Robison, B., and Zimmerman, T. P. (1971), *J. Biol. Chem. 246*, 110.
Sander, C., and Ts'o, P. O. P. (1971), *J. Mol. Biol. 55*, 1.
Schmechel, D. E. V., and Crothers, D. M. (1971), *Biopolymers 10*, 465.
Schmidt, J., Burchardt, B., and Reid, B. R. (1970), *J. Biol. Chem. 245*, 5743.
Skoultchi, A., Ono, Y., Moon, H. M., and Lengyel, P. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 675.
Stryer, L. (1968), *Science 162*, 526.
Tao, T., Nelson, J. H., and Cantor, C. R. (1970), *Biochemistry 9*, 3514.
Tritton, T. R., and Mohr, S. C. (1971), *Biochem. Biophys. Res. Commun. 45*, 1240.
Wacker, W. E. C., and Vallee, B. L. (1959), *J. Biol. Chem. 234*, 3257.
Waring, M. J. (1965), *J. Mol. Biol. 13*, 269.
Waring, M. J. (1968), *Nature (London) 219*, 1320.
Watanabe, K., and Imahori, K. (1971), *Biochem. Biophys. Res. Commun. 45*, 488.
Wickstrom, E. (1971), *Biochem. Biophys. Res. Commun. 43*, 976.
Yang, S. K., Söll, D., and Crothers, D. M. (1972), *Biochemistry 11*, 2311.
Yarus, M. (1972), *Biochemistry 11*, 2050.
Yarus, M., and Rashbaum, S. (1972), *Biochemistry 11*, 2043.

Synthesis of Ribonucleic Acid Containing 6-Thioguanylic Acid Residues†

J. L. Darlix,* P. Fromageot, and E. Reich

ABSTRACT: 6-Thioguanosine was labeled with ^{35}S and then phosphorylated under conditions which avoid oxidation and release of the labile thioketone sulfur to give 6-[^{35}S]thioguanosine triphosphate. s^6GTP substituted only for GTP during RNA synthesis by DNA-dependent RNA polymerase. With calf thymus DNA as template and with s^6GTP, RNA synthesis amounted to 25–30% of that with GTP. s^6GTP initiated only a limited number of RNA chains while it had no effect on RNA chain initiation by ATP. The process of RNA elongation seemed unaffected by incorporation of s^6GMP residues. With poly(C) as template, RNA polymerase synthesized poly(s^6G), but polymerization ceased always after 30 min. Like s^6GMP, poly(s^6G) has two absorption maxima, one at 342 nm, specific of the thioketone, and one at 250 nm. However sulfur of the thioketone moiety became very labile once s^6GMP was polymerized. This fact prevented isolation of pure poly(s^6G). When polynucleotide phosphorylase activity was determined in the presence of s^6GDP an irreversible inhibition of the enzyme was observed.

The nucleoside 6-thioguanosine was first synthesized by Fox *et al.* (1958) and was found to be an inhibitor of tumor growth. As pointed out by Guschlbauer (1972), the behavior of guanosine is often qualitatively quite different from that of the other three normal nucleosides. Thus, studies of guanosine analogs are of particular interest (Darlix *et al.*, 1971). We have studied the utilization of phosphorylated derivatives of 6-thioguanosine by polynucleotide phosphorylase and by DNA-dependent RNA polymerase of *Escherichia coli*, since spectral and chemical properties of 6-thioguanosine are particularly favorable for a variety of physical studies and for possible formation of chemical derivatives of polynucleotides.

In this paper, we report the preparation of [^{35}S]s^6GTP[1] under conditions which avoid oxidation and release of the labile thioketone sulfur (Doerr *et al.*, 1961), in contrast with previously published methods (Roy *et al.*, 1961; Naruse and Fujimoto, 1966). It is shown that s^6GTP substitutes only for GTP during DNA-directed RNA synthesis and that s^6GTP is polymerized by RNA polymerase with poly(C) as template.

Material and Methods

RNA polymerase was prepared according to a previously published method (Darlix *et al.*, 1969). Polynucleotide phosphorylase was a gift of Dr. M. Grunberg-Manago (Paris).

Enzymatic Assay. The concentrations of DNA and enzyme in the reaction mixtures are given in the individual legends. The ionic conditions are: 5×10^{-2} M Tris-HCl (pH 8), 5×10^{-3} M $MgCl_2$, 1×10^{-3} M $MnCl_2$, and 8×10^{-3} β-mercaptoethanol.

The syntheses were followed by precipitation of the RNA with 5% trichloroacetic acid and filtration on HAWP 025 Millipore membranes; direct filtration technique was also used (Sentenac *et al.*, 1968). In both cases radioactivity was determined using a Nuclear-Chicago scintillation counter.

Analysis of RNA synthetized *in vitro* was performed by MAK column chromatography as already described (Mandell and Hershey, 1960; Darlix *et al.*, 1968), in the presence of total nucleic acids extracted from *Escherichia coli* B, as markers.

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[1] Abbreviations used are: s^6GMP, s^6GDP, and s^6GTP, the mono-, di-, and triphosphates of 6-thioguanosine; 8-azaGTP, the di- and triphosphates of 8-azaguanosine; formycin-P_2 and -P_3, the di- and triphosphates of formycin; poly(s^6G), the homopolymer of s^6GMP; MAK, column made of methylated serum albumin adsorbed on kieselghur.

High molecular weight DNAs of calf thymus and *E. coli* were gifts of Dr. Pouyet (Strasbourg). Poly(C) was purchased from Sigma Chemical Inc. Nucleoside triphosphates were obtained from P-L Biochemicals, [^{3}H]UTP from the "Service des molecules marquées" (Saclay), [^{3}H]GTP and [^{3}H]CTP from Schwarz BioResearch. ATP and GTP labeled in the γ-phosphate were prepared as already described (Sentenac *et al.*, 1968). Thioguanosine was a gift of Dr. H. B. Wood, Drug Development Branch, National Cancer Institute, Bethesda, Md. ^{35}S was obtained from the Département des Radioéléments (Saclay).

Synthesis of 6-[^{35}S]Thioguanosine 5'-Triphosphate. LABELING OF THE 6-THIOGUANOSINE. The sulfur of the nucleoside undergoes an exchange reaction with elemental ^{35}S when the two components are dissolved in a mixture of benzene–pyridine. Thioguanosine (19 mg, 65 μmol) dried over P_2O_5 *in vacuo* was dissolved in 5 ml of anhydrous pyridine. ^{35}S (15.4 mCi, 86 μmol) was dissolved in 0.6 ml of anhydrous benzene and added to the solution of thioguanosine. The mixture was heated at reflux temperature for 3.5 hr and kept anhydrous. After removal of the solvent *in vacuo*, a black residue and sulfur were eliminated by successive extractions with benzene. 6-[^{35}S]Thioguanosine was then dissolved in 5 ml of boiling water. The product, consisting of tiny cream-colored needles, was filtered and dried over P_2O_5 *in vacuo*. At this stage, the specific radioactivity was 1.4×10^5 cpm/nmol corresponding to a yield in the exchange reaction of 84%; 17 mg (85%) of 6-[^{35}S]thioguanosine was recovered.

PHOSPHORYLATION OF 6-[^{35}S]THIOGUANOSINE. Protection of the *cis*-Diol. Thioguanosine is unstable at extremes of pH, the sulfur being rapidly released. To ensure high yield of phosphorylation and to prevent any sulfur losses we have protected the 2'- and 3'-OH by condensation with trimethyl orthoformate, a reagent readily removed under mild conditions (Darlix *et al.*, 1967).

β-Cyanoethylphosphoric acid (700 μmol) in anhydrous dimethylformamide was added to a well-stirred suspension of 14 mg of 6-[^{35}S]thioguanosine in 0.750 ml of anhydrous trimethyl orthoformate. (β-Cyanoethylphosphoric acid is the catalyst.) After 2-hr stirring at 4°, thioguanosine had dissolved and the condensation with trimethyl orthoformate was complete. The solution was concentrated *in vacuo* to a syrup to which 3 ml of anhydrous pyridine and 500 mg of dicyclohexylcarbodiimide were added. β-Cyanoethyl phosphate was now the phosphorylating agent (Tener, 1961), and after 18 hr in the dark at 20°, the reaction was stopped by addition of 2 ml of cold water. The mixture was evaporated to dryness, 5 ml of water was added, and dicyclohexylurea and acrylonitrile were eliminated by filtration through glass wool. After β elimination of the cyanoethyl group, the *cis*-diol is still protected. The methoxymethylidene group is very labile at acid pH and its conversion to a formyl ester was complete 35 min after the pH of the filtrate was lowered to 1.5–2 by ion-exchange chromatography on a 2-ml Dowex 50 (H^+) column. The 2'(3')-formyl ester derivative was hydrolyzed upon addition of pyridine to neutralize the solution. 6-[^{35}S]-Thioguanosine 5'-monophosphate (26 μmol) was recovered (60% yield). Identity and purity of the compound were established by its absorption spectra, its extinction coefficient at 260, 320, and 340 nm (Fox *et al.*, 1958), by paper chromatography with ammonia–isobutyric acid–water (7:1:2), and by paper electrophoresis at pH 3.5 (citric acid–citrate, 5×10^{-2} M) and pH 8.7 (borate, 0.2 M).

6-[^{35}S]Thioguanosine 5'-triphosphate was synthesized chemically by the method of Smith and Khorana (1958). [^{35}S]s^6-GTP was purified by preparative electrophoresis on Whatman No. 3MM paper using the solvent pyridine–acetic acid (pH 4.5). s^{6}GTP was stored at −20° *in vacuo* and in the dark; if necessary, it was repurified just before use.

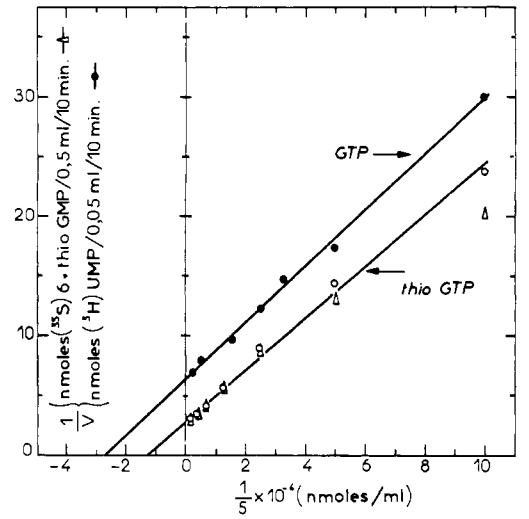

FIGURE 1: Apparent K_m values for s^{6}GTP and GTP with RNA polymerase and calf thymus DNA as template. Conditions of the assays: standard salt; calf thymus DNA, 20 μg; ATP, CTP, and UTP, 10^{-4} M; and RNA polymerase, 10 μg. Final volume, 0.5 ml; various concentrations of [^{35}S]s^{6}GTP from 10^{-5} to 6×10^{-4} M; specific radioactivity, 3.6×10^3 cpm/nmol; or various concentrations of GTP from 10^{-5} to 4×10^{-4} M; specific activity [^{3}H]UTP, 1.8×10^3 cpm/nmol. Incubations lasted 15 min. RNA was recovered by acid precipitation.

Results

Calf Thymus DNA Transcription. In the presence of ATP, UTP, CTP, and calf thymus DNA, RNA polymerase utilized s^{6}GTP for the synthesis of RNA (Table I). As shown in Table I and Figure 1, s^6-GTP substituted only for GTP. This is not surprising since the pairing properties of sG should be the same as those of G. However, replacement of GTP by s^{6}GTP decreased by 75% the total *in vitro* transcription of

TABLE I: s^{6}GTP Substitutes for GTP during DNA Directed RNA Synthesis.[a]

Expts	Nucleoside Triphosphates Added	Incorp of Labeled Nucleotide (nmol)
1	ATP, UTP, CTP, GTP	1.45
2	As 1 without GTP	0.03–0.05
3	As 2 but with s^{6}GTP	0.40
4	As 3 without ATP	0.04
5	UTP, CTP, GTP, s^{6}GTP	0.05
6	GTP, ATP, CTP, s^{6}GTP	0.03
7	GTP, ATP, UTP, s^{6}GTP	0.04

[a] Conditions of the assays: calf thymus DNA, 20 μg; standard salt, nucleoside triphosphates were present at 10^{-4} M. The radioisotopes were either [^{3}H]UTP (1800 cpm/nmol) or [^{35}S]s^{6}GTP (3600 cpm/nmol); RNA polymerase, 10 μg; final volume, 0.5 ml; 15 min at 37°. RNA was recovered by acid precipitation.

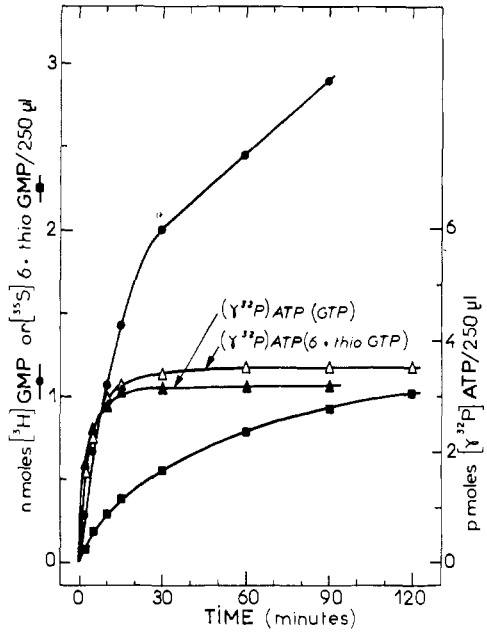

FIGURE 2: Kinetics of RNA sG synthesis and initiation with ATP Two incubation mixtures contained: standard salt; calf thymus DNA, 180 µg; GTP, UTP, [³H]CTP, 10^{-4} M, specific radioactivity 6×10^3 cpm/nmol, or UTP, CTP, 10^{-4} M, and [³⁵S]s⁶GTP, 4×10^{-4} M, specific activity 6×10^3 cpm/nmol and in both cases [γ-³²P]ATP, 4×10^{-5} M, specific activity 800 cpm/pmol; RNA polymerase, 100 µg. Final volume, 2.5 ml. 250-µl aliquots were withdrawn at the times indicated and RNA was recovered by direct filtration.

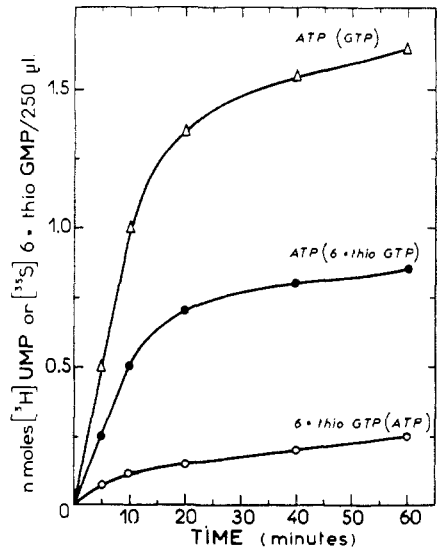

FIGURE 3: Kinetics of RNA chain synthesis initiated with ATP or s⁶GTP. Three incubation mixtures contained: standard salt; calf thymus DNA, 180 µg; UTP, CTP, and one of the purine nucleotide, ATP, GTP, or rs⁶GTP. The label was either [³H]CTP specific activity 10^4 cpm/nmol, or [³⁵S]s⁶GTP, specific activity 6×10^3 cpm/nmol; RNA polymerase, 60 µg. Final volume, 1 ml. After 30-min preincubation at 37°, the fourth nucleotide, in brackets, was added, and NaCl concentration was raised to 0.6 M to prevent further initiation. At the times indicated, 250-µl aliquots were withdrawn and RNA was recovered by acid precipitation.

calf thymus DNA. The apparent K_m value for s⁶GTP is 8×10^{-5} with native calf thymus DNA as template. Under identical conditions the apparent K_m for GTP is 2.5×10^{-5} M (Figure 1). It should be noted that the apparent K_m for GTP is constant with different preparations of RNA polymerase while that of s⁶GTP was somewhat variable. In view of previous correlations (Darlix *et al.*, 1971) between inhibition of total RNA synthesis by analogs and their ability to function as chain initiators, the low overall yield of RNA synthesis in the presence of s⁶GTP raised the question of its ability to initiate transcription (Figure 2).

In general initiation is conveniently studied by measuring the [γ-³²P]nucleoside triphosphate incorporation. In the case of s⁶GTP the high blank values that were obtained excluded the use of γ-³²P-labeled nucleotide. To circumvent this difficulty, s⁶GTP, UTP, and CTP were incubated with RNA polymerase and the template for 30 min, and then ATP and NaCl (0.6 M) were added. Under such conditions the RNA synthesized must originate only from chains initiated by s⁶GTP. Figure 3 shows that s⁶GTP is able to initiate RNA chains, although less effectively than GTP. On the other hand, s⁶GTP did not affect chain initiation by ATP (Figure 2). Nevertheless, while the total number of chains initiated by ATP is normal, the total RNA synthesized with these chains is reduced by approximately one-half (Figure 3). This recalls a qualitatively similar observation with 8-azaGTP (Darlix *et al.*, 1971) and suggests that the chain length of this s⁶GMP containing polynucleotides might be abnormally short.

Accordingly, the chain-length distribution of the RNA formed in presence of s⁶GTP was analyzed by chromatography on MAK columns. RNA chain lengths formed in the presence of s⁶GTP (Figure 4) qualitatively resembled those made with GTP (Darlix *et al.*, 1968); the normal classes of RNA chains were obtained: chains of 1400–1500 (peak II), 2700–2900 (peak III), and 4000–5500 (peak IV) nucleotides. However the ratio of the RNA fractions II plus III to that in fraction IV was equal to unity for the s⁶GTP product whereas it was definitely lower in the GTP control. This indicates that discontinuous kinetics of chain growth occur in the presence of s⁶GTP and that the pauses that intervene at chain lengths of 1400–1500 and 2700–2900 nucleotides are abnormally prolonged when s⁶GTP substitutes for GTP (Darlix *et al.*, 1971; Darlix and Fromageot, 1972). Apart from these pauses, the rate of chain elongation seems unaffected. A similar conclusion was reached when 8-azaGTP substituted for GTP in *in vitro* transcription (Darlix *et al.*, 1971).

Synthesis of Poly(s⁶G) with Poly(C) Template. The utilization of s⁶GTP for *in vitro* transcription of calf thymus DNA yields a mixed polymer and gives no clue to its ability to form homopolymer. Indeed, catalysis of homopolymer synthesis is not possible (Fromageot *et al.*, 1969; Ward *et al.*, 1969). This point has been investigated in the present case with poly(C) as template. Poly(C) directed the synthesis of poly(s⁶G) (Figure 5), the optimum conditions being pH 7.4, 5×10^{-3} M Mn^{2+} and 4×10^{-3} M s⁶GTP. These conditions are somewhat different from those used for poly(G)·poly(C) synthesis (Hirschbein *et al.*, 1967) particularly with regard to the optimal s⁶GTP concentration, which is threefold higher than that of GTP. Rate of poly(s⁶G) synthesis was slow and polymerization always stopped after 20-min incubation (Figure 6). At that point, no active enzyme remained free in the medium, since an additional increment of poly(C) did not stimulate further polymerization. The labeled poly(s⁶G) synthesized in the presence of poly(C) was not retained by filtration on Millipore membrane in the presence of RNA polymerase and remained complexed with the template. This behavior,

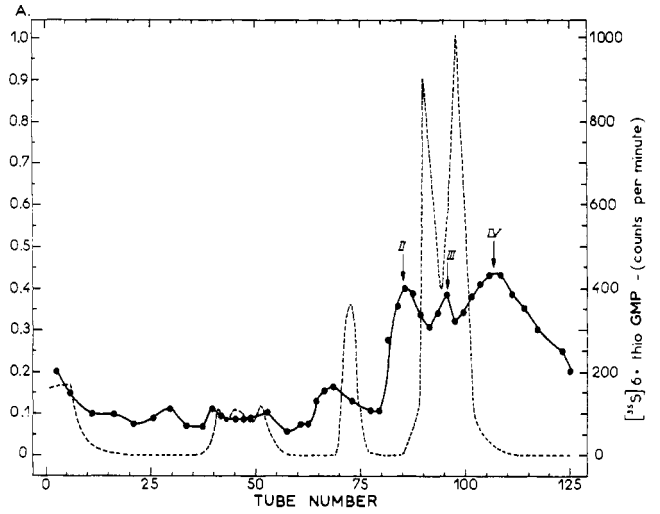

FIGURE 4: Analysis of RNA chains synthesized in the presence of s^{6}GTP and with calf thymus DNA as template. The incubation mixture contained: calf thymus DNA, 45 μg; standard salt; RNA polymerase, 30 μg; UTP, CTP, ATP, 10^{-4} M, and [^{35}S]s^{6}GTP (40,000 cpm/nmol), 2×10^{-4} M. Final volume, 1 ml; 20 min at 37°. Incorporation of [^{35}S]s^{6}GMP is 0.75 nmol. Recovery of radioactivity was 70%. Marker nucleic acids extracted from *E. coli* B(. . .) were added.

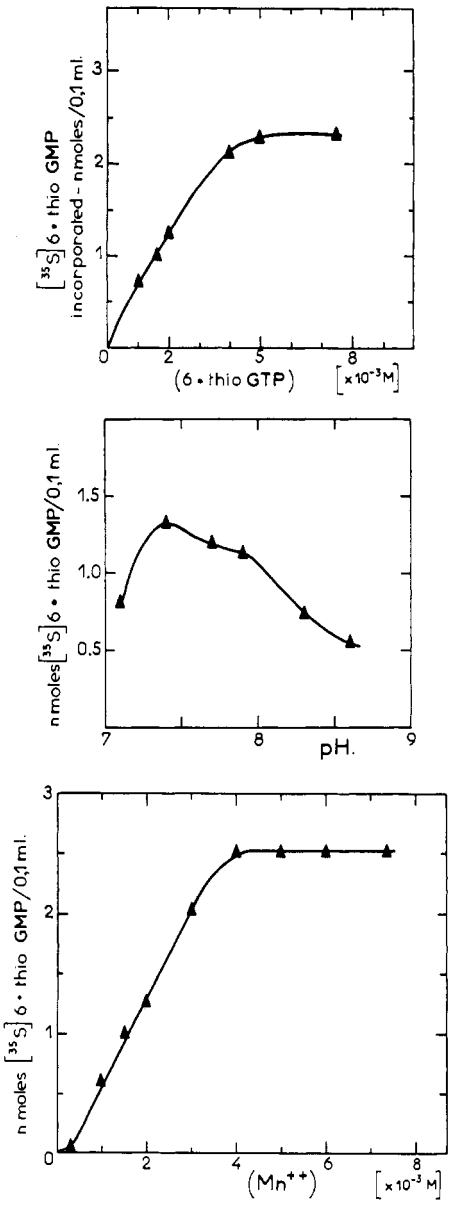

FIGURE 5: Rate of s^{6}GMP polymerization as a function of substrate, Mn^{2+} ion concentrations, and pH, with poly(C) as template. (A, top) s^{6}GTP concentration, poly(C), 6 μg; ionic conditions, Tris-HCl (pH 7.9), 5×10^{-2} M, $MnCl_2$, 2×10^{-3} M, β-mercaptoethanol, 8×10^{-3} M; RNA polymerase, 8 μg. Final volume, 0.1 ml; 20 min at 37°; [^{35}S]s^{6}GTP (1000 cpm/nmol) concentration as indicated. Polymer was recovered by acid precipitation. (B, middle) pH, as above except that [^{35}S]s^{6}GTP, 2×10^{-3} M, and pH of the Tris-HCl buffer was varied from pH 7 to 8.6. (C, bottom) $MnCl_2$ concentration, as in part B except that pH is 7.4 and $MnCl_2$ concentration was varied from 0 to 8×10^{-3} M.

which is identical with that of poly(G)·poly(C), suggests that the newly formed labeled polymer did not bind the enzyme. Furthermore, incubation of s^{6}GTP alone with RNA polymerase did not inactivate the enzyme. Thus the low rate and the spontaneous arrest in the polymerization of s^{6}GTP, even in the presence of an excess of poly(C) seems to be due to some specific but undefined reaction. This could result from an inactivation of the enzyme on the poly(C) template in the presence of s^{6}GTP, or simply an inhibition of dissociation of the enzyme–template complex under the influence of s^{6}GTP or poly(s^{6}G).

Spectral Properties of Poly(s^{6}G) Complexed with Poly(C). RNA (sG) as well as poly(s^{6}G) complexed with poly(C) show an ultraviolet absorption maximum at 342 nm in acid medium (Figure 7). In both cases increasing pH shifted the absorption maximum toward 320 nm. Since s^{6}GMP residues are labeled, one can calculate the molar extinction of the thioketone in both the free (ϵ_m 2.4 × 10^4), and in the polymerized form (log ϵ_m 10^4). Thus, polymerization causes an hypochromicity of 60% at 342 nm. These findings suggest the participation of s^{6}GMP in an ordered structure, a conclusion consistent with the resistance of the polymer to the action of T_1 and pancreatic RNases; treatment with these RNases degrades at most 10–15% of the polymer.

The set of spectra obtained by varying the pH of a solution of free s^{6}GTP present an isosbestic point at 327 nm, and leads to a pK of 8.6 for the thioketone. In contrast, spectra given by titration of poly(s^{6}G) complexed with poly(C) do not show a single isosbestic point in the range of pH investigated (Figure 8). Titration curves plotted at 317 nm as well as at 250 nm show two discontinuities, whereas at 342 nm the transition extends over a range of 4 pH units. Thus several transitions have to be considered, corresponding to structures which remain to be defined. A similar conclusion is reached by following the absorbance as a function of temperature. At pH 6.5, in the presence of 0.1 M NaCl, the melting curve showed two slopes and was not cooperative. T_m for overall process was 87° (Figure 9); under these conditions, poly(G)·poly(C) was not denatured at all.

It is important and of interest to note that the sulfur of poly(s^{6}G) complexed with poly(C) (Figure 9) is remarkably labile compared with free monomer. Heating the polymer at neutral pH, or raising pH to 8.5–9 at room temperature release the sulfur, with loss of the 340-nm absorption peak, a phenomenon that affects the melting behavior of the polymer as well as the titration curves. In contrast, the sulfur in s^{6}GMP under identical conditions is stable.

These characteristics make it difficult to isolate pure poly(s^{6}G) by separation from poly(C) template. Direct polymer-

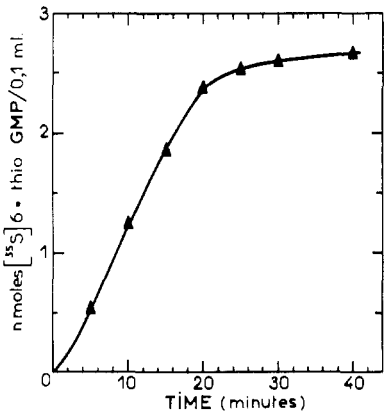

FIGURE 6: Kinetics of s^{6}GMP polymerization with poly(C) as template. Incubation as in Figure 5c. Final volume, 1 ml; 100-μl aliquots were withdrawn and polymer was recovered by acid precipitation.

ization of s^{6}GDP in the presence of polynucleotide phosphorylase was therefore investigated as a potential alternative route to the preparation of a homopolymer. However, no polymer is formed by this enzyme. Moreover, s^{6}GDP did not take part in the exchange reaction s^{6}GDP + [^{32}P]P_i $\rightleftarrows$ [^{32}P]s^{6}GDP + P_i. In addition s^{6}GDP irreversibly inhibits polynucleotide phosphorylase. Polymerization of GDP (at 65°) and of ADP (at 30°) are nearly abolished in the presence of s^{6}GDP, and the enzyme is almost totally inactivated under these conditions.

Discussion

The preceding results with thioguanine nucleotides and polynucleotides raise a number of questions that deserve comment.

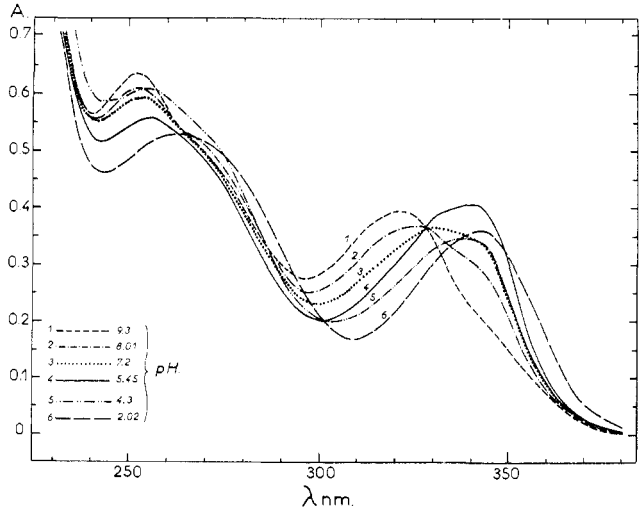

FIGURE 7: Spectra at different pH of poly(s^{6}G) complexed with poly(C). Synthesis as in Figure 6 but [^{35}S]s^{6}GTP (150 cpm/nmol) 4 × 10^{-3} M. Poly(C), 90 μg; RNA polymerase, 400 μg. Final volume, 3 ml; incubation 45 min at 37°. RNA polymerase was eliminated by a phenol extraction (10 min at 0°). s^{6}GTP and phenol were eliminated by repetitive Diaphlo concentration, and finally polymer was dialyzed against water. s^{6}GMP (75 μg) was polymerized.

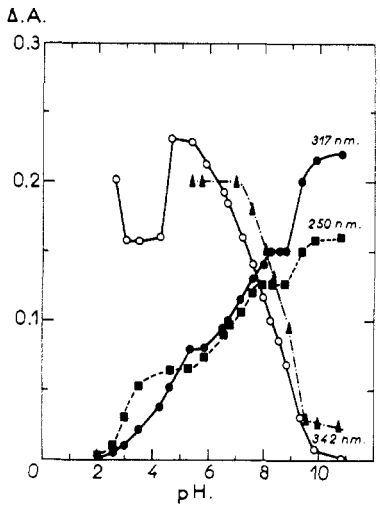

FIGURE 8: Titration curves of poly(s^{6}G) complexed with poly(C) at 342, 317, and 250 nm. Conditions as in Figure 6. (▲) Titration curve of s^{6}GMP at 340 nm. (○, ●, ■) Titration curves of poly(s^{6}G) complexed with poly(C) at 342, 317, and 250 nm, respectively.

From the work of Roy *et al.* (1961), who first studied the biochemical properties of this analog, it is known that 6-thioguanine is metabolized to the corresponding nucleoside 5′-mono-, di-, and triphosphates in mammalian tissue, and that these nucleotides function as efficient substrates for a variety of enzymes including guanylate kinase, phosphoenolpyruvate kinase, and hexokinase. Moreover s^{6}GTP effectively replaces GTP for ribosomal incorporation of amino acid into protein. However, the inhibition of IMP-dehydrogenase of Sarcoma 180 cells (Meich *et al.*, 1967) and of a nucleotide monophosphokinase (specific for the phosphorylation of GMP to GDP and isolated from hog brain) by s^{6}GMP, in addition to the incorporation of this analog into the DNA of tumors (Le Page, 1960; Le Page *et al.*, 1964) may account for the cytotoxicity or antitumor effects of thioguanine.

Our findings suggest additional possible mechanisms for visualizing the basis of thioguanine cytotoxicity. The fact that

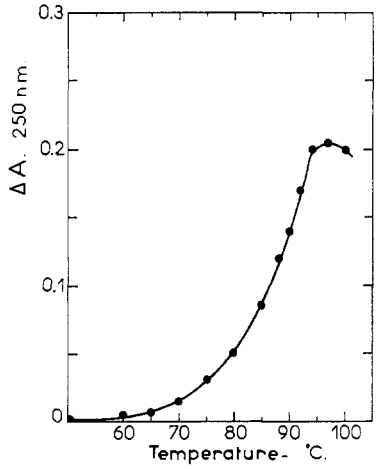

FIGURE 9: Melting curve of poly(s^{6}G) complexed with poly(C) at 250 nm. Buffer was Tris-HCl, pH 6.5 × 10^{-2} M; NaCl, 0.1 M.

desulfurization accompanies either the thermal or pH-mediated denaturation of s(G·C) base pairs suggests that the same might occur during transcription of sG containing segments in DNA. Such an event could destroy the integrity of the template, or the enzyme, or both; indeed, the observation that RNA polymerase is inactivated while functioning in the presence of poly(sG·C) provides some experimental substance for such a possibility, and it is fortified by the rapid irreversible inactivation of polynucleotide phosphorylase produced by sGDP. Another potential contribution toward cytotoxicity arises from the finding that sGTP, like 8-azaGTP and formycin-P_3 (Darlix *et al.*, 1971), limits transcription, predominantly by restricting the number of initiation sites, and therefore the template regions that are functioning during transcription. Both of these potential mechanisms will require much more detailed analysis than that available from results obtained to date.

The substitution of oxygen by sulfur changes the space-filling properties of a molecule due to the increased van der Waal's radius of the sulfur atom; this is also correlated with the increased bond length of the thioketone (1.64 Å) compared with that of the carbonyl (1.25 Å) (Wyckoff and Corey, 1934). In addition, the reactivity of the molecule is modified due to the specific chemical properties of the sulfur. Even with these factors in mind, it remains impossible to provide a plausible explanation for the properties of the thioguanine nucleotides and polymers. This is due in part to insufficient information, and may be remedied by more detailed and systematic study of the thioguanine derivatives. However, even so, the behavior of thioguanine is perplexing when compared with that of the thiopyrimidine compounds (Lezius *et al.*, 1971). In all of these cases, the thiopyrimidines are readily and efficiently polymerized by a variety of enzymes, and the properties of the polymers, while distinctive in a number of respects can easily be reconciled with those of the corresponding monomers (Scheit *et al.*, 1969). The surprising reactivity of the sulfur in thioguanine polymers requires further investigation.

Acknowledgments

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References

Darlix, J. L., and Fromageot, P. (1972), *Biochimie 54*, 47.
Darlix, J. L., Fromageot, H. P. M., and Fromageot, P. (1967), *Biochim. Biophys. Acta 145*, 517.
Darlix, J. L., Fromageot, P., and Reich, E. (1971), *Biochemistry 10*, 1525.
Darlix, J. L., Sentenac, A., and Fromageot, P. (1968), *Biochim. Biophys. Acta 166*, 438.
Darlix, J. L., Sentenac, A., Ruet, A., and Fromageot, P. (1969), *Eur. J. Biochem. 11*, 43.
Doerr, I. L., Wempen, I., Clarke, D. A., and Fox, J. J. (1961), *J. Org. Chem. 26*, 3401.
Fox, J. J., Wempen, I., Hampton, A., and Doerr, I. L. (1958), *J. Amer. Chem. Soc. 80*, 1669.
Fromageot, P., Darlix, J. L., and Sentenac, A. (1969), First Lepetit Colloquium on RNA Polymerase and Transcription, Amsterdam, North Holland, p 46.
Guschlbauer, W. (1972), *Purines–Theory Expt., Proc. Symp. Quantum Chem. Biochem.*, 297.
Hampton, A. (1963), *J. Biol. Chem. 238*, 3068.
Hirschbein, L., Dubert, J. M., and Babinet, C. (1967), *Eur. J. Biochem. 1*, 135.
Le Page, G. A. (1960), *Cancer Res. 20*, 403.
Le Page, G. A., Junga, I. G., and Bowman, B. (1964), *Cancer Res. 24*, 835.
Lezius, A. G., and Scheit, K. H. (1967), *Eur. J. Biochem. 3*, 85.
Mandell, J. D., and Hershey, A. D. (1960), *Anal. Biochem. 1*, 68.
Meich, R. P., Anderson, J. M., and Sartorelli, A. C. (1967), *Biochem. Pharmacol. 16*, 2222.
Naruse, M., and Fujimoto, Y. (1966), *Yakugaku Zasshi 86*, 192.
Roy, J. K., Kvam, D. C., Dahl, J. L., and Parks, R. E. (1961), *J. Biol. Chem. 236*, 1158.
Scheit, K. H., and Gaertner, E. (1969), *Biochim. Biophys. Acta 182*, 1.
Sentenac, A., Simon, E. J., and Fromageot, P. (1968), *Biochim. Biophys. Acta 161*, 299.
Smith, M., and Khorana, H. G. (1958), *J. Amer. Chem. Soc. 80*, 1141.
Tener, G. M. (1961), *J. Amer. Chem. Soc. 83*, 159.
Ward, D. C., Cerami, A., and Reich, E., Acs, G., and Altwerger, L. (1969), *J. Biol. Chem. 244*, 3243.
Wyckoff, R. W. G., and Corey, R. B. (1934), *Krist. 89*, 462.