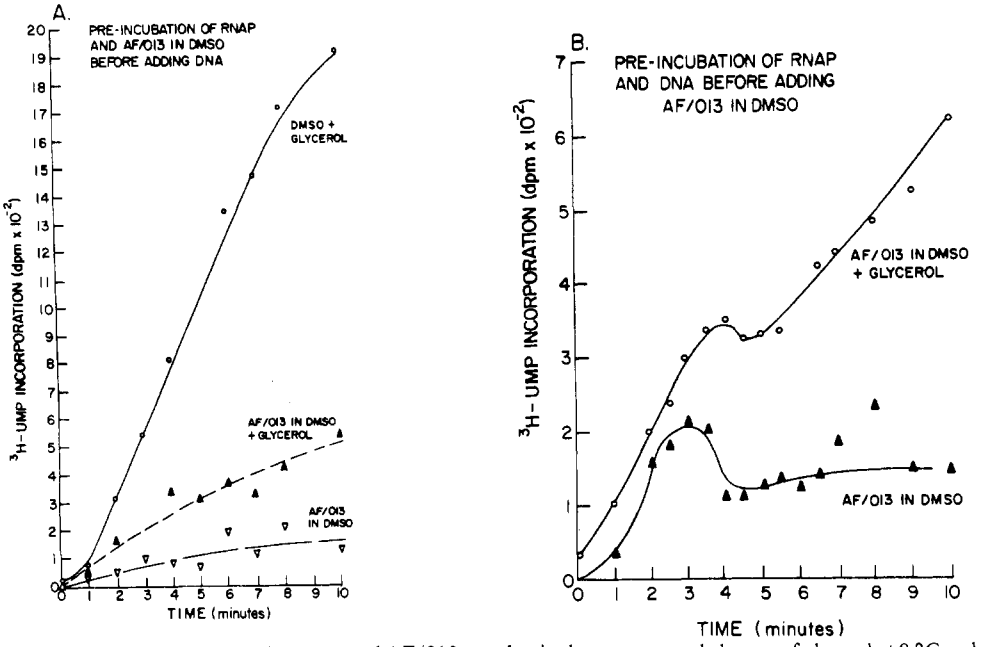

FIGURE 4: (A) Effects of preincubating RNA polymerase and AF/013 together in the presence and absence of glycerol at 0 °C and prior to the addition of calf thymus DNA. RNA polymerase activity was assayed at 37 °C as described in Table I. In flasks containing scaled-up reaction volumes, RNA polymerase (150 μg of protein/assay) was preincubated for 2 min at 0 °C in the presence and absence of glycerol (25%, v/v), followed by the addition of AF/013 (100 μg/mL in 3% Me_2SO). Two minutes later calf thymus DNA (50 μg/assay) was added. Two minutes after the addition of template, [^{3}H]UTP was added and the flasks were transferred to a shaking water bath at 37 °C. Aliquots (0.25 mL) were removed at the times indicated. In the upper control curve, RNA polymerase was preincubated with glycerol and 3% Me_2SO, but AF/013 was not added. Each point is the average of four to six values. (B) Effects of preincubating RNA polymerase and calf thymus DNA in the presence and absence of glycerol at 0 °C and prior to the addition of AF/013, followed by transcription assay at 37 °C. In flasks containing scaled-up reaction volumes, RNA polymerase (150 μg of protein/assay) and calf thymus DNA (50 μg/assay) were preincubated for 2 min at 0 °C in the presence and absence of glycerol (25%, v/v). AF/013 (100 μg/mL in 3% Me_2SO) was then added, and 2 min later [^{3}H]UTP was introduced as the flasks were transferred to a shaking water bath at 37 °C. Aliquots (0.25 mL) were then removed at the times indicated. (DMSO in figure = dimethyl sulfoxide.)

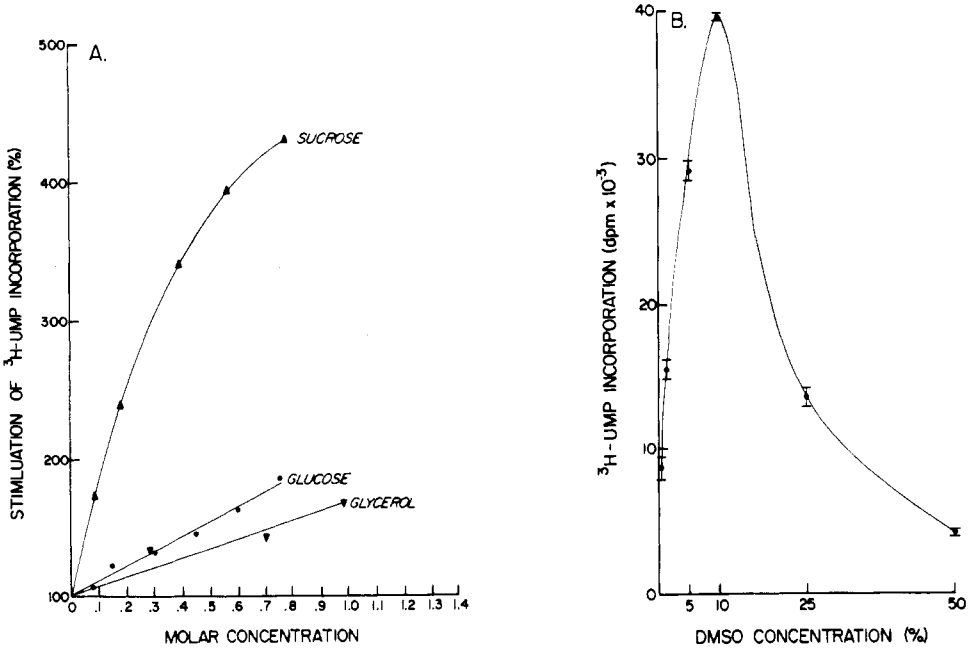

FIGURE 5: (A) Transcription stimulation by polyhydroxylic compounds. RNA polymerase activity was assayed as described in Table I in the presence of various molar concentrations of sucrose, glucose, and glycerol. The stimulatory activity of dimethyl sulfoxide (not shown) was intermediate between glucose and sucrose. Each value is the mean of four to six replicates. (B) Transcription stimulation and inhibition by dimethyl sulfoxide. RNA polymerase activity was assayed in the presence of various concentrations (v/v) of dimethyl sulfoxide (DMSO). Each value is the mean ± SEM of six replicates.

stimulate incorporation as greatly as preincubation of the RNA polymerase and DNA template with glycerol, glycerol must act at least in part to facilitate the interaction of the RNA polymerase and DNA template. Incorporation values for the preincubation of the DNA template + glycerol and RNA polymerase + glycerol were found to sum to the RNA polymerase + DNA template + glycerol curve, suggesting additive glycerol effects on the RNA polymerase and DNA template. An explanation of the glycerol effects that we observe in Figures 1–3 would, therefore, seem to be that glycerol stabilizes the initiation complex and allows RNA polymerase to transcribe through stop signals and subsequent initiation sites.

TABLE II: Temperature-Dependent Stimulation of Transcription by Glycerol.[a]

glycerol (25%, v/v)	incorp (dpm ± SEM)		
	23 °C	37 °C	46 °C
−	4663 ± 247	9617 ± 387	5 430 ± 267
+	6220 ± 1187	45 620 ± 3160	28 407 ± 993
stimulation (%)	133	474	523

[a] Assays were performed as described in the footnote to Table I but at the temperatures indicated. Incorporation values are the mean ± SEM of four replicates.

TABLE III: Glycerol Effects on Endogenous RNA Polymerase Activity in Nuclear Homogenates.[a]

glycerol (25%, v/v)	calf thymus DNA (50 μg)	incorp. (dpm ± SEM)	rel act. (%)
−	−	11 893 ± 490	100
+	−	8 590 ± 510	72
−	+	12 730 ± 147	100
+	+	17 317 ± 463	136

[a] Nuclear homogenate was prepared as described under Methods. Assays were performed as indicated in the footnote to Table I, utilizing 150 μg of nuclear homogenate protein/assay. Glycerol (25%, v/v) and exogenous calf thymus DNA (50 μg) were added as indicated. Incorporation values are the mean ±SEM of four replicates.

The antibiotic AF/013, which blocks formation of the transcription initiation complex in eukaryotic cells (Wu & Goldthwait, 1969), was used in an attempt to determine if glycerol stabilized the initiation complex. When AF/013 (100 μg in 3% Me_2SO) was added to RNA polymerase and prior to the addition of the DNA template, it markedly reduced the extent of glycerol-mediated transcription stimulation (Figure 4A). In contrast to transcription initiation in the presence of glycerol only, there was a 95% inhibition of transcription initiation with 100 μg/mL AF/013 in the absence of glycerol, and a 75% inhibition of transcription initiation with 100 μg/mL AF/013 in the presence of glycerol. While it has been previously reported that 95% inhibition required 400 μg of AF/013 (Juhasz et al., 1972), this may be due to the presence of glycerol, which, as shown in Figure 4A, antagonizes the action of AF/013. The data in Figure 4A demonstrating that AF/013 markedly reduces the magnitude of glycerol-mediated transcription stimulation are consistent with the hypothesis that glycerol acts to stabilize the initiation complex.

When the RNA polymerase and DNA template are preincubated together prior to the addition of AF/013 (Figure 4B), the association of the RNA polymerase with the DNA template protects against AF/013 inactivation. RNA polymerase that dissociates from the DNA template or is released from the DNA template following a round of transcription, however, binds AF/013 and cannot initiate further transcription. In Figure 4B, incorporation during the first minute appears to be increased in the presence of glycerol, which supports the hypothesis that glycerol acts to stabilize the association of RNA polymerase with the DNA template. Incorporation rates between 1 and 3 min do not appear to be significantly different, suggesting that glycerol does not alter the rate of RNA synthesis. In addition, the presence of glycerol appears to permit RNA polymerase to produce a longer average transcript (i.e., incorporation continues beyond 2.5 min). Following an interruption in incorporation at 4.5 min, a new round of transcription appears to occur in the presence of glycerol, supporting the hypothesis that glycerol permits RNA polymerase to transcribe through stop signals or permits reinitiation to occur.

The crucial stage of RNA chain initiation is thought to be the opening of template promoter regions (Travers, 1974a). Glycerol, Me_2SO, and sucrose decrease the T_m of DNA and are thought to alter the conformation of promoter regions by disrupting or reordering the water structure around DNA (Travers, 1974a; Nakanishi et al., 1974). In prokaryotic systems, it has been demonstrated that glycerol may substitute for cyclic AMP and cyclic AMP receptor in stimulating the transcription of specific operons (Nakanishi et al., 1974). The cAMP-CAP complex has been proposed to facilitate the entry of RNA polymerase at an adjacent promoter site by causing destabilization of the GC-rich region between CAP and the polymerase binding site (Dickson et al., 1975). Opening of prokaryotic DNA promoter sites is strongly temperature dependent, and the presence of glycerol or other polyhydroxylic compounds appears to stabilize the open configuration which permits RNA polymerase to attach and initiate transcription (Travers, 1974b). The data shown in Table II demonstrate that glycerol effects in reconstituted eukaryotic systems are also temperature dependent. These data suggest that the mechanism of transcription stimulation by glycerol, ethylene glycol, sucrose, glucose, and Me_2SO involves changes in the DNA template.

Alcohols, glycols, and solvents such as Me_2SO have been found to have a pronounced capacity to denature RNA and DNA. Denaturation is thought to derive from solvent destabilization of hydrophobic bonding, which has been demonstrated to be important in maintaining the secondary structure of polynucleotides (Herskovits et al., 1961; Herskovits, 1962; Fasman et al., 1964). Glycols (e.g., ethylene glycol), which are equivalent to water in terms of hydrogen-bonding properties, provide a medium which weakens polynucleotide hydrophobic interactions and leads to structural alterations (Brahms & Kay, 1962). The effectiveness of alcohols in polynucleotide denaturation increases with solvent chain length and hydrocarbon content. Increasing the solvent hydroxyl group content has no effect on polynucleotide denaturation (Herskovits, 1962). Alkyl-substituted solvents such as Me_2SO are more effective polynucleotide denaturants than alcohols or glycols (Herskovits, 1962). While DNA denaturation determined by increases in absorbance at 259 nm occurred rather abruptly at approximately 80% (v/v) methanol or ethylene glycol (Herskovits, 1962), loss of the helical conformation of poly(cytidylic acid) was dependent upon ethylene glycol concentration, increasing from a slight loss of helical structure at the first glycol concentration examined (10%, v/v) to complete loss of helical structure at 90% ethylene glycol (Fasman et al., 1964). While DNA denaturation in alcohol was reversible, denaturation in ethylene glycol was only partially reversible, and denaturation in Me_2SO was irreversible (Herskovits, 1962).

While polynucleotide denaturation appears to require 80–90% (v/v) alcohol or glycol, partial loss of DNA structure induced by lower concentrations of glycerol and polyhydroxylic compounds could explain, at least in part, the transcription stimulation observed in our experiments. Partial structural loss in DNA could produce opening of promoter regions and facilitate initiation by RNA polymerase. As shown in Figure 5B, Me_2SO produces a dramatic transcription stimulation followed by transcription inhibition. These results are consistent with the observation that Me_2SO is a more effective polynucleotide denaturant than the alcohols or glycols (Herskovits, 1962).

Increasing solvent hydroxyl group content, however, while without effect on polynucleotide denaturation, produces increasing transcription stimulation in our experiments.

Proteins may also be denatured as protein hydrophobic regions are solvated by alcohols, glycols, and solvents such as Me_2SO (Herskovits et al., 1970). While increasing the hydrocarbon content of the solvent increases solvent-denaturing capacity toward both proteins and polynucleotides, increasing the hydroxyl group content tends to decrease the protein-denaturing ability of the alcohols. Thus, both ethylene glycol and propylene glycol are less effective protein denaturants than ethanol and propanol (Herskovits et al., 1970). The loss of some secondary and tertiary protein structure induced by low concentrations of an organic solvent may act to increase or decrease enzyme activity, while high concentrations result in denaturation (Elödi, 1961a,b). In studies on the effects of ethylene glycol on ATPase activity, ATPase activity increased as ethylene glycol concentration was raised to 45% (v/v) and then declined rapidly at higher glycol concentrations. Ethylene glycol disrupted hydrophobic bonding and resulted in a progressive loss of the native ATPase α-helical structure, resulting in ATPase activation followed by inactivation (Brahms & Kay, 1962; Kay & Brahms, 1963).

As in the case of polynucleotide denaturation by alcohols and glycols, protein denaturation generally required higher alcohol and glycol concentrations than those used in our experiments. Moreover, protein-denaturing capacity declined with increasing hydroxyl group content, in contrast to our demonstration of increasing transcription stimulation with increasing hydroxyl group content. Low concentrations of glycerol and related polyhydroxylic compounds, however, could produce alterations in RNA polymerase structure that result in an increased capacity for transcription. In support of this hypothesis is the observation that glycerol, Me_2SO, glucose, and sucrose at concentrations of 20% are used as solvents to perturb surface chromophores (Herskovits & Sorensen, 1968a,b; Herskovits & Greenblatt, 1969).

E. coli premature core RNA polymerase was found to be activated by the σ subunit, by DNA, and by enhancers such as glycerol or high salt concentrations (Saitoh & Ishihama, 1976). This study suggested that preincubation with glycerol or high salt concentrations fostered reassociation and refolding of denatured and dissociated core RNA polymerase. The presence of DNA or the σ subunit could also produce activation, and, although these latter conditions appear unfavorable for protein refolding, they may provide spatial organization that permits RNA polymerase activation. Preincubation of the σ subunit with core RNA polymerase activated RNA polymerase to the same extent in the presence or absence of glycerol. In contrast, DNA-promoted reactivation was higher in the presence of glycerol than in the absence of glycerol. These findings support the hypothesis that glycerol may alter both the DNA template and RNA polymerase and may produce transcription stimulation by facilitating the association of these two components to form an active transcriptive complex.

Glycerol has been previously reported to inhibit purified and chromatin-associated mouse liver hepatoma RNA polymerase II activity (Smith & Duerksen, 1975). Purified hepatoma RNA polymerase II activity was inhibited by glycerol in a concentration-dependent fashion when single- or double-stranded DNA or sheared chromatin was used as template (Smith & Duerkson, 1975). Mouse liver, RA3 adenocarcinoma, and Ehrlich ascites carcinoma RNA polymerase II were inhibited by glycerol, but only Ehrlich ascites carcinoma RNA polymerase I was inhibited (Blair, 1977). While our data indicate that endogenous rat thymic RNA polymerase activity is inhibited by glycerol (Table III), we find glycerol stimulation when crude or purified thymic RNA polymerase II is used to transcribe the calf thymus DNA template. These findings suggest that glycerol affects endogenous transcription differently than transcription in reconstituted transcriptive systems. In addition, glycerol may affect transcription by RNA polymerases I and II differently, depending upon the tissue of origin. It may be hypothesized that increases and decreases in transcriptive capacity may result from a loss of structure due to weakened hydrophobic bonding in the interaction of RNA polymerase and DNA or chromatin templates.

In prokaryotic transcriptive systems, transcription was maximally stimulated in the presence of 20% glycerol. In contrast, we find that maximal stimulation in eukaryotic transcriptive systems is not obtained until 50–60% glycerol is added. This suggests that the eukaryotic RNA polymerase–DNA template interaction is more complex than the prokaryotic interaction. For example, there could be classes of eukaryotic transcriptive regions which require different levels of activation energy to initiate transcription or which have different RNA polymerase association constants under the experimental conditions used. Nonetheless, the similarity of effects of polyhydroxylic compounds in both prokaryotic and eukaryotic transcriptive systems strongly suggests that transcription is regulated in a similar fashion in both systems. The interpretation that we make from the data presented is that the polyhydroxylic compounds stabilize the RNA polymerase–DNA template interaction at promoter regions of eukaryotic templates and permit transcript elongation. While it appears unlikely that polyhydroxylic compounds regulate transcription at the concentrations studied in this paper [e.g., 25% (v/v) glycerol is 3.06 M], transcription regulation may involve polyhydroxylic compounds that induce local structural changes. This regulation may include inhibitory or stimulatory effects dependent upon the conformation of the template and the presence or absence of specific protein regulatory components.

We feel that it is of importance to define the mechanism of glycerol- and sucrose-mediated eukaryotic transcription stimulation because of its capacity to alter transcription kinetics when present at concentrations as low as 1–2%. As virtually every published RNA polymerase isolation and storage procedure utilizes glycerol and/or sucrose, we conclude that virtually all reported data regarding isolated eukaryotic RNA polymerase demonstrates altered transcription kinetics and overestimates RNA polymerase activity. This finding explains repeated observations of increased soluble RNA polymerase activity in the presence of glycerol relative to levels measured endogenously and increases in activity seen upon purification on sucrose gradients. Increases in RNA polymerase activity have been previously ascribed to the loss of subunits or cofactors which act to decrease RNA polymerase activity and as the result of using less repressed templates. Furthermore, observations on the degree of repression of endogenous chromatin in relation to DNA as template for transcription studies are inaccurate in view of our findings that glycerol stimulates transcription on DNA templates but inhibits endogenous RNA polymerase activity.

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