Gallin [9-(2′-Carboxyphenyl)-3,4,5,6-tetrahydroxyxanthene], a New Inhibitor of *Escherichia coli* Ribonucleic Acid Polymerase†

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ABSTRACT: The triphenylmethane dye, gallin, inhibits *Escherichia coli* RNA polymerase by 50% at a concentration of 8 μM. Gallin is a competitive inhibitor of DNA in the RNA polymerase reaction with a K_i of 3.5 μM; the dye affects the apparent K_m of GTP and ATP but not that of UTP and CTP. The apparent K_m and K_i expressed in micromolar concentrations are as follows: ATP, 80 and 16; GTP, 20 and 8.7; CTP, 8.5 and 20; UTP, 34 and 19. Measurement of γ-^{32}P-labeled GTP and ATP incorporation into RNA indicates that low concentrations of gallin inhibit initiation of RNA chains but do not affect RNA chain elongation. The dye binds to RNA polymerase and, at low concentrations, inhibits association of the enzyme with its DNA template. These results suggest that gallin acts by binding to DNA-dependent RNA polymerase, thereby preventing attachment of DNA and the subsequent synthesis of RNA.

Gallin [9-(2′-carboxyphenyl)-3,4,5,6-tetrahydroxyxanthene] (Figure 1) is a member of a family of triphenylmethane dyes that prevent attachment of messenger RNA to ribosomes and, as a result, inhibit protein synthesis in cell-free extracts prepared from bacteria or animal cells (Grollman and Stewart, 1968; Stewart *et al.*, 1971; Siegelman and Apirion, 1971; Huang and Grollman, 1972; Tai *et al.*, 1973; Smith *et al.*, 1973). Gallin and other triphenylmethane dyes inhibit globin synthesis in cell-free extracts prepared from rabbit reticulocytes and the reverse transcriptase activity of Rauscher murine leukemia virus (Liao *et al.*[1]). The present paper describes the effects of gallin on the activity of *Escherichia coli* RNA polymerase. Our results indicate that the dye, at low concentrations, blocks association of RNA polymerase with the DNA template, thereby inhibiting synthesis of RNA.

Experimental Section

Materials. [^{3}H]GTP (14.5 Ci/mmol) and [^{3}H]UTP (12.6 Ci/mmol) were obtained from Schwarz BioResearch; ATP and GTP, labeled in the γ position with ^{32}P, from New England Nuclear; and calf thymus DNA from Worthington. T_3 DNA and [^{14}C]thymidine-labeled adenovirus DNA were generous gifts from Dr. U. Maitra and Dr. M. Horwitz, respectively, of the Albert Einstein College of Medicine. Gallin and [^{3}H]gallin (3.4 Ci/mol) were synthesized by Dr. J. Martin of the Eastern Regional Laboratory, Dow Chemical Co. Actinomycin D and [^{3}H]actinomycin D (3.4 Ci/mmol) were obtained from Schwarz BioResearch. Proflavine and Congo Red were obtained from Nutritional Biochemicals Corp.; 1,10-phenanthroline monohydrochloride from Baker. Rifampicin was a gift from Dow Chemical Co. Frozen *E. coli*, harvested in mid-log phase, were purchased from General Biochemicals.

Preparation and Assay of E. coli RNA Polymerase. RNA polymerase was extracted from *E. coli* and purified by centrifugation on glycerol gradients according to the procedure of Burgess (1969). Protein was measured by the method of Lowry *et al.* (1951), using crystalline bovine serum albumin (fraction V) as a standard. Solutions of DNA were prepared by dissolving calf thymus DNA at a concentration of 1.2 mg/ml in 0.1 M Tris-HCl buffer (pH 7.9) containing 0.5 mM EDTA; the concentration of DNA was determined spectrophotometrically, using an extinction coefficient of 6600 at 260 mμ (Mahler *et al.*, 1964) or by measuring phosphate content (Ames, 1966).

The standard reaction mixture for the assay of RNA polymerase activity contained, in a final volume of 0.25 ml: 0.04 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 120 μg of bovine serum albumin, 12 μg of calf thymus DNA, 0.15 mM each of ATP, GTP, CTP, and [^{3}H]UTP (specific activity, 13.3 Ci/mol), and 5 μg of purified *E. coli* RNA polymerase. Reactions were initiated by addition of enzyme, incubated for 10 min at 37°, and terminated by chilling in ice and adding 3 ml of 5% trichloroacetic acid containing 0.01 M sodium pyrophosphate. After standing at 4° for 15 min, precipitates were collected on 2.5-cm Whatman GF/C glass filters and washed with 2% trichloroacetic acid containing 0.01 M sodium pyrophosphate. Radioactivity was determined by liquid scintillation counting in 10 ml of toluene containing 0.5 ml of Triton X-100 and 57 mg of 2,5-diphenyloxazole.

Incorporation of γ-^{32}P-Labeled Ribonucleoside Triphosphates. Initiation of RNA chains was determined by incorporation of γ-^{32}P-labeled ATP or GTP into acid insoluble material. Conditions were similar to those described above for the assay

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[1] L. L. Liao, S. B. Horwitz, D. Steward, J. Martin, M. T. Huang, and A. P. Grollman, unpublished observations.

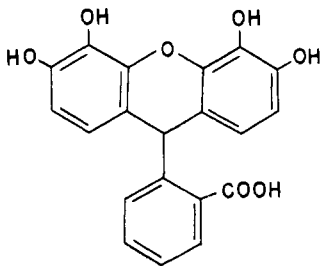

FIGURE 1: Structural formula of gallin.

of RNA polymerase. The assays contained, in a final volume of 0.25 ml: 0.04 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 120 μg of bovine serum albumin, 23 μg of calf thymus DNA (when labeled GTP was used), or 56 μM T_3 DNA (when labeled ATP was used), 12.5 μg of RNA polymerase, and 80 μM ATP, CTP, UTP, and [γ-^{32}P]GTP (1.46×10^6 cpm/nmol) or 80 μM GTP, CTP, UTP, and [γ-^{32}P]ATP (1.73×10^6 cpm/nmol). After incubation for 60 min at 37°, the reaction mixtures were chilled in ice, and 0.05 ml of bovine serum albumin solution (5 mg/ml) was added, followed by 0.3 ml of 7% $HClO_4$. The resulting precipitate was centrifuged for 5 min at 1500*g*, and the pellet was dissolved in 0.3 ml of ice-cold 0.2 N NaOH. This was followed by addition of 5 ml of cold 5% trichloroacetic acid solution containing 0.01 M sodium pyrophosphate. After 5 min, the acid-insoluble material was collected by centrifugation, and the washing procedure was repeated twice more (Maitra *et al.*, 1967). The final acid-insoluble material was prepared for determination of radioactivity as described above. Nonspecific background, determined by adding perchloric acid at zero time, was less than 0.2 pmol of ^{32}P.

Formation of Enzyme–DNA Complex. Formation of complex between RNA polymerase and [^{14}C]adenovirus DNA was determined by filtration through nitrocellulose membranes (Jones and Berg, 1966). Incubation mixtures contained, in a final volume of 0.25 ml: 0.04 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 5 μg of RNA polymerase, and 0.25 μg of [^{14}C]adenovirus DNA (3300 cpm). Following incubation for 5 min at 37°, reaction mixtures were diluted with 2 ml of ice-cold 0.01 M Tris (pH 7.9) containing 0.05 M NaCl, and filtered under gentle suction. Filters were washed with 40 ml of the Tris-NaCl buffer and radioactivity was determined as described above.

Sucrose Density Gradients. Twelve-milliliter 5–20% Linear sucrose gradients, containing 0.05 M KCl, 0.01 M Tris (pH 7.9), 5 mM 2-mercaptoethanol, and 0.5 mM EDTA (Wehrli *et al.*, 1968), were centrifuged in an SW 41 rotor at 32,000 rpm for 17 hr at 4°. The tubes were punctured, and 0.8-ml fractions were collected from the bottom of the gradient. Each fraction was mixed with 10 ml of scintillation fluid and the radioactivity determined as described above.

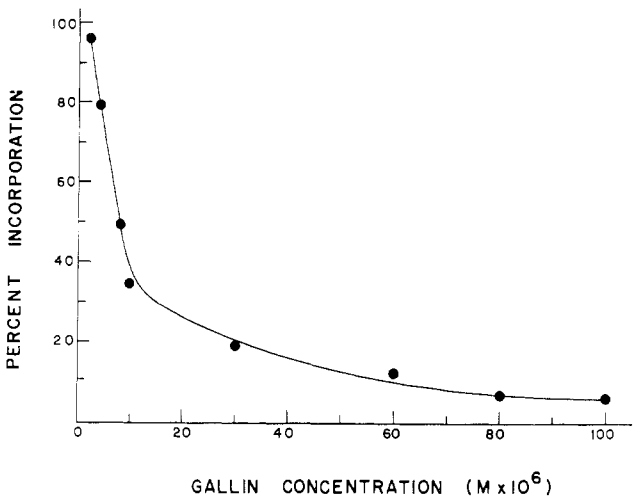

FIGURE 2: Effect of gallin on the activity of *E. coli* RNA polymerase. Enzyme activity was assayed by measuring incorporation of [^{3}H]UMP into trichloroacetic acid insoluble material as described in the Experimental Section; gallin was present at the final concentrations indicated in the figure. The per cent incorporation shown is expressed relative to the control in which 650 pmol of [^{3}H]UMP was incorporated into acid-insoluble material in 10 min.

Results

Effect of Gallin on RNA Polymerase Activity. Gallin inhibits the synthesis of RNA by approximately 50% at a concentration of 8 μM (Figure 2). A similar degree of inhibition is observed when gallin is preincubated with DNA and the four ribonucleoside triphosphates prior to addition of the enzyme (Table I). Preincubation of gallin with RNA polymerase, four ribonucleoside triphosphates, and other components of the reaction mixture for 5 min at 37°, prior to addition of DNA, enhances inhibition.

Binding of [^{3}H]Gallin to E. coli RNA Polymerase. [^{3}H]Gallin was incubated with RNA polymerase for 10 min at 37°, then sedimented through a 5–20% neutral sucrose gradient (Figure 3). Fractions of the gradient were assayed for radioactivity and RNA polymerase activity. Most of the radioactive dye remains at the top of the gradient; however, a small but significant fraction of radioactivity sediments with the enzyme. Under similar conditions, [^{3}H]actinomycin does not bind to the RNA polymerase. Based on a molecular weight of 500,000 for RNA polymerase (Burgess, 1971), we calculate that 132

TABLE I: Effect of Preincubation on Inhibition of RNA Synthesis.[a]

Components Present During First Incubation	Components Present During Second Incubation	Incorporation of [^{3}H]UMP (pmoles)	Inhibition (%)
None	DNA, enzyme, triphosphates	660	
None	DNA, enzyme, triphosphates, gallin	290	56
DNA, triphosphates, gallin	Enzyme	280	57
Enzyme, triphosphates, gallin	DNA	90	86

[a] RNA synthesis was measured as described in the Experimental Section. First incubation was at 27° for 5 min; second incubation was at 37° for 10 min. Gallin was present at a concentration of 10 μM. Ribonucleoside triphosphates used were ATP, GTP, UTP, and CTP.

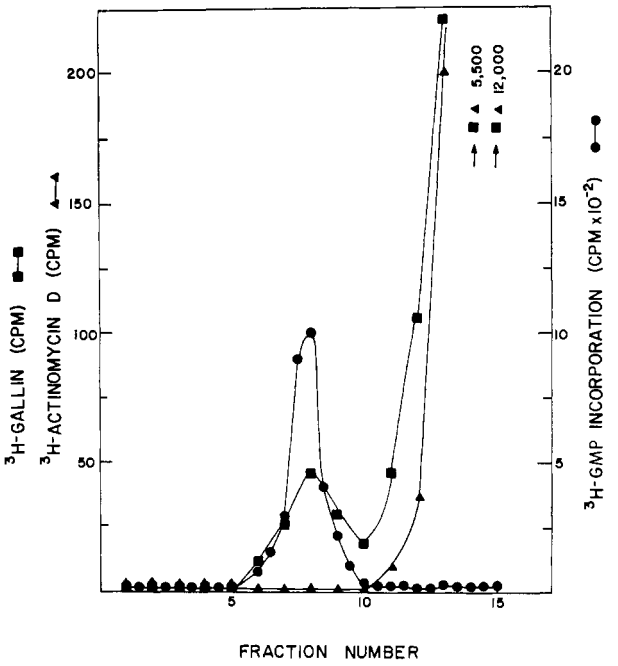

FIGURE 3: Binding of [^{3}H]gallin to *E. coli* RNA polymerase. Reaction mixtures contained, in a final volume of 0.1 ml: 0.04 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 660 μg/ml of RNA polymerase, and either 200 μM [^{3}H]gallin (18,000 cpm) or 200 μM [^{3}H]actinomycin D (18,000 cpm). The mixtures were incubated at 37° for 10 min, chilled in an ice bath, and centrifuged on sucrose density gradients as described in the Experimental Section. The position of the enzyme in the gradient was determined by assaying individual fractions for enzymatic activity in a second tube containing 330 μg/ml of RNA polymerase and no gallin: (●) control, RNA polymerase activity ([^{3}H]GMP incorporated/0.4-ml fraction); (■) [^{3}H]gallin (cpm/0.8-ml fraction); (▲) [^{3}H]actinomycin (cpm/0.8-ml fraction).

pmol of enzyme bind 140 pmol of [^{3}H]gallin; thus, the molar ratio of bound gallin to enzyme is approximately 1.

In a similar experiment, the activity of RNA polymerase was determined after separation of the complex from the free dye by sucrose density gradient centrifugation (Table II). Compared to an uninhibited control, the activity of enzyme containing bound gallin was reduced by 34%.

TABLE II: Activity of Gallin-Treated RNA Polymerase Following Sucrose Gradient Centrifugation.[a]

Inhibitor Added	[^{3}H]UMP Incorporation (nmol)	Total Protein (μg)	Specific Activity (nmol/μg)	Inhibition (%)
None	24	66	0.36	
Gallin	16	66	0.24	34

[a] Experimental conditions were described in the legend to Figure 3. Fractions corresponding to numbers 6, 7, 8, and 9 (Figure 3) were pooled (3.2 ml), and the protein concentration and enzyme activity were determined. The enzyme activity was assayed in a reaction mixture (0.5 ml) containing 0.04 M Tris-HCl (pH 7.9), 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 0.2 mM ATP, GTP, CTP, and [^{3}H]UTP (specific activity, 13.3 Ci/mol), 23 μg of calf thymus DNA, and 0.4 ml of the pooled fractions. After 20 min incubation at 37°, incorporation of [^{3}H]UMP into RNA was determined as described in the Experimental Section.

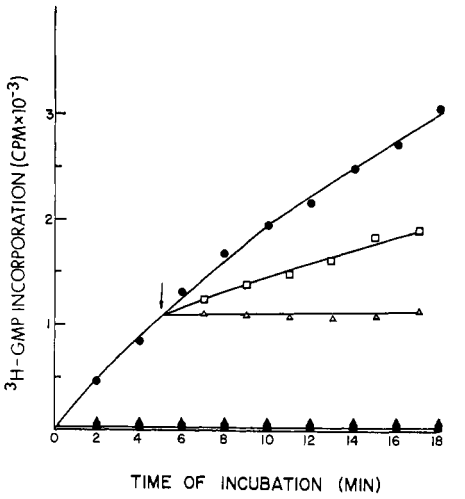

FIGURE 4: Effect of gallin and actinomycin on RNA synthesis. Incubation mixtures contained, in a final volume of 3 ml: 100 mM Tris-HCl (pH 7.9), 200 mM KCl, 12 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol, 0.75 mM ATP, CTP, UTP, and [^{3}H]GTP (13.3 Ci/mol), 60 μg/ml of calf thymus DNA, and 20 μg/ml of RNA polymerase. After incubation at 35° for the indicated time, 0.1-ml samples were removed and assayed for [^{3}H]GMP incorporation into trichloroacetic acid insoluble radioactivity. The standard reaction mixture described in the Experimental Section was used except that [^{3}H]GTP was substituted for [^{3}H]UTP: (●) uninhibited control; (□) 100 μM gallin was added at time indicated by arrow; (△) 10 μM actinomycin was added at time indicated by arrow; (■) reaction started at zero time by addition of DNA following 25 min preincubation at 4° with 100 μM gallin and all other components of assay mixture; (▲) reaction started at zero time by addition of DNA following 25 min preincubation at 4° with 10 μM actinomycin and all other other components of assay mixture.

Effect of Gallin on Initiation of RNA Synthesis and Elongation of RNA Chains. Preincubation of enzyme with 100 μM gallin for 25 min at 4° prior to addition of DNA resulted in complete inhibition of RNA synthesis (Figure 4). Addition of the same concentration of gallin 5 min after the start of the enzyme reaction partially reduced the rate of RNA synthesis. In contrast, actinomycin markedly inhibits elongation of RNA chains (Richardson, 1966; Maitra *et al.*, 1967) and immediately stops the synthesis of RNA when added after the reaction has begun.

Incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP into RNA was used to elucidate the mechanism by which gallin inhibits RNA synthesis (Table III). At concentrations of 10 and 20 μM, gallin inhibits initiation of RNA synthesis (measured by the incorporation of [γ-^{32}P]GTP) to the same extent as total RNA synthesis (measured by incorporation of [^{3}H]UMP). Calf thymus DNA was used as template in these experiments; similar results were observed with T_3 DNA as template when [γ-^{32}P]ATP was used to measure RNA chain initiation (Table IV). At a concentration of 100 μM, gallin inhibits total RNA synthesis or the elongation of RNA chains to a greater extent than the initiation of new RNA chains. At concentrations of 0.4 and 0. 8 μM, actinomycin inhibits RNA chain elongation significantly more than RNA chain initiation.

Kinetics of Inhibition. Inhibition of RNA polymerase activity by gallin is diminished by increasing the concentration of DNA (Figure 5A). A double reciprocal plot of the rate of RNA synthesis *vs.* DNA concentration suggests that gallin is a competitive inhibitor of DNA (Figure 5B). The apparent K_m for DNA and K_i for gallin are 8.6 and 3.5 μM, respectively.

Incorporation of four nucleoside triphosphates into RNA in the absence and presence of 10 μM gallin is shown in Figure

TABLE III: Effect of Gallin and Actinomycin D on Incorporation of [γ-^{32}P]GTP and [^{3}H]UMP into RNA.[a]

Inhibitor Added (M)	Nucleotide Incorporated [γ-^{32}P]GTP (pmol)	[^{3}H]-UMP (nmol)	Ratio [^{3}H]/[^{32}P]	Inhibition of Incorporation [γ-^{32}P]-GTP (%)	[^{3}H]-UMP (%)
None	1.41	2.93	2075		
Gallin					
1×10^{-5}	1.07	2.19	2045	24	25
2×10^{-5}	0.89	1.82	2045	37	38
1×10^{-4}	0.73	1.16	1606	49	60
Actinomycin D					
4×10^{-7}	1.08	0.92	854	24	68
8×10^{-7}	1.06	0.57	542	25	80

[a] The reaction mixture (0.25 ml) was described in the Experimental Section.

TABLE IV: Effect of Gallin and Actinomycin on Incorporation of [γ-^{32}P]ATP and [^{3}H]UMP into RNA.[a]

Inhibitor Added (M)	Nucleotide Incorporated [γ-^{32}P]ATP (pmol)	[^{3}H]-UMP (nmol)	Ratio [^{3}H]/[^{32}P]	Inhibition of Incorporation [γ-^{32}P]-ATP (%)	[^{3}H]-UMP (%)
None	2.22	5.13	2330		
Gallin					
1×10^{-5}	1.60	3.98	2480	27	23
2×10^{-5}	1.27	3.00	2362	43	42
1×10^{-4}	0.90	1.52	1696	60	70
Actinomycin D					
4×10^{-7}	1.27	0.77	600	43	85
8×10^{-7}	1.24	0.48	389	44	91

[a] The reaction mixture (0.25 ml) was described in the Experimental Section.

6. Gallin appears to be a noncompetitive inhibitor of incorporation of UTP and CTP into RNA chains. The V_{max} is decreased in the presence of the dye, but the apparent K_m for UTP and CTP remains unchanged. The effect of gallin on incorporation of ATP and GTP into RNA chains is reflected in both the V_{max} and apparent K_m for these triphosphates. The apparent values for K_m and K_i, expressed in micromolar concentrations, are: ATP 80 and 16; GTP, 20 and 8.7; CTP, 8.5 and 20; UTP, 34 and 19.

Effect of Gallin and Other Inhibitors of RNA Polymerase on Binding of DNA to RNA Polymerase. The effect of gallin on the formation of an adenovirus DNA–RNA polymerase complex was measured by the Millipore membrane filter technique (Jones and Berg, 1966). This method is based on the observation that the DNA–enzyme complex is quantitatively retained by the filter. At a concentration of 3 μM, gallin inhibits by 50% the formation of a complex between *E. coli* RNA polymerase and [^{14}C]adenovirus DNA (Figure 7). The amount of adenovirus DNA used in this experiment (0.25 μg) results in the synthesis of 70 pmol of RNA in 10 min. In control experiments, neither actinomycin nor rifampicin prevented the formation of the DNA–enzyme complex even when these inhibitors of RNA polymerase activity were present at a concentration of 40 μM.

Several established inhibitors of RNA synthesis were assayed to determine the concentration at which they inhibited RNA synthesis and the formation of the DNA–enzyme complex (Table V). All of the compounds tested, except 1,10-phenanthroline, inhibited the synthesis of RNA by 50% at a concentration of 15 μM or less; however, only gallin and

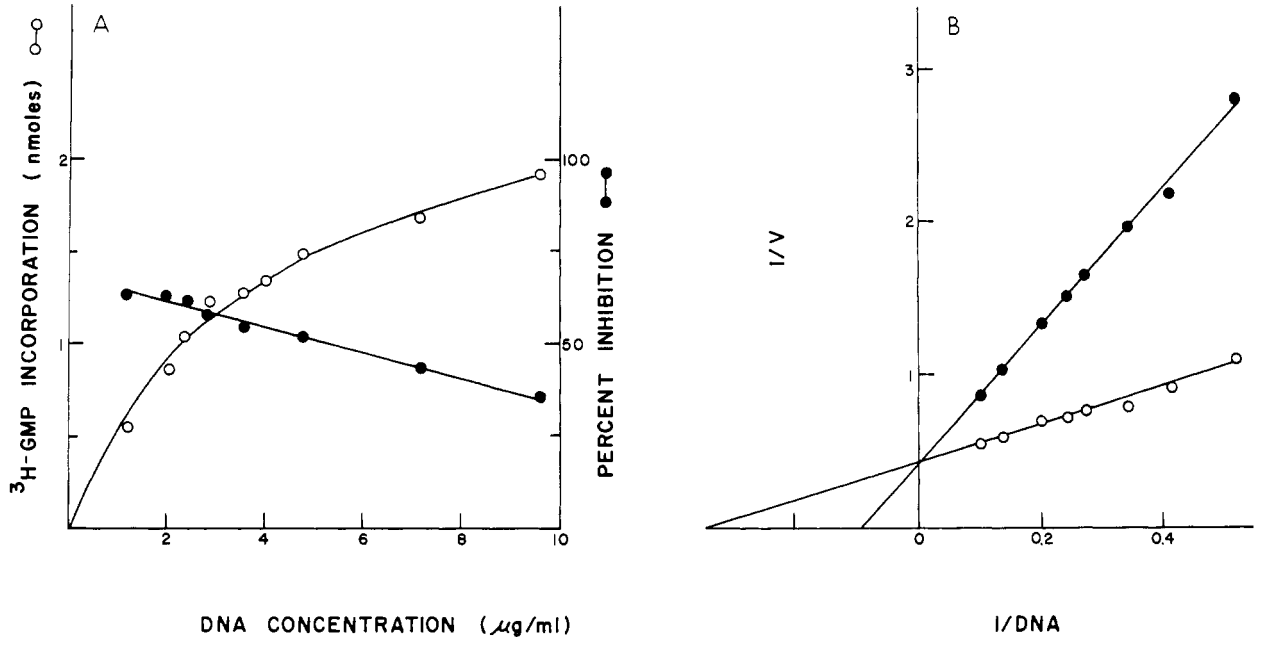

FIGURE 5: Effect of varying concentrations of DNA on inhibition of RNA polymerase by gallin. RNA polymerase activity was determined by measuring incorporation of [^{3}H]GMP into trichloroacetic acid insoluble material during a 20 min incubation at 37°. The standard reaction mixture described in the Experimental Section was used except that [^{3}H]GTP was substituted for [^{3}H]UTP. Calf thumus DNA was present at the final concentration indicated in panel A. These data are plotted in panel B according to the method of Lineweaver–Burk. The method of least squares was used to determine the best fitting line: (○) RNA synthesis; (●) RNA synthesis in the presence of 10 μM gallin.

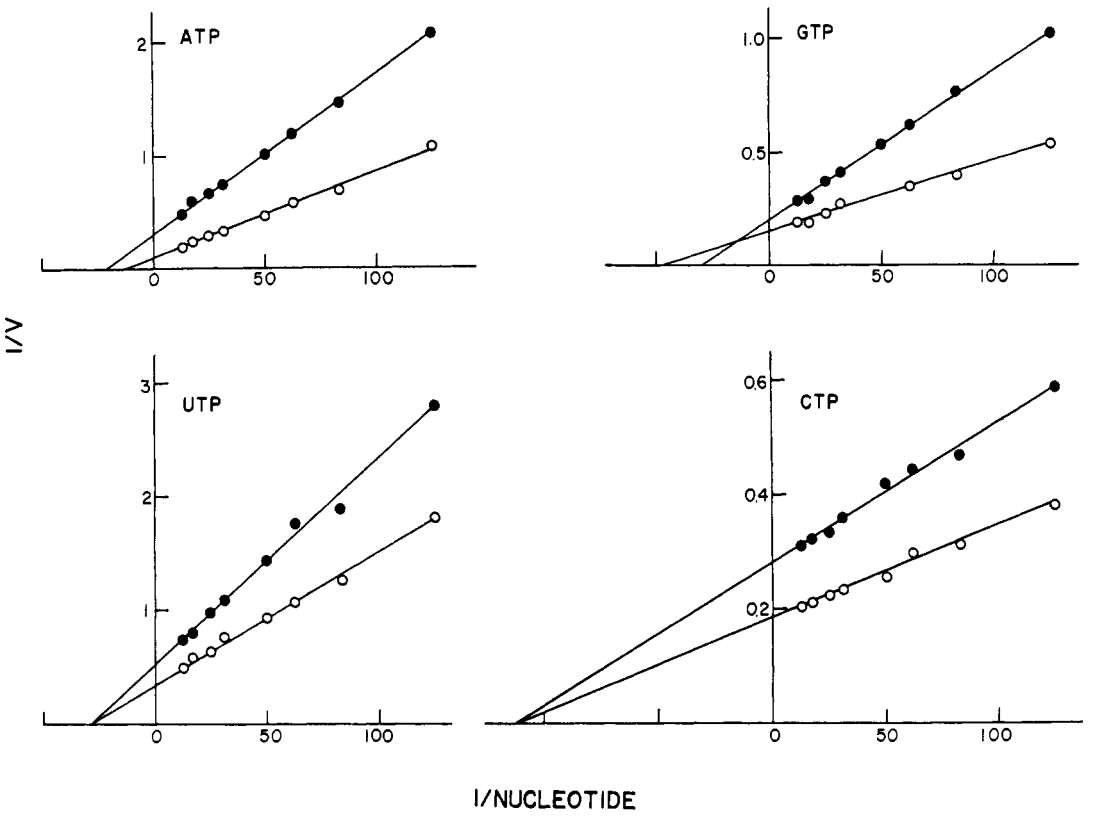

FIGURE 6: Effect of gallin on the kinetics of RNA synthesis while varying the concentration of a single ribonucleoside triphosphate. The general conditions of the reaction are described in the Experimental Section, except that the concentration of one ribonucleoside triphosphate was varied and the concentration of the other three ribonucleoside triphosphates was fixed at 160 μM. Incorporation of [^{3}H]UMP was determined when the concentration of ATP, GTP, or CTP was varied, and the incorporation of [^{3}H]GMP was determined when the concentration of UTP was varied. The reciprocal of the rate of RNA synthesis expressed as cpm $\times 10^{-3}$ is shown on the Y axis. The X axis shows the reciprocal of the ribonucleoside triphosphate concentration expressed as mM. The method of least squares was used to determine the best fitting line: (○) RNA synthesis; (●) RNA synthesis in the presence of 10 μM gallin.

Congo Red inhibited the formation of the DNA–enzyme complex by 50% at concentrations less than 40 μM.

Since it has been reported that proflavine sulfate (10 μg/ml) inhibits the binding of *E. coli* RNA polymerase to T_7 DNA (Richardson, 1966), the effect of different concentrations of proflavine on the binding reaction and on the synthesis of RNA were compared (Figure 8). There is no indication that binding of RNA polymerase to adenovirus DNA is inhibited even at concentrations that totally inhibit synthesis of RNA with calf thymus DNA as template.

TABLE V: Effect of Inhibitors on RNA Synthesis and on Formation of a DNA–RNA Polymerase Complex.[a]

Inhibitor Added	Concentration Required for 50% Inhibition: RNA Synthesis (μM)	DNA–Enzyme Complex (μM)
Gallin	8.0	3
Actinomycin D	0.2	>40
Congo Red	3.0	0.2
1,10-Phenanthroline	1100	>1000
Proflavine	15	>40
Rifampicin	0.1	>40

[a] RNA synthesis was measured as described in the Experimental Section. In the control reaction, 650 pmol of [^{3}H]UMP was incorporated into acid-insoluble material in 10 min. DNA–enzyme complex formation was measured as described under the Experimental Section.

Effect of Gallin and Congo Red on Dissociation of the DNA–RNA Polymerase Complex. The DNA–RNA polymerase complex was prepared as described in the Experimental Section, and the effect of gallin and Congo Red on the dissociation of this complex was measured by determining the amount of radioactive DNA retained on a nitrocellulose membrane filter after a 30-min incubation at 37° of complex and dye (Table VI). Although both compounds dissociated the complex in 30 min, Congo Red was approximately 100 times more active in this assay than gallin.

Discussion

The primary inhibitory effect of gallin on the activity of *E. coli* RNA polymerase appears to be on initiation of RNA chains. Evidence supporting this conclusion includes the following experimental observations: (a) gallin inhibits incorporation of γ-labeled GTP and ATP into RNA, (b) inhibition of enzyme activity is enhanced if the dye is incubated with the enzyme prior to initiation, and (c) the rate of chain elongation is unaffected at concentrations of dye that significantly inhibit RNA synthesis.

Kinetic studies of RNA polymerase inhibition provide further insight into the action of the dye. Gallin is a competitive inhibitor of DNA and a noncompetitive inhibitor of incorporation of UTP and CTP into RNA. Inhibition of incorporation of ATP and GTP into RNA is of the "mixed" type; that is, both V_{max} and K_m are altered. The K_m for ATP is

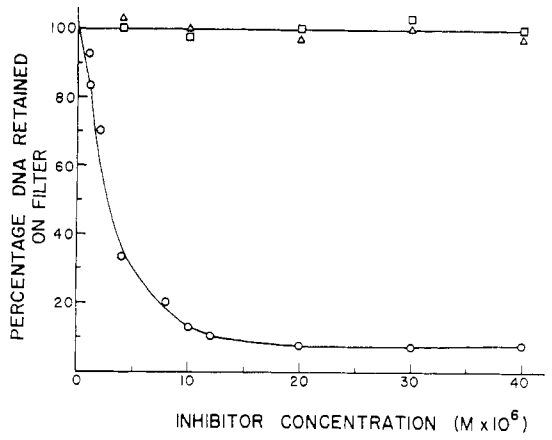

FIGURE 7: Effect of gallin on the formation of DNA–RNA polymerase complex. Incubation mixtures were described in the Experimental Section. Gallin, actinomycin, or rifampicin were present at the final concentrations indicated in the figure: (○) gallin; (□) actinomycin; (△) rifampicin.

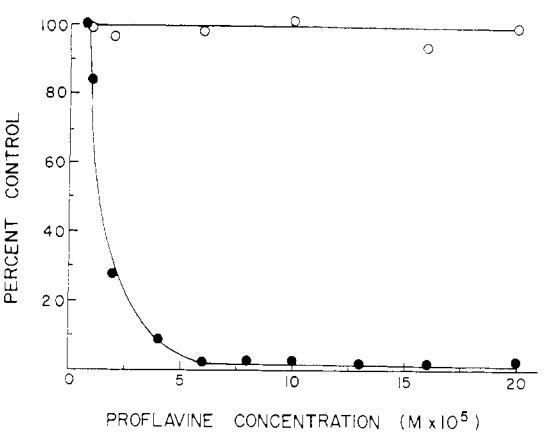

FIGURE 8: Effect of proflavine on RNA synthesis and on formation of DNA–RNA polymerase complex. RNA synthesis was measured as described in the Experimental Section. In the control reaction, 650 pmol of [^{3}H]UMP was incorporated into acid-insoluble material in 10 min. DNA–enzyme complex formation was measured as described in the Experimental Section: (●) RNA synthesis; (○) DNA–enzyme complex formation.

increased in the presence of the dye while that for GTP is decreased, possibly reflecting selective changes in the affinity of the enzyme for these substrates, both of which are involved in initiation.

We envision the mechanism of the inhibitory effect of gallin on RNA synthesis as follows: gallin binds, in an equimolar complex, to RNA polymerase; this dye–enzyme complex is unable to bind DNA, an early step in initiation. Gallin acts as a competitive inhibitor of DNA binding and preferentially blocks initiation of new chains of RNA by preventing formation of the initial phosphodiester bond. This mechanism of action is analogous to that previously demonstrated for aurintricarboxylic acid and related triphenylmethane dyes as inhibitors of protein synthesis. In this instance, the dyes also prevent binding of a polynucleotide template (mRNA), thereby blocking initiation of protein synthesis on the ribosome (Grollman and Stewart, 1968). A similar mechanism may account for the observed inhibition of reverse transcriptase activity of Rauscher leukemia virus by gallin (Liao *et al.*[1]).

The effects of other drugs and dyes that inhibit initiation of RNA synthesis by bacterial RNA polymerases may be contrasted with those of gallin. Rifampicins (Umezawa *et al.*, 1968; Neuhoff *et al.*, 1969) and streptovaricins (Mizuno *et al.*, 1968) do not affect formation of the DNA–enzyme complex. Proflavine inhibits initiation of RNA chains (Maitra *et al.*, 1967; Richardson, 1966) and readily forms a complex with DNA (Lerman, 1961), but the possibility that it also binds to polymerase cannot be excluded (Richardson, 1966). Unlike gallin, we find that proflavine does not inhibit formation of an adenovirus DNA–polymerase complex. Luteoskyrin (Ruet *et al.*, 1973) and kanchanomycin (Joel *et al.*, 1970) interact with the transcription complex and affect chain elongation as well as initiation. 1,10-Phenanthroline inhibits initiation of RNA synthesis by RNA polymerase but only at very high (1 mM) concentrations of drug (Scrutton *et al.*, 1971).

6-Chloro-8-aza-9-cyclopentylpurine is similar to gallin in that it shares an affinity for the enzyme as well as preventing DNA binding and chain initiation (Cranston and Ruddon, 1973). However, the effect of 6-chloro-8-aza-9-cyclopentylpurine resembles more closely that of *p*-chloromercuribenzoate and *N*-ethylmaleimide (Ishihama and Hurwitz, 1969) in that it interacts with sulfhydryl groups in the enzyme. In contrast to gallin, 6-chloro-8-aza-9-cyclopentylpurine inhibits incorporation of all nucleoside triphosphates in a noncompetitive manner.

The effects of gallin are most reminiscent of those reported for Congo Red (Krakow and von der Helm, 1970). Among the many reported inhibitors of RNA polymerase, only gallin and Congo Red block formation of a DNA–enzyme complex at concentrations lower than those required to inhibit RNA synthesis. The two dyes differ somewhat in their ability to dissociate the complex. Approximately the same concentration of Congo Red that inhibits DNA binding by 50% is required to achieve half-dissociation of the complex; a higher concentration of gallin is required to dissociate the complex than to block its formation. Although chemically distinct, both gallin and Congo Red are anionic dyes that share an affinity for divalent metals. Zinc is a component part of

TABLE VI: Effect of Gallin and Congo Red on the Dissociation of DNA–RNA Polymerase Complex.[a]

Compound Added (M)	DNA Retained on Filter (%)
None	100
Gallin	
3.7×10^{-6}	72
3.7×10^{-5}	46
3.7×10^{-4}	35
7.4×10^{-4}	20
Congo Red	
3.7×10^{-8}	100
3.7×10^{-7}	41
3.7×10^{-6}	4

[a] The DNA–enzyme complex was formed as described in the Experimental Section. At the completion of the 5 min incubation at 37°, either gallin or Congo Red was added to the assay mixture and incubation was continued for 30 min. The effect of these compounds on the stability of the DNA–enzyme complex was determined by measuring the amount of DNA retained on the nitrocellulose membrane filter as described in the Experimental Section.

E. coli RNA polymerase (Scrutton *et al.*, 1971), and it is possible that both dyes act by binding to this metal at the active site.

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Added in Proof

In a related study, Blumenthal and Landers (1973) recently reported that a commercial sample of aurintricarboxylic acid inhibits initiation of RNA synthesis catalyzed by $\alpha\beta$ replicase, *E. coli* RNA polymerase, and T7 RNA polymerase. Binding of polynucleotide templates to these enzymes was prevented by this inhibitor.

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