Interaction of Kanchanomycin with Nucleic Acids. III. Contrasts in the Mechanisms of Inhibition of Ribonucleic Acid and Deoxyribonucleic Acid Polymerase Reactions*

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ABSTRACT: Kanchanomycin, an antibiotic produced by a *Streptomyces* species, interacts with polynucleotides if Mg^{2+} is present.

The ability of a kanchanomycin–calf thymus DNA complex and kanchanomycin–d(A-T)$_n$ complex to serve as templates for RNA and DNA synthesis by polymerases from *Escherichia coli* has been studied. Kanchanomycin, polynucleotide, and Mg^{2+} were preincubated to form a complex prior to addition of the remaining components necessary for RNA and DNA synthesis. Both DNA and RNA synthesis are strongly inhibited by the presence of kanchanomycin. The inhibition of DNA synthesis by kanchanomycin is overcome by increasing the concentration of template but is unaffected by increasing the concentration of DNA polymerase. In sharp contrast, the inhibition of RNA synthesis by kanchanomycin is *not* overcome by increasing the concentration of template preincubated with a fixed amount of kanchanomycin, although it is reversed by addition of template which has not been exposed to kanchanomycin prior to assay. The inhibition of RNA synthesis by kanchanomycin *can* be overcome by increasing the concentration of RNA polymerase. Kanchanomycin is as effective an inhibitor of RNA synthesis when added after RNA synthesis has commenced as when added before formation of the initiation complex. These data suggest that the RNA polymerase is attracted to sites on the DNA which possess antibiotic but that the physical state of the treated DNA is changed by kanchanomycin so as to allow enzyme to attach to unexposed DNA and to begin RNA synthesis. DNA synthesis, however, is inhibited by virtue of an antibiotic-induced alteration of the template activity of the DNA itself.

Kanchanomycin, an antibiotic of unknown structure produced by a *Streptomyces* species, is bactericidal and tumoricidal at very low concentrations (Liu *et al.*, 1963). Kanchanomycin has been shown to interact with polynucleotides if Mg^{2+} is present (Friedman *et al.*, 1969a,b). An initial complex which forms immediately changes with time to a second complex. There is no apparent base specificity nor requirement for a given secondary structure in the polynucleotide for the initial complex formation. Of all polynucleotides studied, only poly A did not change with time to the second form.

Complex formation is inhibited by high concentrations of NaCl. Once formed, the initial complex can be readily dissociated by EDTA and partially dissociated by NaCl. The second complex is only slowly dissociated by EDTA and is unaffected by addition of NaCl. Kanchanomycin (in the second complex) decreases the buoyant density and increases the sedimentation rate of d(A-T)$_n$,[1] and increases the viscosity of calf thymus DNA.

The initial complex formed between kanchanomycin, Mg^{2+}, and polynucleotide presumably involves in part an electrostatic interaction between the negatively charged phosphate backbone of the polynucleotide and a positively charged kanchanomycin–Mg^{2+} species. The nature of the second complex is obscure but more intimately involves the bases of the polynucleotide.

In this paper we present the results of experiments using polynucleotides complexed with kanchanomycin as template for the RNA and DNA polymerase of *Escherichia coli*.

Materials and Methods

Kanchanomycin was a gift from Chas. Pfizer, Maynard, N. J. A stock solution was prepared containing 1 mM kanchanomycin (600 μg/ml) in 100% dimethylformamide. The stock solution was stored in the dark at −20°. All solutions containing kanchanomycin including samples for enzyme assays were prepared in dim light and kept in the dark during incubations or storage.

[^3H]UTP and [^3H]dTTP were purchased from Schwartz BioRearch, Inc. Calf thymus DNA was purchased from Sigma Chemical Co. Stock solutions of 5 mM DNA-P in 0.01 M Tris, pH 7.5, were stored at 4°. Heat-denatured DNA was prepared by heating the DNA at 90° for 10 min and then immediately placing the DNA in an ice bath. d(A-T)$_n$ was prepared by unprimed synthesis using *Escherichia coli* DNA polymerase (Schachman *et al.*, 1960). The d(A-T)$_n$ product was deproteinized with phenol and exhaustively dialyzed against a solution with 1 mM EDTA–0.5 M NaCl–0.01 M Tris, pH 7.5, and finally against 0.01 M Tris, pH 7.5.

The *Escherichia coli* DNA polymerase (fraction VII) prepared according to Richardson *et al.* (1964) and the *Esche-*

* From the Department of Medicine, Harvard Medical School, and the Beth Israel Hospital, Boston, Massachusetts 02215. *Received June 15, 1970.* This work was supported by grants from the National Institutes of Health, U. S. Public Health Service (GM 12573 and CA 10736), and the American Cancer Society.

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[1] Abbreviation used is: d(A-T)$_n$, the strictly alternating deoxyadenylate–deoxythymidylate copolymer.

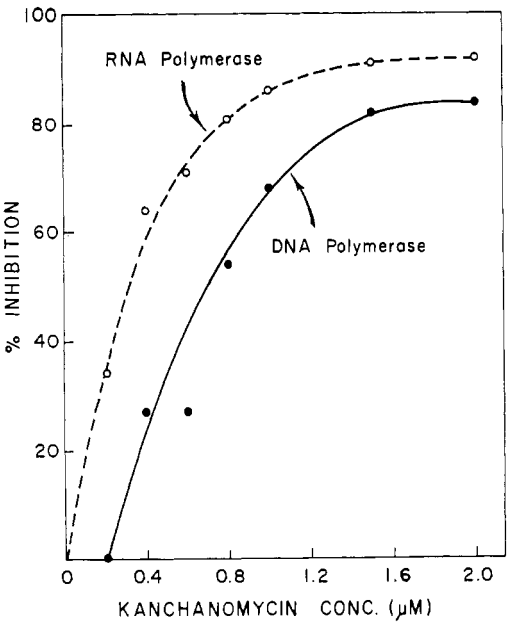

FIGURE 1: Effect of kanchanomycin on RNA and DNA synthesis directed by d(A-T)$_n$. The preincubation mixtures contained 80 μM (d(A-T)$_n$, 0.16 mM $MgCl_2$, and kanchanomycin at a concentration 4 times the final concentration given on the graph. The preincubation period was 4 hr before measurement of RNA synthesis and 15 min before measurement of DNA synthesis. Each assay for RNA synthesis contained 0.7 unit of RNA polymerase. Each assay for DNA synthesis contained 0.1 unit of DNA polymerase and was incubated 30 min. See Methods for details. The control assay for RNA synthesis incorporated 0.66 mμmole of [^{3}H]UTP, and that for DNA synthesis incorporated 0.21 mμmole of [^{3}H]TTP.

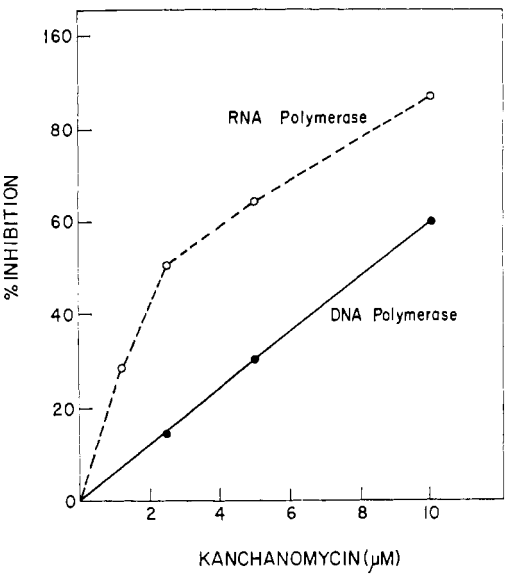

FIGURE 2: Effect of kanchanomycin on RNA and DNA synthesis directed by calf thymus DNA. The preincubation mixtures contained 0.80 mM native or heat-denatured calf thymus DNA, 80 μM $MgCl_2$, and kanchanomycin at a concentration 20 times the final concentration given on the graph. Heat-denatured DNA was used as template for the DNA polymerase and native DNA for the RNA polymerase. Preincubation was for 1 hr at 25° in both assays. Each assay for RNA synthesis contained 4 units of RNA polymerase. Each assay for DNA synthesis contained 2.1 units of DNA polymerase and was incubated for 30 min. Final volume in each assay was 110 μl. See Methods for details.

richia coli RNA polymerase (fraction IV) prepared according to Chamberlin and Berg (1962) were generously donated by A. Cerami. RNA polymerase was stored and added to the assays in 1 mM potassium phosphate (pH 8.4)–5 mM $MgCl_2$–5 mM β-mercaptoethanol–0.05 mM EDTA–50% glycerol. The solution contained 400 units per ml with one unit equal to 1 mμmole of UTP incorporated/hr at 37°. DNA polymerase was stored in 0.02 M potassium phosphate (pH 7.4)–0.001 M glutathione and diluted in 0.07 M potassium phosphate, pH 7.0, containing 1 mg/ml of bovine serum albumin. The stock solution contained 700 units per ml with one unit equal to 10 mμmoles of dATP incorporated per 30 min at 37°.

Preincubation and Assay Conditions. In all experiments, except those reported in Tables II and III, the polynucleotide template (calf thymus DNA or d(A-T)$_n$) was preincubated with and without kanchanomycin before the remaining components necessary for RNA or DNA synthesis were added. The preincubation mixtures contained DNA or d(A-T)$_n$, kanchanomycin, and $MgCl_2$ in the concentrations indicated in the legends in 0.01 M Tris, pH 7.5, and 10% dimethylformamide for DNA or 8% dimethylformamide for d(A-T)$_n$. The $MgCl_2$ was added last to the preincubation mixture. The mixtures were preincubated at 37° unless otherwise indicated for DNA or 25° for d(A-T)$_n$ for the time indicated in the legends.

The preincubation mixture (25 μl) was assayed for RNA synthesis in a final volume of 100 μl unless otherwise indicated. The components added for the assay were 6 μmoles of Tris, pH 7.9, 0.6 μmole of $MgCl_2$, 0.15 μmole of $MnCl_2$, 1.8 μmoles of β-mercaptoethanol, and 40 mμmoles each of ATP, CTP, GTP, and [^3H]UTP with DNA as template or ATP and [^3H]-UTP only with d(A-T)$_n$ as template. The [^3H]UTP was uniformly labeled with a specific activity of 4 × 10^3 to 22 × 10^3 cpm per mμmole. The units of *Escherichia coli* RNA polymerase added are indicated in the legends. The enzyme was added last. After the assay mixtures had incubated for 30 min at 37°, the reactions were stopped with 1.5 ml of cold 5% trichloroacetic acid. Each precipitate was collected on a Millipore filter (0.45 μ) and washed with approximately 20 ml of cold 5% trichloroacetic acid. The filters were dried under a heat lamp, placed in vials with 10 ml of scintillator solution (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per l. of toluene), and counted in a Packard scintillation spectrophotometer. Background radioactivity obtained from assays with no enzyme present have been subtracted from all results.

The preincubation mixture (25 μl) was assayed for DNA synthesis in a final volume of 100 μl unless otherwise indicated. The components added for the assay were 7 μmoles of potassium phosphate, pH 7.0, 1 μmole of β-mercaptoethanol, 0.7 μmole of $MgCl_2$, and 10 mμmoles each of dATP, dCTP, dGTP, and [^3H]dTTP for DNA as primer or dATP and [^3H]dTTP with d(A-T)$_n$ as primer. The [^3H]dTTP was uniformly labeled with a specific activity of approximately 1 × 10^4 cpm per mμmole. The units of *Escherichia coli* DNA polymerase used are indicated in the legends. The assay mixtures were incubated at 37° for the time indicated in the legends. The reactions were stopped and the precipitates collected, washed, and counted as indicated above in the RNA polymerase assay.

TABLE I: Influence of Mg^{2+} Concentration and Time of Preincubation of Kanchanomycin and DNA upon the Inhibition of RNA Synthesis by Kanchanomycin.[a]

	Mg^{2+} Concn (mM)	Preincubation Time (hr), Temperature (°C)	% Inhibition of RNA Synthesis
A	0.4	0.25, 25	46, 58
B	0.4	4, 37	68, 71, 74
C	0.4	24, 37	68, 78
D	0.04	4, 37	57, 60

[a] The results are the composite of several experiments. In each experiment the preincubation mixtures contained 0.4 mM calf thymus DNA, 0 or 0.04 mM kanchanomycin, and $MgCl_2$ as indicated. Each assay for RNA synthesis contained 2 units of RNA polymerase. See Methods for details. The controls incorporated 0.23 mμmole of [^3H]UTP.

TABLE II: The Effect of Nucleoside Triphosphates on the Inhibition of DNA-Directed RNA Synthesis by Kanchanomycin.[a]

	Components Added First to DNA (mμmoles)	Components Added Second (mμmoles)	mμmole of UTP Incorporated	% Inhibition
1		25 each of the 4 NTP	0.538	
2		50 each of the 4 NTP	0.655	
3	1 KM	25 each of the 4 NTP	0.234	57
4	1 KM	50 each of the 4 NTP	0.275	58
5	25 each of 4 NTP	1 KM	0.416	23
6	50 each of 4 NTP	1 KM	0.510	22

[a] Solutions of native calf thymus DNA, containing 6 μmoles of Tris (pH 7.9)–0.6 μmole of $MgCl_2$–0.15 μmole of $MnCl_2$–1.8 μmoles of β-mercaptoethanol, had the components listed in the table added in the order indicated. There was no preincubation. Kanchanomycin (KM) was added within 2 min following the four nucleoside triphosphates (NTP) or *vice versa*, and the reaction was begun with the addition of 6 units of RNA polymerase. Final volume was 125 μl.

Results

Effect of Kanchanomycin on RNA and DNA Synthesis Directed by d(A-T)$_n$ and Calf Thymus DNA. Varying concentrations of kanchanomycin were preincubated with d(A-T)$_n$ or calf thymus DNA in the presence of Mg^{2+} as described in Methods to permit binding of the antibiotic to the polynucleotide. Subsequently the abilities of the kanchanomycin–Mg^{2+}–polynucleotide complexes (having varying ratios of kanchanomycin to nucleotide) and of the control polynucleotide without kanchanomycin to serve as templates for RNA and DNA synthesis were examined using *Escherichia coli* RNA and DNA polymerases. Results with d(A-T)$_n$ are given in Figure 1 and with calf thymus DNA in Figure 2. With both templates kanchanomycin proved to be a strong inhibitor of both RNA and DNA synthesis, the former being somewhat more susceptible than the latter. Kanchanomycin inhibited native and heat-denatured DNA-directed RNA synthesis to a similar extent.

Earlier studies (Friedman *et al.*, 1969a,b) indicated that the initial complex formed between kanchanomycin and polynucleotides changed slowly over a period of a few hours to a second complex. Further, whereas changes in the spectral properties of kanchanomycin accompanying complex formation required a Mg^{2+} concentration equal only to the kanchanomycin concentration, certain changes in the physical properties of polynucleotides upon complex formation (*e.g.*, viscosity) required a Mg^{2+} concentration equal to the nucleotide concentration. It was of interest, therefore, to determine whether the degree of inhibition of RNA and DNA synthesis by kanchanomycin depended upon either the length of time the kanchanomycin and polynucleotide were preincubated or upon the Mg^{2+} concentration during preincubation. In Table I, the experiments are presented in which these two factors varied. There appeared to be only a slight increase in the degree of inhibition of RNA synthesis by kanchanomycin as the preincubation time was extended from 15 min to 4 hr. Prolonging the preincubation to 24 hr gave no further increase in inhibition. Increasing the Mg^{2+} concentration during the preincubation from a concentration equal to the kanchanomycin concentration (Table I,D) to a concentration equal to the DNA-P concentration (B) also increased the degree of inhibition only slightly. It should be noted, however, that any differential effect of varying Mg^{2+} concentration in the preincubation may be masked during the assay for RNA synthesis where the Mg^{2+} is considerably higher.

Nucleoside triphosphates will interact directly with kanchanomycin leading to immediate precipitation and thus precluding quantitative measurements (Friedman *et al.*, 1969b). When the nucleoside triphosphates normally added for the assay of RNA synthesis were added to a DNA solution and kanchanomycin then was added, the level of inhibition of RNA synthesis by kanchanomycin during the subsequent assay was somewhat less than one-half that seen when nucleoside triphosphates were added after kanchanomycin (Table II). Presumably, the nucleoside triphosphates compete with DNA for kanchanomycin. Once kanchanomycin has interacted with DNA, however, nucleoside triphosphates are unable to reverse the binding as indicated by the virtually identical per cent inhibition observed with either 0.2 mM or 0.4 mM of the four triphosphates added after kanchanomycin.

Effect of Increasing Template and Enzyme Concentration on the Inhibition of RNA and DNA Synthesis by Kanchanomycin. Figure 3 presents experiments designed to determine whether increasing the template concentration could overcome the inhibition of RNA and DNA synthesis by kanchanomycin. Increasing concentrations of d(A-T)$_n$ were preincubated with a constant concentration of kanchanomycin and subsequently assayed for their priming capacity for RNA and

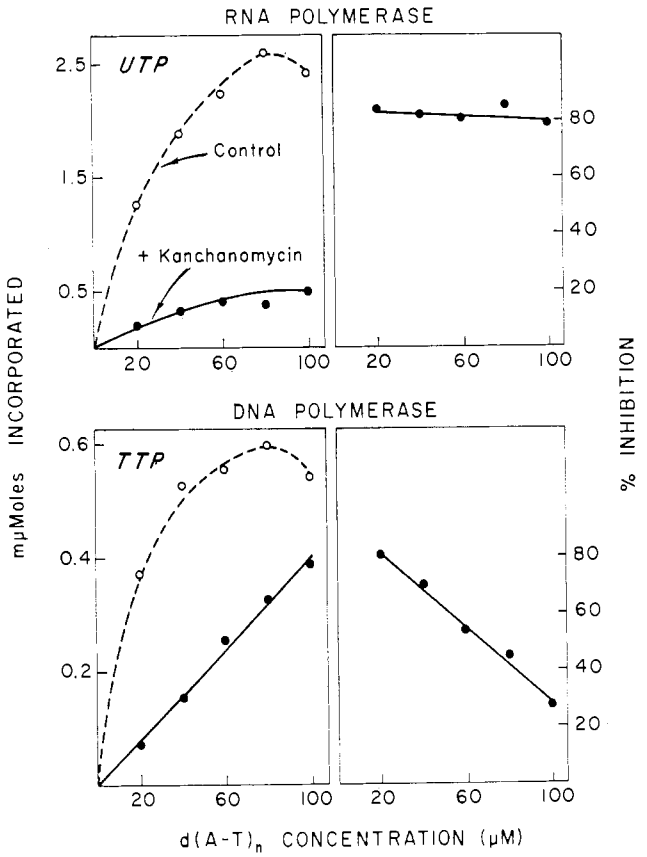

FIGURE 3: Effect of increasing d(A-T)$_n$ concentration on the inhibition of RNA and DNA synthesis by kanchanomycin. The preincubation mixtures contained 0 or 6 μM kanchanomycin, 0.48 mM $MgCl_2$, and d(A-T)$_n$ at a concentration 4 times the final concentration given on the graph. The preincubation period was 2 hr. Each assay for RNA synthesis contained 2 units of RNA polymerase. Each assay for DNA synthesis contained 0.42 unit ofDN A polymerase and was incubated for 10 min. See Methods for details.

DNA synthesis. The inhibition of *DNA* synthesis by kanchanomycin was sharply overcome by increasing concentrations of d(A-T)$_n$. In contrast, the inhibition of *RNA* synthesis was not reduced by increasing the concentration of template. Similarly, when calf thymus DNA was used as template increasing the concentration of DNA preincubated with kanchanomycin did not overcome the inhibition of RNA synthesis by the antibiotic (Figure 4).

In the above experiments, all of the templates used had been preincubated with kanchanomycin and presumably most, if not all, of the DNA molecules had bound at least one molecule of kanchanomycin. In another series of experiments (Figure 5), increasing amounts of DNA (10 to 40 mμmoles) which had *not* been exposed to kanchanomycin were combined with DNA (10 mμmoles) which had been preincubated with kanchanomycin, and assayed for RNA synthesis.[2] Increasing the amount of DNA unexposed to kanchanomycin did overcome the inhibition of RNA synthesis due to antibiotic complexed to a fixed amount of DNA. The extent to which the in-

[2] Kanchanomycin binds extremely tightly to polynucleotides and does not redistribute when more polynucleotide is added (Friedman *et al.*, 1969a).

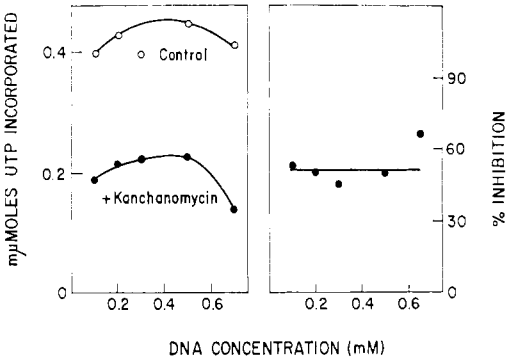

FIGURE 4: Effect of increasing calf thymus DNA concentration on the inhibition of RNA synthesis by kanchanomycin. The preincubation mixtures contained 0 or 20 μM kanchanomycin, 40 μM $MgCl_2$, and DNA at a concentration 2 times the final concentration given on the graph. The preincubation period was 30 min. The assay for RNA synthesis contained 50 μl of the preincubation mixture and 2 units of RNA polymerase in a final volume of 100 μl.

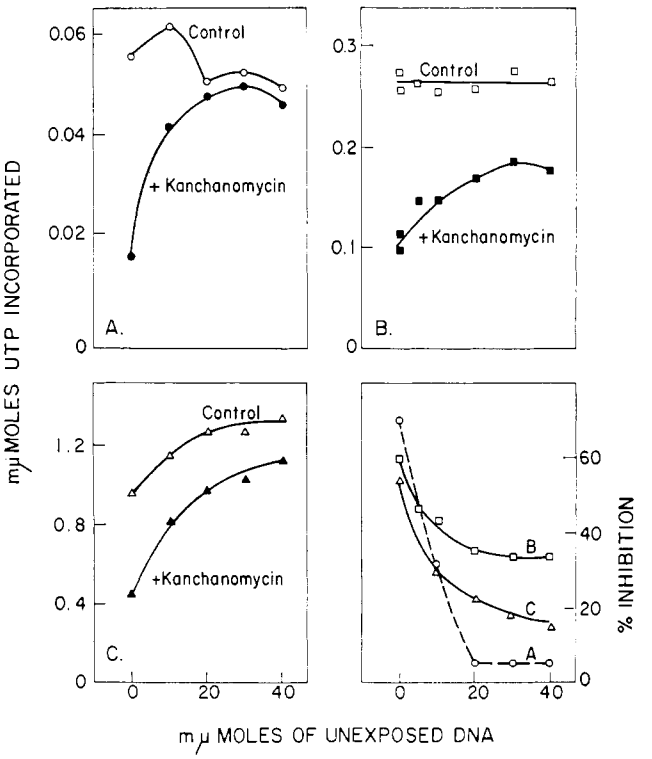

FIGURE 5: Effect of increasing calf thymus DNA which has not been exposed to kanchanomycin on the inhibition of RNA synthesis by kanchanomycin. The standard preincubation mixtures for A and C contained 0.4 mM DNA, 0 or 40 μM kanchanomycin, and 0.4 mM $MgCl_2$ and were preincubated 24 hr. The preincubation mixtures for B were the same except that they contained 0.04 mM $MgCl_2$ and were preincubated 4 hr. The "unexposed DNA" was preincubated under the same conditions as the standard mixtures except that no kanchanomycin was present and the DNA concentrations were such as to give the appropriate mμmoles of "unexposed DNA" in a volume of 10 μl. Each subsequent assay in a total volume of 100 μl contained 25 μl of one of the standard preincubation mixtures (10 mμmoles of DNA) and 10 μl of one of the "unexposed DNA" preincubation mixtures (0–40 mμmoles DNA). RNA polymerase was added as follows: A, 0.7 unit; B, 2 units; C, 8 units. The data in A are the average of two experiments with very similar results. The data in A–C are calculated as per cent inhibition in the lower right-hand corner of the figure.

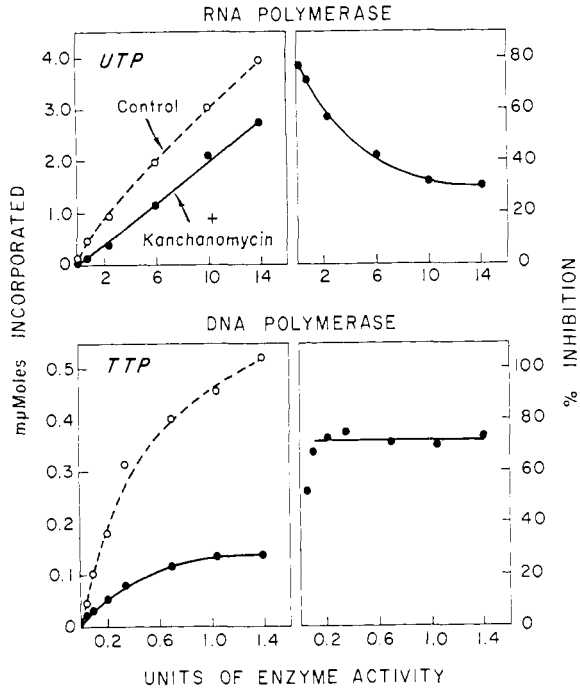

FIGURE 6: Effect of increasing enzyme concentration on the inhibition of RNA and DNA synthesis by kanchanomycin with d(A-T)$_n$ as template. The preincubation mixtures contained 80 μM d(A-T)$_n$, 0 or 3.2 μM kanchanomycin, and 0.16 mM $MgCl_2$. The preincubation period was 2 hr. The units of RNA or DNA polymerase present in the subsequent assays are indicated on the graph. Assays receiving less than the maximum volume of enzyme-containing solution received appropriate volumes of the medium (minus enzyme) in which the enzyme was stored or diluted.

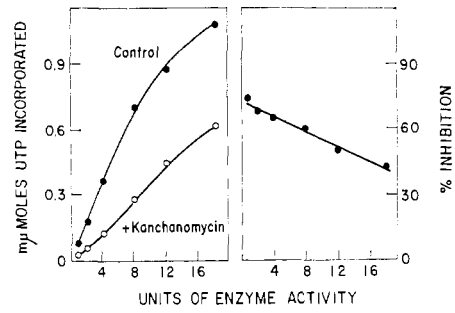

FIGURE 7: Effect of increasing enzyme concentration on the inhibition of calf thymus DNA-directed synthesis of RNA. The preincubation mixtures contained 0.4 mM DNA, 0 or 40 μM kanchanomycin, and 0.4 mM $MgCl_2$. The preincubation period was 4 hr. The units of RNA polymerase present in the subsequent assays are indicated on the graph. Assays receiving less than the maximum volume of enzyme-containing solution received appropriate volumes of the medium (minus enzyme) in which the enzyme was stored.

hibition was overcome varied and depended in part upon the amount of RNA polymerase used in the assay.

Since the inhibition of RNA synthesis was not overcome by increasing the concentration of template *preincubated* with a constant amount of kanchanomycin, the effects of increasing the RNA and DNA polymerase concentrations upon inhibition was studied (Figure 6). Each assay received a constant amount of d(A-T)$_n$ preincubated with or without kanchanomycin and increasing amounts of DNA or RNA polymerase. Increasing the concentration of DNA polymerase did not overcome the inhibition of DNA synthesis by kanchanomycin. In contrast, increasing the amount of RNA polymerase did overcome the inhibition of RNA synthesis by kanchanomycin. Similarly, when calf thymus DNA was used as template for RNA synthesis, increasing the concentration of RNA polymerase reduced the inhibition of RNA synthesis by kanchanomycin (Figure 7).

Effect of Kanchanomycin on RNA Synthesis as a Function of the Length of Incubation and Time of Addition of Kanchanomycin. In an attempt to determine whether the effect of kanchanomycin might be preferentially upon initiation of RNA synthesis or preferentially upon polymerization, the extent of inhibition of RNA synthesis was followed as a function of incubation time (Figure 8). The per cent inhibition of RNA synthesis changed little between 5 and 40 min of incubation with both d(A-T)$_n$ and calf thymus DNA. With calf thymus DNA the inhibition at the earliest time point (2 min) was much less than at later time points. This brief lag in inhibition was not seen with d(A-T)$_n$ as template.

To test more directly whether kanchanomycin inhibits polymerization (apart from initiation) kanchanomycin was added to an assay after RNA synthesis had been in progress

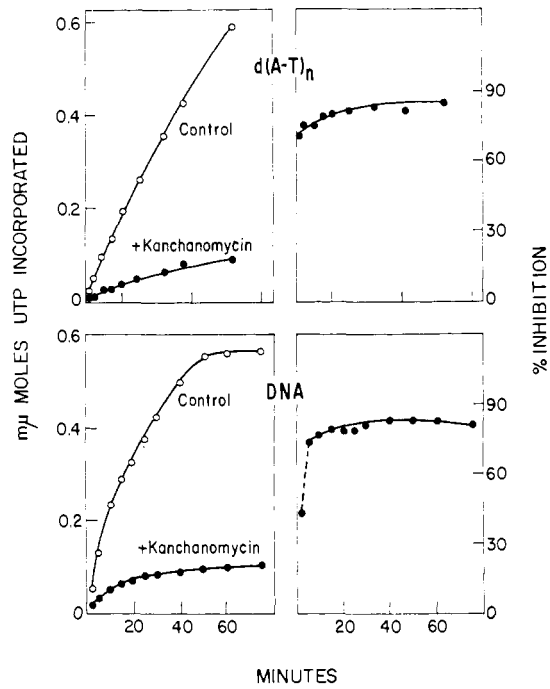

FIGURE 8: The inhibition of RNA synthesis by kanchanomycin as a function of the length of incubation. The two preincubation mixtures for the experiment with d(A-T)$_n$ as template contained 20 μM d(A-T)$_n$, 0 or 3 μM kanchanomycin, and 0.16 mM $MgCl_2$. The preincubation period was 2 hr. The two preincubation mixtures for the experiment with calf thymus DNA as template contained 0.416 mM DNA, 0 or 40 μM kanchanomycin, and 0.4 mM $MgCl_2$. The preincubation period was 4 hr. Each preincubation mixture of 0.25 ml was diluted to 1.0 ml for the assay of RNA synthesis and contained 8 units of RNA polymerase with d(A-T)$_n$ as template or 40 units of RNA polymerase with calf thymus DNA as template. Samples (50 μl) were taken at each time point. A zero time value was subtracted from each subsequent time point.

TABLE III: Comparison of the Effect of Kanchanomycin Added before and After Formation of a d(A-T)$_n$–RNA Polymerase Complex and Initiation of RNA Synthesis.[a]

	Components Present During First Incubation	Components Added before Second Incubation	% Inhibition of UTP Incorporation Due to KM
1	d(A-T)$_n$, ATP, UTP, ± KM	Enzyme	18, 20
2	d(A-T)$_n$, enzyme	ATP, UTP, ± KM	42, 42
3	d(A-T)$_n$, enzyme, ATP, UTP	± KM	50, 50

[a] Each sample contained 2 mμmoles of d(A-T)$_n$ and 6 μmoles of Tris (pH 7.9)–0.6 μmole of $MgCl_2$–0.5 μmole of $MnCl_2$–1.8 μmoles of β-mercaptoethanol in 70 μl; 40 mμmoles each of ATP and [^3H]UTP, 0.2 mμmole of kanchanomycin (KM) in 20% dimethylformamide or 20% dimethylformamide alone, and 1.2 units of RNA polymerase (enzyme) were each added in additional 10-μl volumes as indicated in the table. The first incubation was 5 min at 37°. The second incubation was 30 min at 37°. In experiments 1 and 3, the second incubation was begun immediately following completion of the first incubation and addition of the indicated components. In experiment 2, the samples were cooled in ice water for 3 min after the first incubation; the ATP and [^3H]UTP were added and the samples mixed before addition of kanchanomycin and incubation at 37°. In each experiment, trichloroacetic acid was added to one sample immediately prior to the second incubation and the cpm of this sample subtracted as a blank from the final cpm of the corresponding samples. The incorporation of [^3H]UTP in the control assays were 1.04, 0.61, and 0.70 mμmole in experiments 1, 2, and 3, respectively.

for 5 min (Table III). When d(A-T)$_n$ and RNA polymerase were together before addition of kanchanomycin (experiment 2) or when RNA synthesis was in progress before addition of kanchanomycin (experiment 3), the per cent inhibition of RNA synthesis by kanchanomycin was twice as great as when kanchanomycin was complexed to d(A-T)$_n$ before initiation of RNA synthesis (experiment 1). Kanchanomycin is able therefore to inhibit polymerization after RNA synthesis is already initiated.

In the above experiments, UTP and ATP were present in the medium before kanchanomycin was added. As indicated in Table II, competition between nucleoside triphosphates and d(A-T)$_n$ for kanchanomycin accounts for the low per cent of inhibition seen in experiment 1. Why experiments 2 and 3 should actually give greater inhibition of RNA synthesis by kanchanomycin is not clear. Perhaps the d(A-T)$_n$–RNA polymerase complex and the d(A-T)$_n$–RNA polymerase-growing $_r$(A-U)$_n$ chain complex compete more effectively against UTP and ATP for kanchanomycin than the d(A-T)$_n$ alone. These data taken together with that showing competition between the RNA polymerase and kanchanomycin (Figures 6 and 7) suggest that the enzyme and the antibiotic interact, probably on the template DNA.

Discussion

Of all the reported antibiotic inhibitors of nucleic acid synthesis, kanchanomycin resembles luteoskyrin most closely. Luteoskyrin, a potent hepatotoxic pigment with carcinogenic properties (Uraguchi *et al.*, 1961), has been shown to bind to nucleic acids (Ueno *et al.*, 1967a), to preferentially inhibit RNA synthesis *in vivo* (Ueno *et al.*, 1967b), and to inhibit the DNA-dependent RNA polymerase *in vitro* (Sentenac *et al.*, 1967). Features of luteoskyrin's binding to nucleic acids include: a requirement for stoichiometric quantities of divalent cation; the formation of two distinct types of complexes— I, a polynucleotide-specific complex rapidly formed with the purine moieties of heat-denatured DNA and synthetic polynucleotides, and II, a nonspecific complex slowly formed with native DNA and synthetic polynucleotides; a time-dependent conversion from I into II; and dissociation of either complex by EDTA (Ohba and Fromageot, 1967, 1968). Kanchanomycin requires stoichiometric quantities of divalent cation for complex formation with nucleic acids and forms two complexes (I and II), both of which are reversible by EDTA although II is much more stable than I to this treatment (Friedman *et al.*, 1969a,b). Kanchanomycin, however, gives initially the same complex I with all polynucleotides followed by a time-dependent conversion into the second complex II, except for that formed with polyadenylic acid which is stable in the initial complex. Complex II formation with kanchanomycin requires ring unsaturation in the bases of the polynucleotide. Further, while kanchanomycin's initial complex is largely electrostatic in nature, it is luteoskyrin's second complex which is considered to be primarily dependent on electrostatic interactions (Ohba and Fromageot, 1968).

Although luteoskyrin, unlike kanchanomycin, inhibits *in vitro* RNA synthesis promoted by heat-denatured DNA to a greater degree than that promoted by native DNA (Sentenac *et al.*, 1967), in some other respects the two agents are alike in their inhibition of the DNA-dependent RNA polymerase. As we have also found for kanchanomycin, increasing the concentration of DNA template relative to luteoskyrin does not overcome the inhibition (Sentenac *et al.*, 1967). Thus, the inhibition of RNA synthesis is not due solely to the binding to the agent to the DNA, but must in addition involve inactivation of the polymerase in the complex. Further, it was suggested that the enzyme might alter the DNA structure locally so as to lead to the selective binding of luteoskyrin (Sentenac *et al.*, 1967). The data in Table III are compatible with such a mechanism for kanchanomycin. It is also possible that the enzyme is attracted to the sites (not necessarily the same as those for the initiation of RNA synthesis) on the DNA where the inhibitor is located. When more enzyme is added, inhibition due to kanchanomycin is overcome because enzyme is able to go to initiation sites free of inhibitor. A Lineweaver–Burk plot of these data shows competition between antibiotic and enzyme but not DNA (Figure 9). Why then was it possible to overcome the inhibition by adding unexposed DNA? Unexposed DNA may be an effective competitor of DNA possessing antibiotic for the RNA polymerase. This could be the result of a change in the physical state of the complexed DNA which makes it less accessible to the enzyme. Presumably ex-

posed DNA contains no molecules free of antibiotic. In fact, it has been found from sedimentation and viscosity measurements that DNA treated with kanchanomycin at the Mg^{2+} concentrations used in this work is probably in an aggregate state (Friedman *et al.*, 1969a).

In contrast with the RNA polymerase, the DNA polymerase is not attracted preferentially to sites on the DNA possessing kanchanomycin. Instead it is the template function of the DNA which is altered by the antibiotic. As shown in Figure 9, a Lineweaver–Burk plot of the data from Figures 3 and 6 show competition between antibiotic and DNA template but not enzyme.

By directly inactivating the polymerase on the template, kanchanomycin differs in its action as an inhibitor of RNA synthesis from most other drugs which bind to DNA. In this effect kanchanomycin is like the rifamycins (Hartmann *et al.*, 1967; Sippel and Hartmann, 1968; Wehrli *et al.*, 1968) and streptovaricins (Mizuno *et al.*, 1968), which do not bind to DNA, but differs from them in its ability to inhibit RNA synthesis even after its initiation.

The molecular and structural nature of the interaction of kanchanomycin with DNA which leads to its interference with the function of both the RNA and DNA polymerase is not yet known. Kanchanomycin does not inhibit DNA replication by preventing separation of the DNA strands, since it does not increase the temperature of thermal transition of DNA (Friedman *et al.*, 1969a). Whatever the nature of the interaction may be, *i.e.*, intercalation, binding to the sugar–phosphate backbone, or attachment to the grooves of the DNA, which interferes with the function of the DNA polymerase, the direct inactivation of the RNA polymerase by kanchanomycin bound to template suggests that functional groups of the antibiotic may reside in the minor groove of DNA where this enzyme is thought to function (Reich and Goldberg, 1964).

It is not the intention of this study to imply that kanchanomycin exerts its toxic effect on cells primarily by a direct action on nucleic acid synthesis. In fact, we have found that this antibiotic rapidly shuts off many synthetic reactions in cells simultaneously (K. Mitsugi and I. H. Goldberg, 1966, unpublished results). This may result from the inhibition by kanchanomycin of oxidative phosphorylation such as has been found with mammalian mitochondria (P. A. Friedman and I. H. Goldberg, 1967, unpublished results).

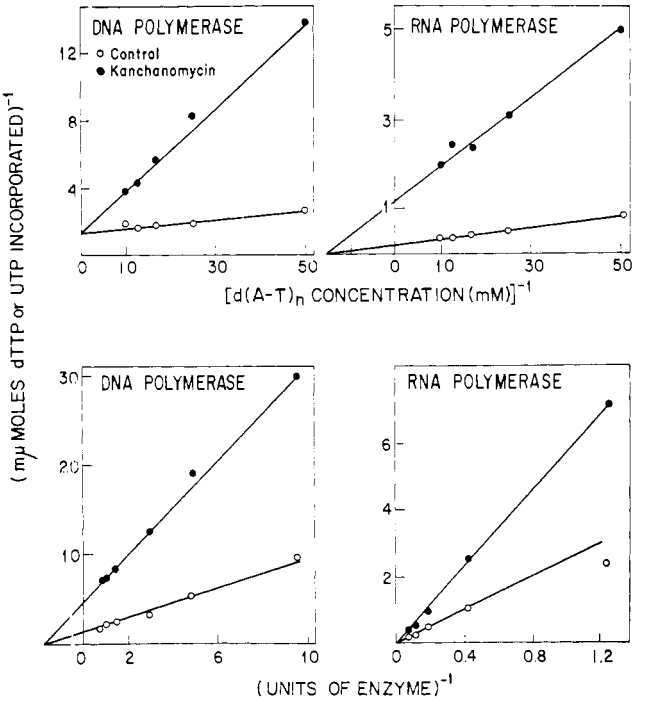

FIGURE 9: Lineweaver–Burk plot of data from Figures 3 and 6.

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