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INHIBITION OF DNA AND RNA POLYMERASE REACTIONS BY TRYPANOCIDAL --ETC(U)

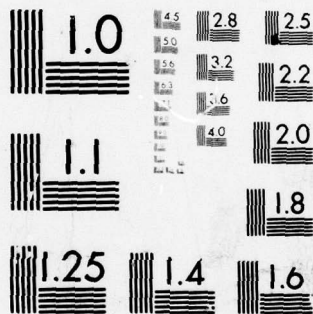
MAR 79 A C ZAHALSKY, D G BROWN, N F NELSON

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6 INHIBITION OF DNA AND RNA POLYMERASE REACTIONS
BY TRYpanocIDAL DRUGS*
Effect of Aromatic Diamidine and Phenanthridinium Compounds,

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Abstract

Several trypanocidal drugs were tested for possible inhibitory activity towards DNA (pol. I) and RNA polymerase. These chemotherapeutically active agents are thought to inhibit nucleic acid syntheses in African bloodstream trypanosomes. In the isolated DNA and RNA reactions those compounds that were most inhibitory include Isometamidium, Berenil (diminazene) and Hydroxystilbamidine. In some cases inhibition of the DNA polymerase reaction could be relieved by the input of additional enzyme and/or template-primer. RNA polymerase activity appeared to be inhibited in direct proportion to the amount of drug bound to the template.



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Introduction

Some chemotherapeutic agents active against bloodstream forms of the African trypanosomiases in man and livestock include phenanthridinium drugs (Ethidium, Isometamidium and Prothidium), diamidine derivatives (Berenil, Hydroxystilbamidine and Pentamidine) and the aminoquinoline compound, Antrycide. In field use these agents exhibit varying degrees of prophylactic activity against members of the congolense-vivax, evansi-equinum-equiperdum and brucei subgroup organisms¹.

Studies on the mode of action of these metal-free trypanocides on insect trypanosomatids includes evidence that Antrycide inactivates Crithidia ribosomes in vitro² and selectively affects utilization of exogenous adenine by C. oncopelti³. Berenil induces dyskinetoplasty in Trypanosoma evansi⁴, is rapidly taken up by the kinetoplast of T. brucei and T. mega⁵ and appears to bind preferentially to DNA of high A+T content⁶. Other evidence indicates that Berenil reversibly inhibits DNA and RNA synthesis in T. brucei in vivo⁷ and may induce a state of unbalanced growth in the bloodstream forms. The effect of some trypanocides on the RNA polymerase reaction has previously been noted⁸ and related to the probable in vivo action of these compounds.

At the ultrastructure level Berenil treated T. rhodesiense show fragmentation of the kinetoplast DNA (K-DNA)⁹ as do organisms exposed to Hydroxystilbamidine¹⁰. Also, an increase in the proportion of doubly branched structures of the small circular-K-DNA of T. cruzi was detected following exposure to Berenil¹¹, possibly indicating blockage by the drug of replication at presumably regularly distributed AT-rich regions of the K-DNA minicircles.

These and other findings^{12,13} have led to the suggestion that trypanocidal aromatic diamidine and phenanthridinium derivatives exert their effect(s) by inhibiting nucleic acid metabolism or synthesis. The present studies examine the activity of some trypanocidal drugs on the isolated DNA and RNA polymerase reactions. Supportive evidence for the action of drugs and other molecules on replication and transcription can be drawn from the effects of these drugs on polymerase reactions in vitro. Such studies have shown that purified DNA(s) and heterologous polymerase(s) (31,32,33) are useful in assessing potential drug action on intact cells in culture (32,34,35), isolated nucleic (31,32), and endogenous enzymes (31,36). The results obtained are discussed with reference to their likely mode of action in vivo.

Materials & Methods

A. Labeled Compounds and Biochemicals: [methyl-³H-dTTP] and [5-³H-UTP] (sp. act. 57 Ci/mole and 21 Ci/mole respectively) were purchased from the New England Nuclear Corp., Boston, Mass. Unlabeled nucleoside-5'-triphosphates and reaction mix components were obtained from the Sigma Chemical Co., St. Louis, Mo. as the highest purity compounds available. Glassfiber or paper discs (Whatman #3, 25mm) were used in these assays. It was determined that the counting efficiency of radioactive DNA on the paper discs was 30% in comparison to 45% on glass fiber discs.

B. DNA Polymerase Assays: (i) E. coli DNA polymerase. (E.C. 2.7.7.7). Highly purified Fraction VII (General Biochemicals, Chagrin Falls, Ohio) in the presence of heat-denatured template was used to monitor the effects of drug additives on the incorporation of [³H]dTTP into acid-insoluble DNA. The standard assay system contained the following constituents in a final

volume of 0.1 ml: TRIS-HCl, 10 μ mole; MgCl₂, 0.7 μ mole; 2-mercaptoethanol, 0.1 μ mole; 20nmoles each of dCTP, dGTP, dATP; 20 nmoles dTTP containing 1 μ Ci [³H]-dTTP; E. coli DNA polymerase, 0.2 Units and heat denatured Calf Thymus (CT) DNA, 0-10 μ g, pH 7.4. Incubation was carried out at 37°C for 60 min. after which the mixture was assayed by the filter paper disc procedure of Bollum¹⁴. The filters were batch washed 2X (30 min/wash) with 5% trichloroacetic acid (TCA) containing 1% pyrophosphate (Na₄P₂O₇), 2X with TCA containing 0.1% ATP, then 2X with abs. EtOH (5 min/wash) and finally with ether for 5 min. The total volume of wash fluid for each disc was 10 ml. Subsequent to the ether wash the discs were dried for 10 min at 30-50 C, placed in vials to which 10 ml toluene based scintillation fluid was added (5g PPO + 0.15g POPOP/liter toluene) and counted in a Packard TriCarb Liquid Scintillometer.

(ii) *Calf-Thymus DNA polymerase* (replicative deoxynucleotidyl transferase). CT-DNA polymerase (General Biochemicals, Chagrin Falls, Ohio) in the presence of heat denatured template was used to monitor the effects of drug additives on the incorporation of [³H]-dTTP into acid-insoluble DNA. The standard assay system contained the following in a final volume of 0.1ml: KH₂PO₄, 4 μ moles; MgCl₂, 0.8 μ moles; 2-mercaptoethanol, 0.1 moles; 10nmoles each of dCTP, dGTP, dATP; 10nmoles dTTP containing 0.5 μ Ci [³H]-dTTP; CT-DNA polymerase, 0.06-0.6 Units and heat denatured CT-DNA, 0-10 μ g, pH 7.0. Incubation was carried out at 37°C for 60 min and the discs treated as described in (i).

C. RNA Polymerase Assays: E. coli K12 RNA polymerase (E.C. 2.7.7.6). (General Biochemicals, Chagrin Falls, Ohio) was used to monitor the effects of drug additives on the DNA-Dependent incorporation of [³H]-UTP into

acid-insoluble RNA. The standard assay system contained the following in a final volume of 0.1 ml: TRIS-HCl, 4 μ mole; 2-mercaptoethanol, 1.2 μ mole; 40nmole each of ATP, CTP, GTP; 40 nmole UTP containing 0.5 μ Ci of [³H]-UTP, pH 7.9. Immediately prior to performing the assay(s) the following components were added: MgCl₂, 0.4 μ mole; MnCl₂, 0.1 μ mole; E. coli RNA polymerase, 0.1-0.8 Units and 0-10 μ g DNA template. Incubation was carried out at 37°C for 30 min and the discs treated as described in (i) above except that the last 5% TCA wash contained 0.01% UTP.

D. Drug Solutions: All drug solutions were made up in .015M, NaCl, pH 6.0. Freshly made up solutions were kept in the dark at 4°C until used. Under these conditions solubility problems were encountered with Antrycide and Bayer 2502.

Results

The dependence of the DNA polymerase assay on both template-primer and enzyme is seen in Fig. 1. The rate of incorporation of [^3H]-dTTP with increasing amount of enzyme is nearly linear over the range used, 0-0.4 Units/assay. A maximal rate of incorporation of [^3H]-dTTP occurs at a concentration of $\sim 80\mu\text{g/ml}$ heat-denatured CT-DNA. These results indicate that in the DNA polymerase assay system used in these studies ($\sim 90\mu\text{g/ml}$ DNA + 2 Units enzyme/ml) the DNA is at saturating level and the enzyme is limiting. The DNA concentration of $9\mu\text{g}$ per assay is equivalent to ~ 29 μmoles as deoxyribonucleotides. At this level any drug interfering with the ability of the DNA to act as a template-primer would be expected to cause considerable inhibition of the reaction rate.

In this and all other assays duplicate determinations were performed; the agreement between the two values was almost always within 5% of the mean. The reaction was completely dependent upon the addition of DNA. When dGTP, dATP or dCTP were omitted from the reaction mixture, either singly or in combination the incorporation of ^3H -dTTP was reduced to less than 5% of control values. In the presence of $9\mu\text{g}$ DNA/assay the reaction proceeded at a constant rate for 90 min, and the effect of enzyme concentration was found to be linear up to a concentration catalyzing the incorporation of 400-450 picomoles of [^3H]-dTTP during a 60 min incubation.

Effects of Trypanocidal Agents on the DNA Polymerase Reaction

Figures 2A, 2B and 2C illustrate the effects of various drugs on DNA polymerase activity. Isometamidium and Ethidium completely inhibit at $100\mu\text{g/ml}$, indicating high affinity for the template and/or enzyme. Berenil, Hydroxystilbamidine, Propamidine and Stilbamidine are almost equally

effective as inhibitors, all approaching 80-90% inhibition at the concentrations tested. Other evidence (see Table 1) suggests that these compounds interact with the enzyme but that binding is not as tight as that which occurs with Isometamidium and Ethidium. Although Primaquine has been reported to inhibit DNA polymerase activity¹⁵, this drug stimulated the rate of incorporation of [³H]-dTTP. The somewhat reduced effectiveness of Antrycide and Bayer 2502 may be a consequence of solubility problems, although Antrycide was almost as effective an inhibitor as the diamidines. Similar results were obtained using E. coli DNA polymerase (Fraction IV) or calf thymus DNA polymerase.

Dependence of the RNA Polymerase Assay on Template and Enzyme

As shown in Fig. 3 the rate of incorporation of [³H]-UMP with arithmetic increase in the amount of enzyme/assay is very nearly linear over the range, 0-12 Units/assay. With heat-denatured CT-DNA as template the incorporation of [³H]-UMP is highest at 100µg/ml. Under the conditions employed in the standard assay system the DNA is saturating.

Effects of Trypanocidal Agents on the RNA Polymerase Reaction

In Figs. 4A-B, 4C-D, and 4E-F drug inhibitions are compared using heat-denatured or native CT-DNA. Both Ethidium and Isometamidium show a >80% inhibition of activity, probably reflecting a tight template binding. At higher concentrations Berenil inhibits activity with heat-denatured DNA, contrasting with a 10% or less inhibition when native DNA is used. At lower concentrations Antrycide stimulates activity with native DNA whereas at higher concentrations inhibition approaches 100% with both sources of template. Propamidine appears to stimulate activity with

heat-denatured DNA and exhibits only about 20% inhibition at higher concentrations with either template. Primaquine and Bayer 2502 both stimulate activity somewhat at low concentration with heat-denatured DNA but inhibit activity by 40-50% at higher drug concentrations using either template. Hydroxystilbamidine and Stilbamidine exhibit similar curves of inhibition. At low Stilbamidine levels polymerase activity is stimulated in the presence of heat-denatured DNA.

Effect of a Constant Amount of Drug(s) on E. coli DNA Polymerase Activity Using Alternate Enzyme and DNA Concentrations

Figure 5A reveals that at the lower DNA concentration inhibition of polymerase activity is somewhat alleviated by the higher enzyme level in the case of Ethidium (38% of control at 0.3 units vs. 19% at 0.1 units). The extent of inhibition with Berenil is the same (~30%) at both enzyme levels, whereas no increase in activity is seen with either Antrycide or Isometamidium.

At the higher DNA level (Fig. 5B) incorporation in the presence of Ethidium or Berenil is similar, i.e. activity at either enzyme level is near 30% of the control value. Similarly, the inhibition by Isometamidium is near 25% of the control value. With Antrycide (55% of control at 0.1 unit), additional enzyme slightly enhances incorporation, to the extent of 27% of the control value at 0.3 units.

In these experiments the presence of additional enzyme generally resulted in increased incorporation. However, with the exception of Ethidium (Fig. 5A), alleviation of inhibition did not occur. In the case of Antrycide or Isometamidium additional enzyme either resulted in no increase in incorporation (5A) or relatively diminished incorporation (5B), suggesting that their interaction with DNA polymerase is greater than the affinity shown by Berenil or Ethidium.

Effect of Trypanocidal Agents on the Time Dependence of RNA Synthesis.

As noted in Fig. 6 the extent of drug inhibitions increased somewhat during the first 20 min. Subsequently the rates of synthesis tended to increase and the rate achieved in the presence of Berenil nearly equaled control values after 45 min. These results suggest that the observed decrease in inhibition may be due to the formation of a drug complex with the product macromolecule as this accumulates, thereby releasing template sites which then become available for polymerase activity. It should be noted that in this Figure the ordinate designation as '% of Control' is applicable to all time points on the abscissa.

Recovery of DNA Polymerase Activity With Template Primer or Enzyme Addition.

The results noted in Table 1 attempt to determine to what extent [³H]-TMP incorporation could be recovered by the input of additional template-primer or enzyme at 2X and 3X normal assay levels. The annulment of drug inhibition by the addition of DNA or enzyme appeared to identify four categories of effects: (i) fully alleviated [Antrycide, Isometamidium, Stilbamidine] or by no less than 70% of control values [Bayer 2502, Berenil] by addition of either 9.25 or 18.5 μ g DNA/assay, (ii) recovery of 50-60% of activity by input of additional enzyme [Antrycide, Berenil, Isometamidium, Propamidine and Stilbamidine], (iii) no recovery of activity upon addition of DNA [Propamidine] and (iv) stimulatory effect by the addition of DNA [Hydroxystilbamidine, Primaquine] or enzyme [Bayer 2502, Primaquine]. When the alleviation of inhibition of a particular drug is compared for added DNA vs. added enzyme the 70-95% effectiveness of DNA,

in the case of Antrycide, Berenil, Isometamidium and Stilbamidine contrasts with a recovery of only 35-60% of activity when additional polymerase is added.

These data appear to be consistent with the findings of Waring⁸ who noted that inhibition by Ethidium and Suramin in the RNA polymerase reaction was related in a competitive fashion to the concentration of DNA. In those cases noted here, where recovery of approximately half normal incorporation was obtained by the input of additional polymerase, competitive annulment of [drug:enzyme] interaction and/or possible displacement of charged drug molecules from the primer terminus or template binding sites on the enzyme may account for the observed alleviation(s).

The results in Table 1 prompted an examination of the consequence of the order of addition of components in the assay procedure. In Table 2 it may be noted that when the template-primer was added as the final component the inhibitions obtained were significantly higher than when drug or enzyme was added last.

DISCUSSION

In the absence of pharmacologic barriers to the establishment of effective blood titres in the animal host¹⁶, and barring problems in the transport of drug molecules across the trypanosome cell boundary¹⁷, the distinction between slow (~24-48 hr onset) and the more rapid acting (~2-10 hr onset) trypanocidal agents¹⁸ could reflect differential uptake and binding affinity by multiple intracellular loci, e.g. nucleus, kinetoplast, lysosomes, cytosol. The lethal effect (clearing) by these compounds on trypanosomes may also reflect a composite of damaging events rather than a select chemotherapeutic target. Such effects could include: (i) release and/or activation of lysosomal enzymes, (ii) inhibition of replicative and transcriptive enzymes in situ and (iii) stoppage of nucleic acid synthesis due to non-covalent interaction with the DNA template⁶. Interference with glycolysis, the sole energy deriving pathway of the pathogenic bloodstream forms¹⁹, also represents a possible additional target of these metal-free trypanocides²⁰.

Under field conditions the demonstrated efficacy of the less toxic diamidine and phenanthridinium derivatives for different subgroup species of African bloodstream trypanosomes¹ suggests that differences in species susceptibility may be a consequence of subtle differences in membrane and enzyme structure, hence the possibility of altered binding affinities within these organisms. Slight alterations in molecular composition or conformation, could also explain the sensitivity of some drug-resistant strains to other trypanocides, frequently of a different chemical class²¹.

Some lines of evidence which support a binding affinity by cationic trypanocides for intact trypanosomes and organelles in other cells include reports of tight cellular binding²² and stabilization of lysosomal membranes by Suramin²³, marked sensitivity of kinetoplast DNA to Antrycide²⁴, the appearance of lysosomes filled with Stilbamidine (chemotherapy granules)^{25,26}, combination of nuclear DNA but not RNA with Hydroxystilbamidine^{26,27} and the shared ability by Ethidium Bromide and Antrycide to block nucleic acid synthesis^{28,29}. Although insect trypanosomatids have a demonstrated usefulness in determining the uptake of antiprotozoal drugs by intact cells and their particulate fractions³⁰ investigations on the mechanisms of drug action may more reliably lie with direct in vivo studies on bloodstream forms in the trypanosome parasitized host⁷.

The present studies confirm the findings by Waring⁸ on the potent inhibitory effect by Ethidium and Prothidium on RNA polymerase, and extend this line of investigation to other trypanocidal agents and their effect(s) on the DNA polymerase reaction. Our findings also correlate with the known ability by these compounds to complex with template-primer. The inhibitions in incorporation of deoxynucleoside monophosphate by both slow (Antrycide, Isometamidium, Hydroxystilbamidine) and more rapid acting trypanocides (Berenil), and the inability to recover control levels of incorporation by the input of additional enzyme with either class of drug, suggests that in the isolated state DNA polymerase exhibits a somewhat non-specific affinity for these compounds. The cationic character of the drug molecules at the assay pH, and therefore their potential for interaction with anionic polymers, also suggests the formation of several equilibria in solution during the

reaction period. The most significant of these would seem to be the association-disassociation for [drug:template] vs. [drug:enzyme] at saturating drug level. In the case of Antrycide, Isometamidium and Stilbamidine the 70-95% relief of inhibition upon input of additional template-primer, in contrast with the results obtained with Propamidine, may signal drug displacements resulting from shifts in binding affinities. Consequently, a resumption of incorporating activity could occur. We are currently attempting to isolate DNA polymerase from bloodstream organisms to extend these initial studies to the trypanosome enzyme.

Table 1. Alleviation of Drug-Inhibited DNA Polymerase Activity by Addition of Template-Primer or *E. coli* DNA Polymerase I

Drug	mM	Standard DNA & Enzyme Level 92.5 µg DNA/ ml & 0.2 unit	Additional DNA		Additional Enzyme	
			185 µg/ml	277.5 µg/ml	0.4 unit	0.6 unit
Control	-	250	225	162	720	1175
Antrycide (W570)	0.112	55	229	215	451	792
Bayer 2502	0.348	94	158	218	790	1466
Berenil	0.097	10	59	135	202	624
Ethidium bromide	0.025	61	160	145	283	557
Hydroxystil- bamidine Isethionate	0.198	125	250	180	300	370
Stilbamidine Isethionate	0.193	72	139	194	374	578
Isometami- dium methane sulfonate	0.035	77	205	205	352	419
Primaquine	0.440	293	427	430	987	1173
Propamidine Isethionate	0.354	69	89	79	251	531

All values are picomoles of dTMP incorporated in 60 min. The standard DNA polymerase reaction contained 92.5 µg heat-denatured DNA/ml and 0.2 units of *E. coli* DNA polymerase I as described in the text. Additional experiments were performed using two and three times the standard amount of DNA or enzyme. The drug concentrations used resulted in a 50 to 80% inhibition of the standard 'Control' assay with the exception of primaquine which resulted in a 20% stimulation.

Table 2. Effect of the Sequence of Addition of Components on DNA Polymerase Activity

DRUG	mM	DNA Added Last	Drug Added Last	Enzyme Added Last	Turbidity
Control	-	100	100	100	
Antrycide W570	0.224	0.7	11	0.6	±
Antrycide W570	0.336	1.1	2.2	1.3	+
Bayer 2502	0.348	40	88	82	0
Bayer 2502	0.522	34	91	92	0
Berenil	0.039	30	64	63	0
Berenil	0.058	25	61	50	0
Ethidium Br.	0.025	15	114	16	0
OH-Stilbamidine	0.188	36	50	50	0
Isometamidium	0.035	22	42	33	±
Primaquine	0.440	67	119	131	0
Propamidine	0.354	18	38	33	0
Stilbamidine	0.097	27	41	59	0
Stilbamidine	0.145	25	45	54	0

All values shown are percent of control. The Controls represent assay with *E. coli* DNA polymerase (pol. I) and heat-denatured CT-DNA in the absence of drug. The 100% values represent approximately 400 picomoles [³H]-dTTP incorporated in 60 min. Components of the assay are as described under 'Methods'. After buffer and appropriate precursors were mixed, the DNA, drug and enzyme were added. The mixtures were incubated at 0-4° for 10 min between each of these three final additions.

Turbidity was assessed visually in the reaction mixtures in the absence or presence of DNA polymerase: there was no change upon the addition of enzyme. 0 means no turbidity or precipitate; ± means perceptible turbidity but no visible precipitate; + means moderate turbidity and perceptible precipitate.

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Figure 1.

Dependence of *E. coli*. DNA polymerase activity on heat-denatured CT-DNA and enzyme. Increasing amounts of CT-DNA were added to 0.2 unit of DNA polymerase (pol I) (o—o) and increasing amounts of enzyme were added to 9.5 μ g CT-DNA (Δ — Δ). Assay conditions and components are described in the "Methods". Subsequent assays contained 0.2 unit of enzyme and 9 to 10 μ g of heat-denatured or native CT-DNA in a total reaction volume of 100 μ l.

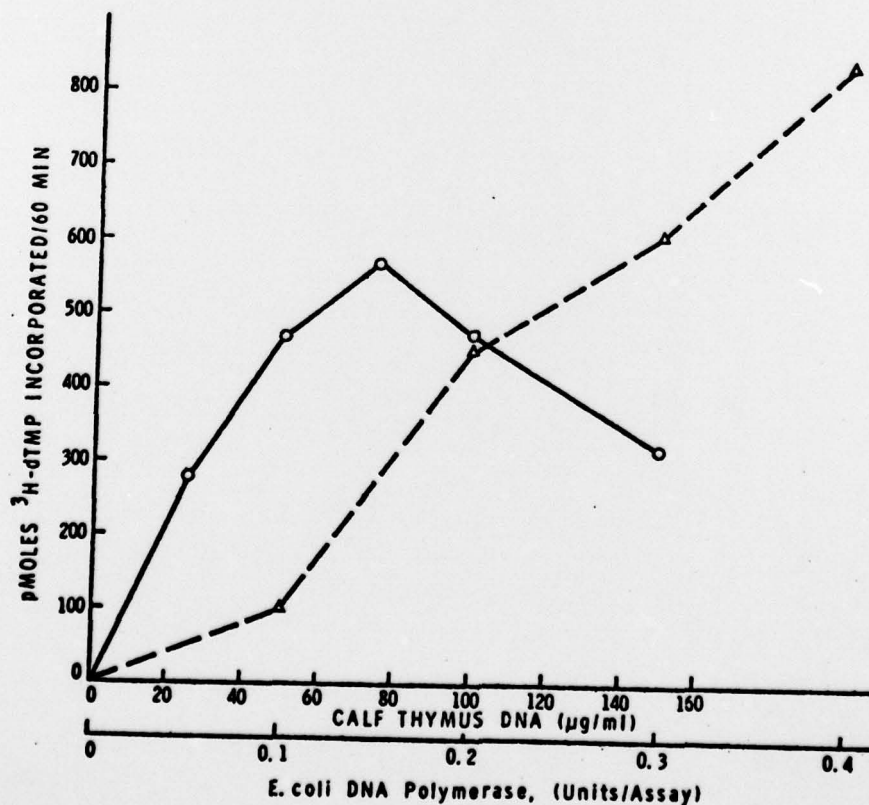


Figure 2A.

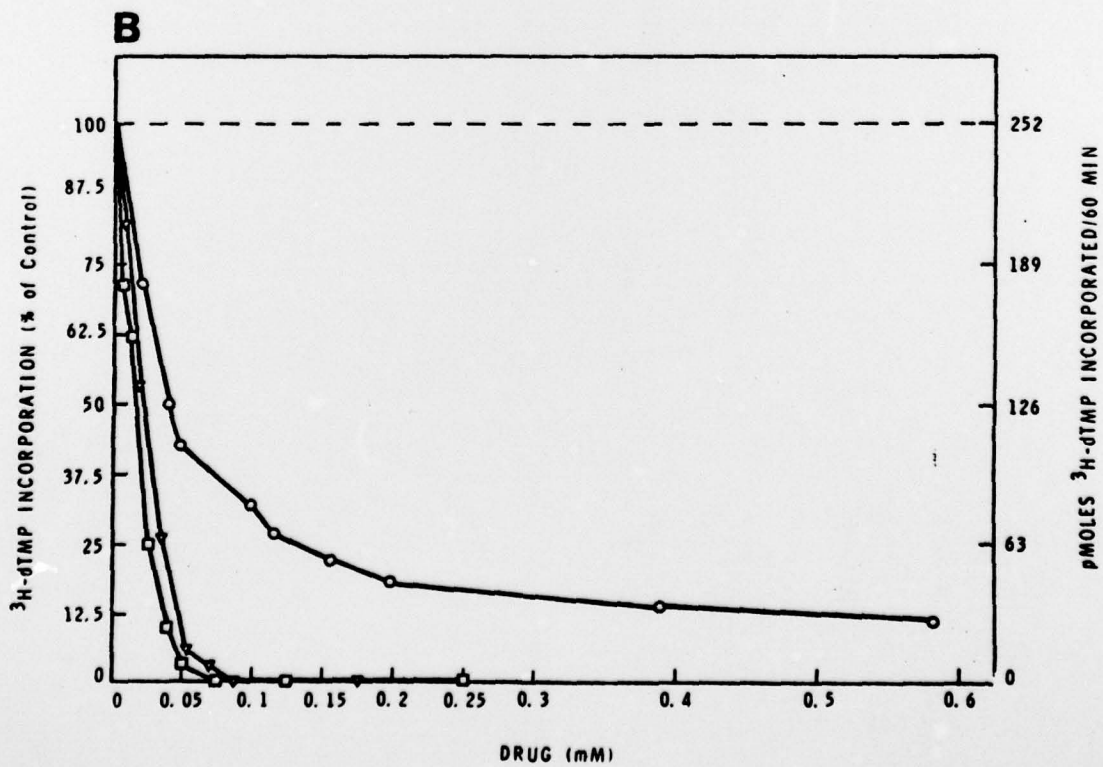
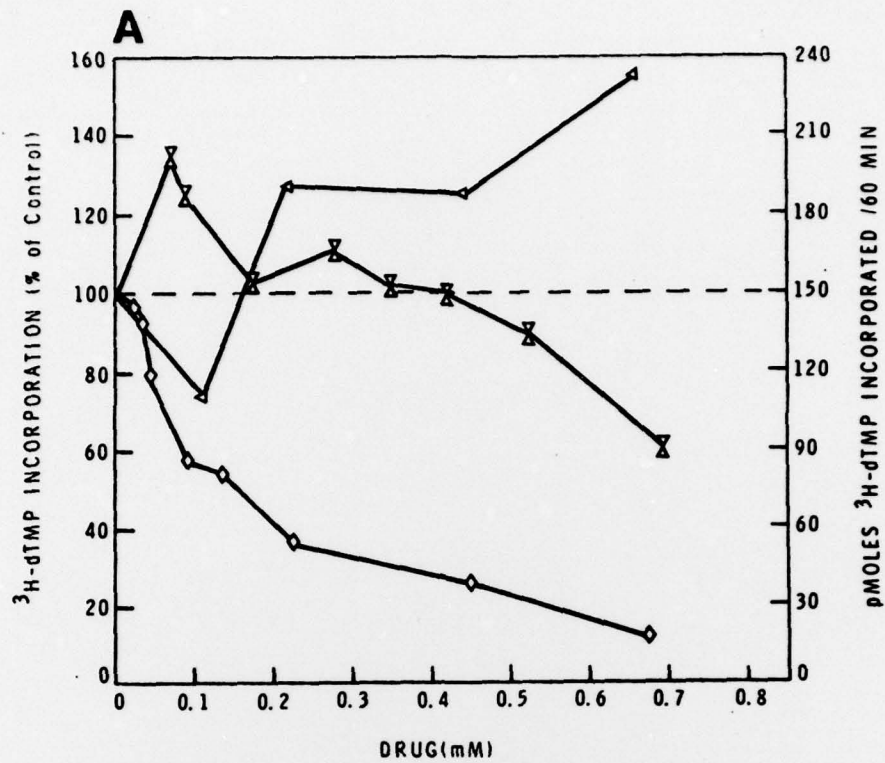
Inhibition of *E. coli* DNA polymerase activity by Antrycide (quinapyramine, dimethosulfate form) \diamond — \diamond , Bayer 2502 \times — \times and Primaquine \leftarrow — \leftarrow . Each assay contained 0.2 unit enzyme and 9.3 μ g heat-denatured DNA. The Control assay in this experiment incorporated 150 pmoles of ^3H -dTTP in 60 min at 37°.

Figure 2B.

Inhibition of *E. coli* DNA polymerase activity by Berenil (diminazene) \circ — \circ , Ethidium bromide \square — \square and Isometamidium ∇ — ∇ . Assay components and conditions were as in Fig. 2A. The Control assay in this experiment incorporated 252 pmoles ^3H -dTTP in 60 min at 37°.

Figure 2C.

Inhibition of *E. coli* DNA polymerase activity by diamidine class trypanocidal agents: Hydroxystilbamidine Δ — Δ , Propamidine \triangleright — \triangleright and Stilbamidine \diamond — \diamond . Assay components and conditions were as in Fig. 2A. The Control assay in this experiment incorporated 350 pmoles ^3H -dTTP in 60 min at 37°.



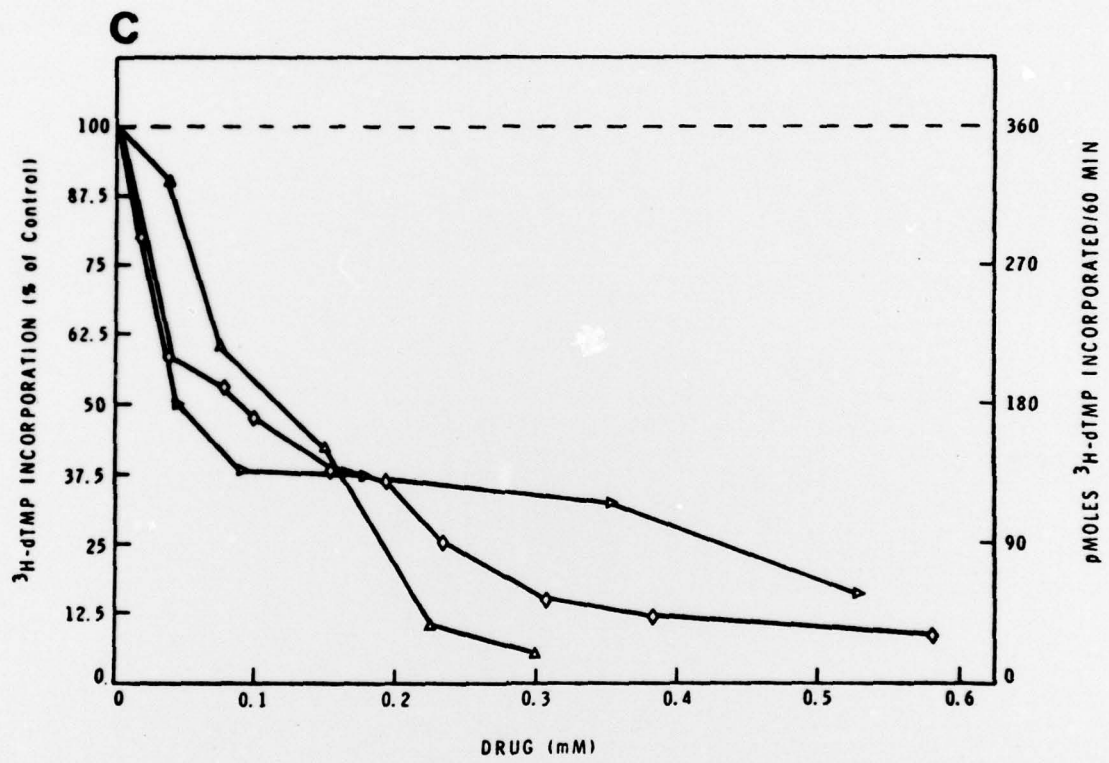


Figure 3.

Dependence of the *E. coli* RNA polymerase activity on heat-denatured CT-DNA and enzyme. Increasing amounts of CT-DNA were added to 3 units of *E. coli* K12 RNA polymerase (o—o) and increasing amounts of enzyme were added to 9.1 μ g CT-DNA (Δ — Δ). The assay conditions and components are described in "Methods".

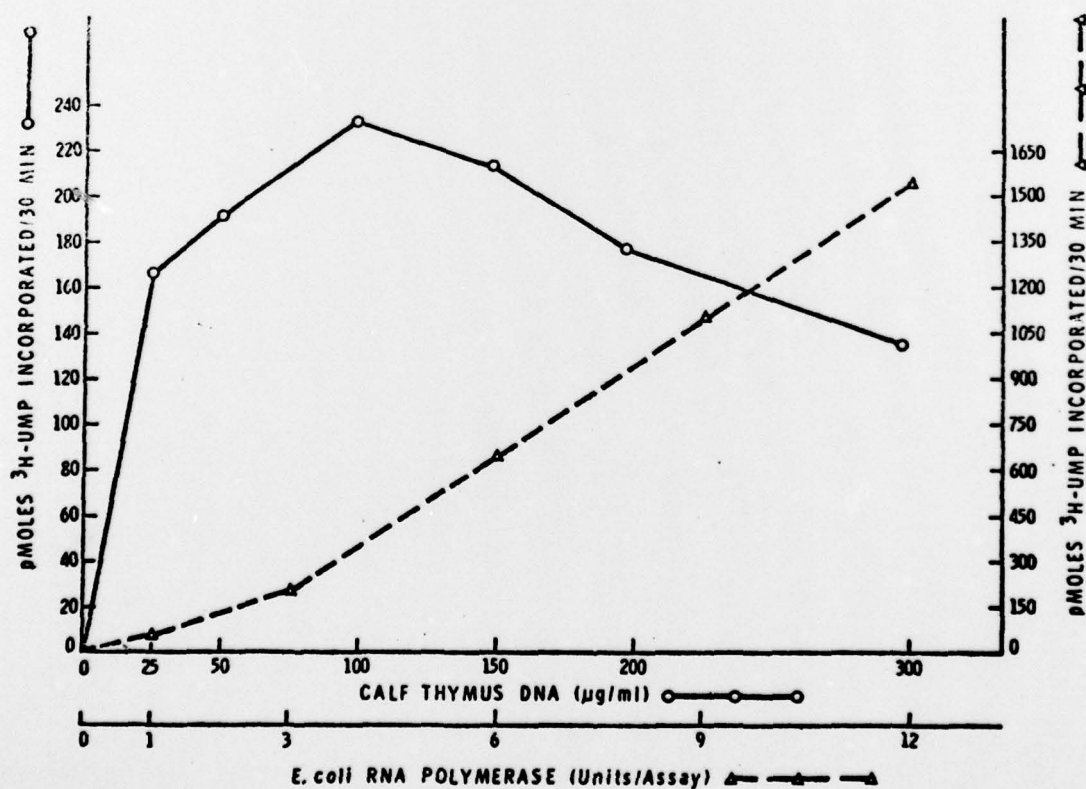


Figure 4 (A,B).

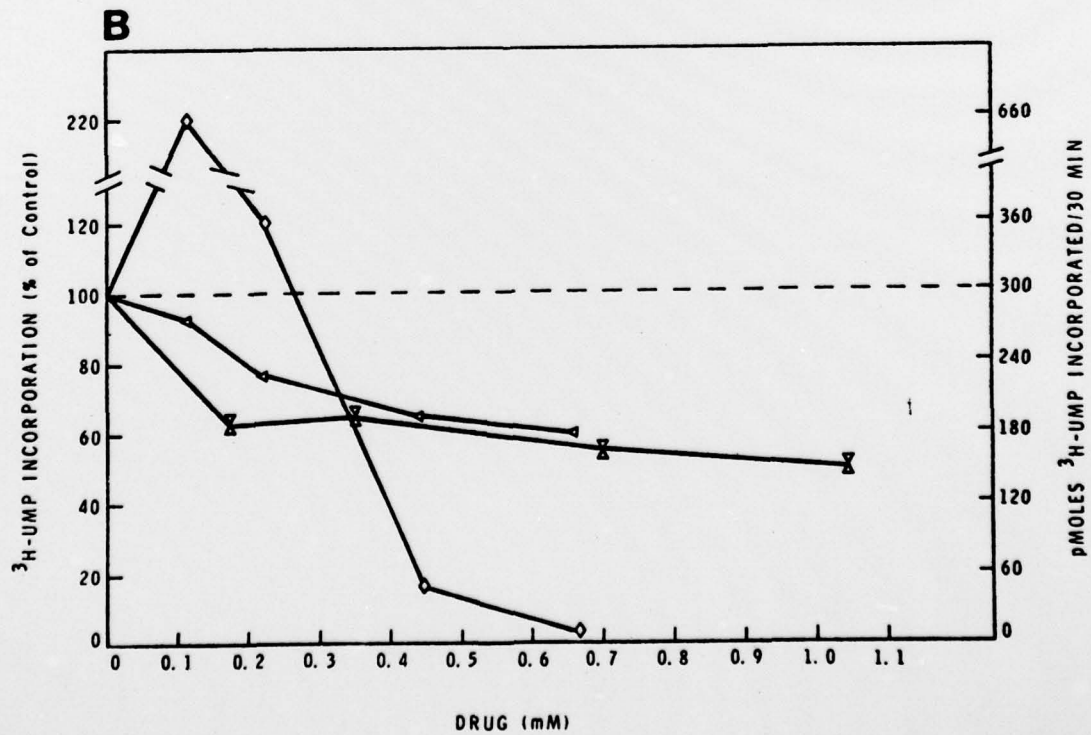
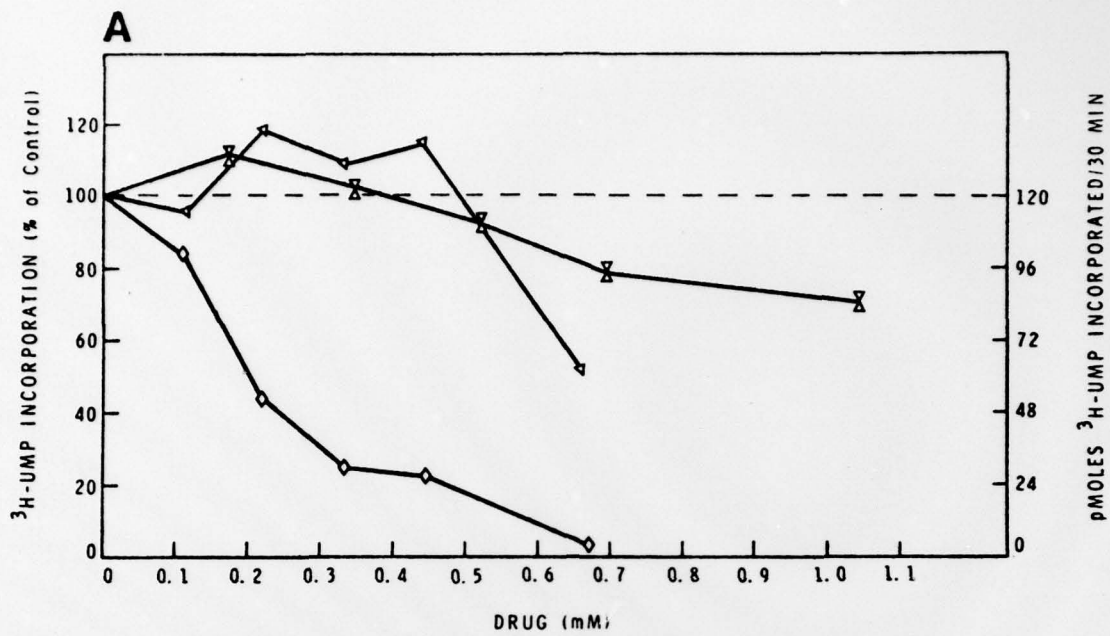
Inhibition of E. coli RNA polymerase activity by Antrycide \diamond — \diamond , Bayer 2502 \boxtimes — \boxtimes , or Primaquine \triangleleft — \triangleleft . Experiments were performed using both heat-denatured CT-DNA [4A] and native CT-DNA [4B] at 9 μ g per assay.

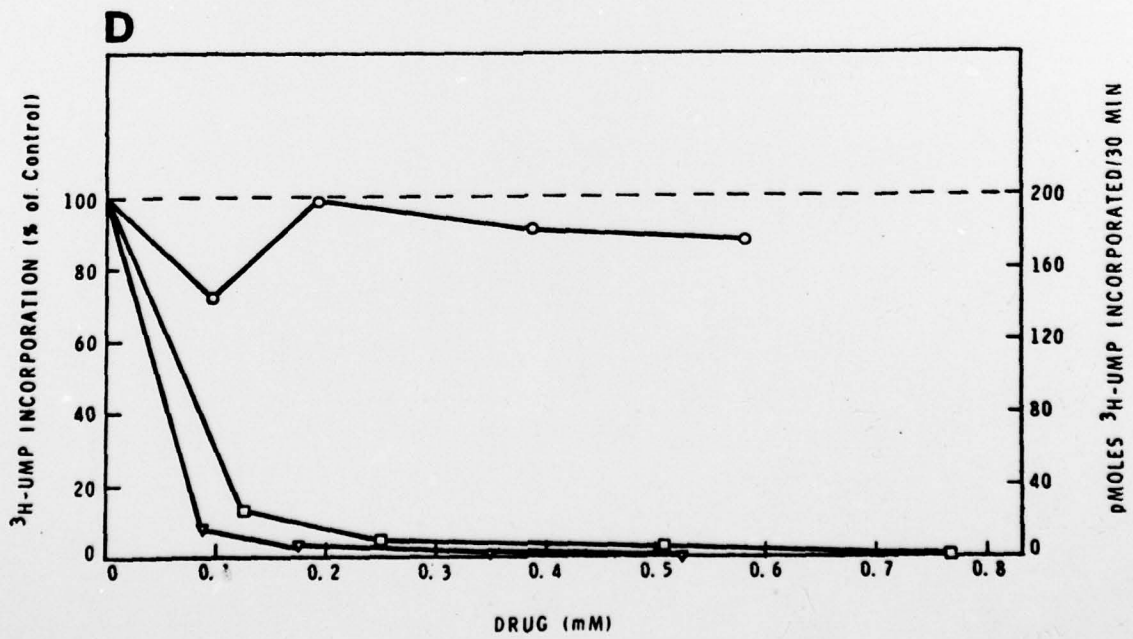
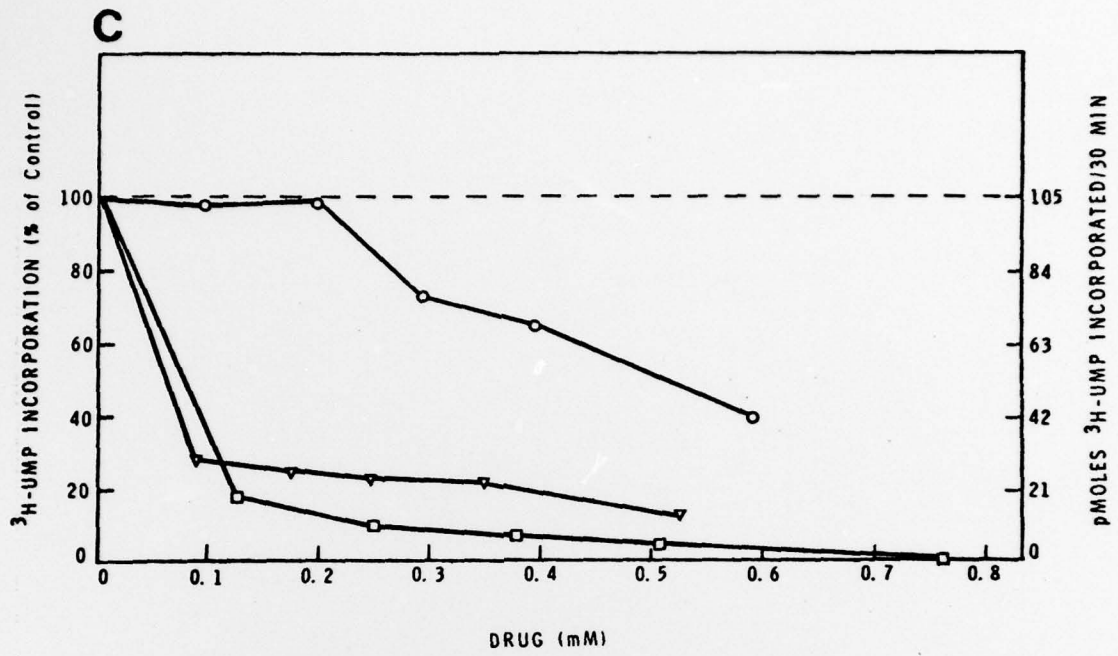
Figure 4 (C,D).

Inhibition of E. coli RNA polymerase activity by Berenil o — o , Ethidium bromide \square — \square or Isometamidium ∇ — ∇ . 4C, heat-denatured CT-DNA; 4D, native CT-DNA.

Figure 4 (E,F).

Inhibition of E. coli RNA polymerase activity by Propamidine \triangleright — \triangleright , Hydroxystilbamidien \triangle — \triangle , or Stilbamidine \diamond — \diamond . 4E, heat-denatured CT-DNA; 4F, native CT-DNA. Assay conditions and components were as in Figure 3. The control assays for these experiments incorporated approximately 200 pmoles (3300cpm) ^3H -dTTP per 60 min at 37°.





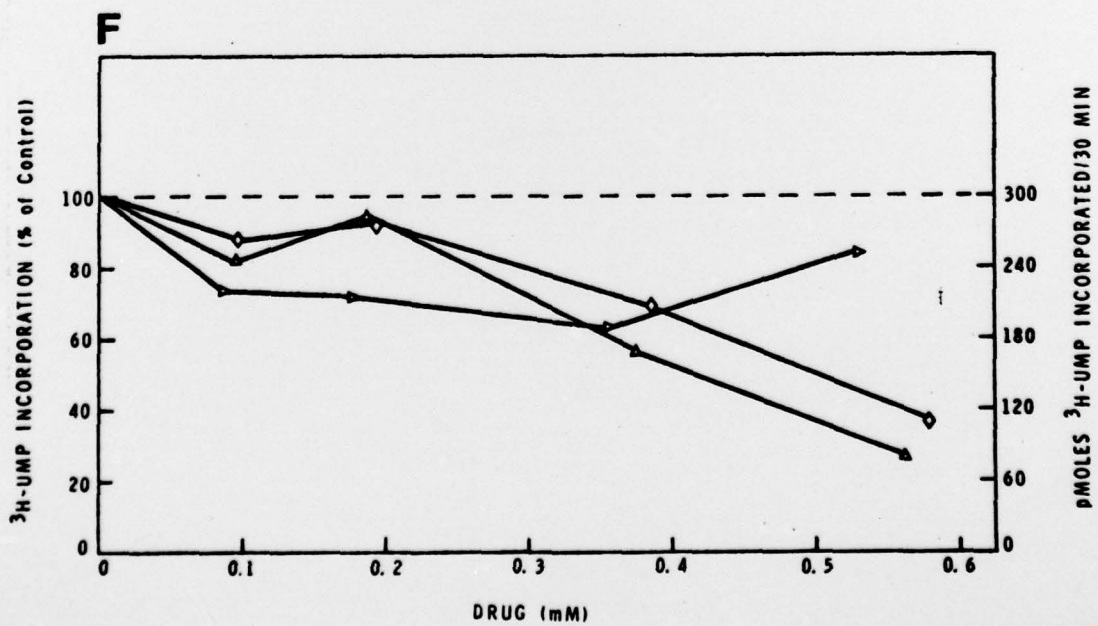
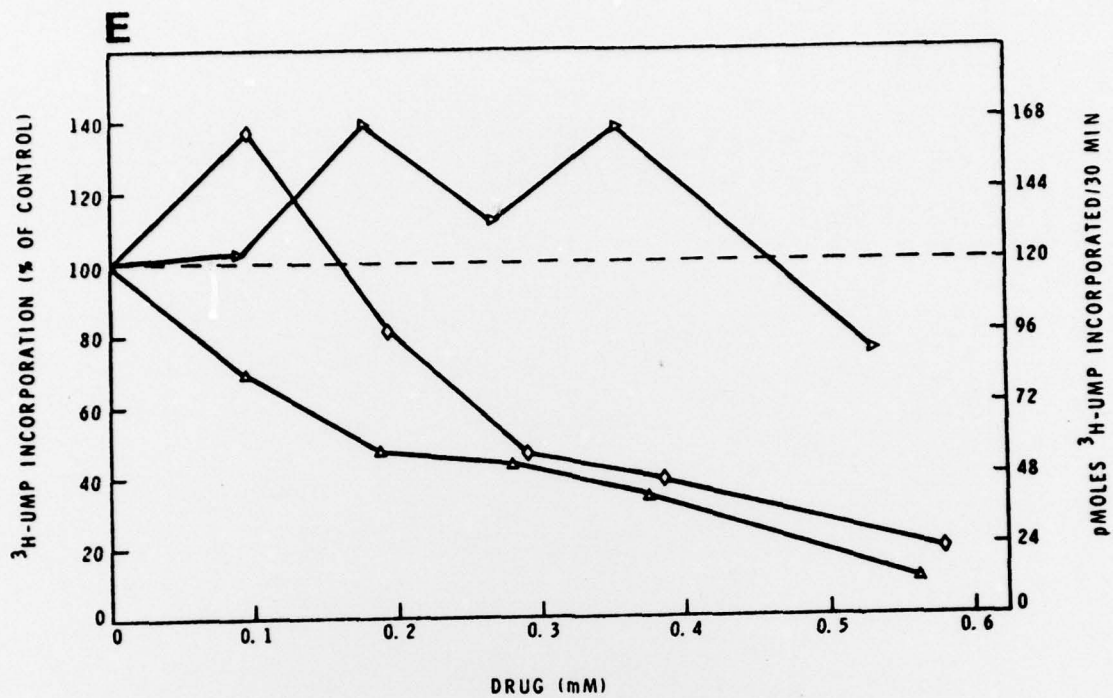


Figure 5.

Effect of increasing amounts of enzyme and heat-denatured CT-DNA on drug inhibited *E. coli* DNA polymerase activity. 0.1 and 0.3 unit of enzyme was tested in the presence of a constant amount of drug and 22.6 μg DNA/ml (A) and 90.5 μg DNA/ml (B). Antrycide [\diamond — \diamond] 50 $\mu\text{g}/\text{ml}$; Berenil [\circ — \circ] 100 $\mu\text{g}/\text{ml}$; Ethidium [\square — \square] 10 $\mu\text{g}/\text{ml}$; and Isometamidium [∇ — ∇] 25 $\mu\text{g}/\text{ml}$. Controls [\times — \times] did not contain drug.

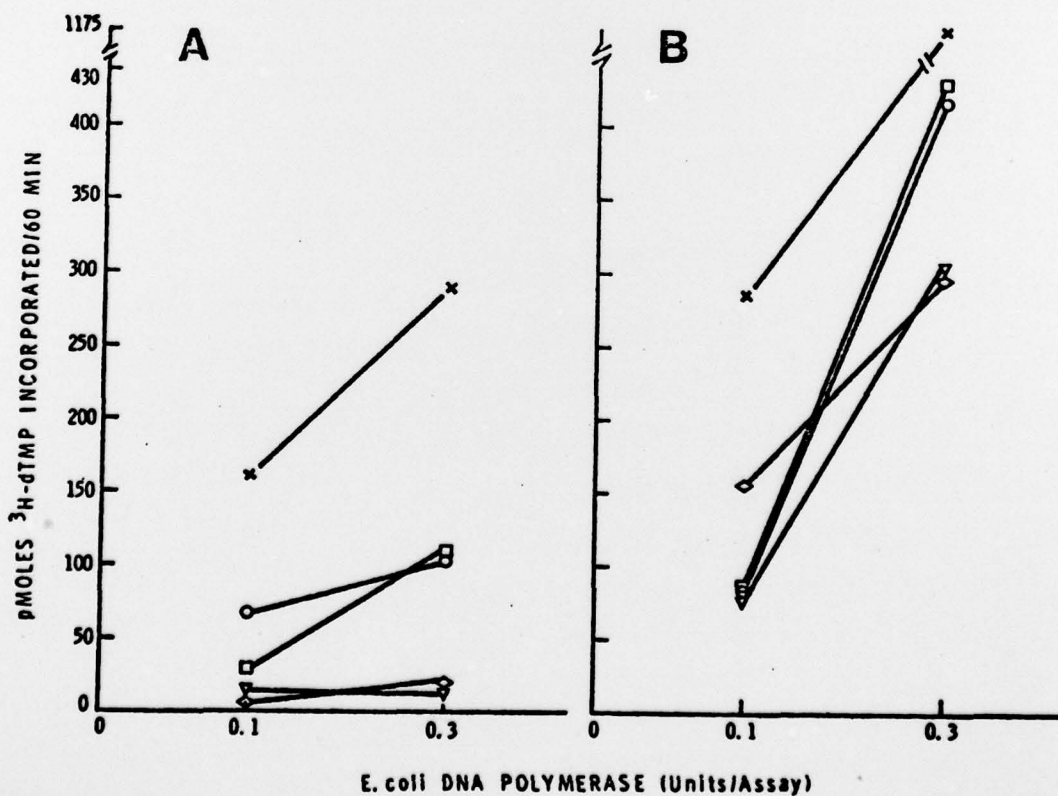


Figure 6.

Time dependence of *E. coli* RNA polymerase activity in the presence of trypanocidal drugs. The assay conditions were as described in the "Methods" and contained 9.1 μg CT-DNA and 3 units of enzyme. Drugs were tested in the following amounts: Berenil [o—o] 40 μg ; Isometamidium [v—v] 5 μg ; Antrycide [\diamond — \diamond] 20 μg and Hydrozystalbamidine [Δ — Δ] 30 μg . The dashed line (---) represents the 100% incorporation level at each time point in the absence of any drug.

