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*REACTIONS OF QUININE, CHLOROQUINE, AND QUINACRINE WITH DNA AND THEIR EFFECTS ON THE DNA AND RNA POLYMERASE REACTIONS**

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The synthetic antimalarial compounds, chloroquine (Resochin[1]) and quinacrine (Atebrin[2]), as well as the tetra-amine, spermine, which is structurally related to the aliphatic side chain of the two drugs,[3] form complexes with DNA[3–5] and interact with the double helix prominently by ionic attraction. We are reporting that quinine also interacts with DNA but binds to the polymer apparently through the formation of urea-sensitive hydrogen bonds.

A variety of effects can be attributed to the formation of complexes of DNA with these substances, for example: stabilization of the double helix by spermine,[5] chloroquine,[3] and quinacrine;[6] inhibition of bacterial type transformation by chloroquine;[7] antimutagenic effects of spermine[8] and quinacrine;[9] inhibition of nucleic acid biosynthesis in two plasmodia by quinine, chloroquine, and quinacrine[10] as well as in bacteria by chloroquine[11] and quinacrine;[12] and finally, inhibition by chloroquine of DNA-primed polymerase reactions *in vitro*.[13]

We distinguish two types of interactions between DNA and these complexing agents. One involves the chromophoric quinoline or acridine moieties of these

molecules and is manifested by changes in their absorption spectra.[4, 14, 15] Such changes in the chloroquine spectrum depend critically on the presence of the 2-amino group of guanine in DNA. No base specificity has been found to determine the interaction between DNA and quinacrine. In the second type of interaction the substituted diaminobutane side chain of the synthetic drugs stabilizes the double helix as indicated by the protection of native DNA against thermal-strand separation.[3, 6, 15] This stabilization is also observed for a copolymer of dA and dT,[3] although we find that dAdT does not change the absorption spectrum of chloroquine. Additionally, spermine, an analogue of the side chain,[3] does not carry a chromophoric ring system, yet it stabilizes DNA[5] as effectively as chloroquine and quinacrine.

We are also reporting that quinine, quinacrine, and spermine, like chloroquine,[13] inhibit the DNA and RNA polymerase reactions *in vitro* and are relating these observations to certain structural properties of the complexes of priming DNA and these substances.

Materials and Methods.—DNA polymerase was prepared from *E. coli* K12 by the method of Billen;[16] the 35–60 per cent ammonium sulfate precipitate of the DEAE-cellulose eluate was used. The enzyme (40 mg protein/ml) was stored at −20°C. RNA polymerase was prepared from *E. coli* K12 by the method of Chamberlin and Berg[17] except that the cells were disrupted in a French pressure cell (Aminco), fraction 4 eluates pooled, precipitated with 1.2 vol saturated ammonium sulfate (saturated at 25°C, pH 8.0), dissolved in buffer A with 50 per cent glycerol, and stored at −20°C (1 mg protein/ml).

The polymerase assays measured the incorporations of C^{14}-labeled adenosine triphosphate or thymidine triphosphate into polynucleotides. DNA polymerase assays were incubated for 20 min and RNA polymerase assays for 10 min; for these periods of time the reaction velocities were linear. The polymerase reactions were interrupted with cold trichloroacetic acid, and the precipitates formed were collected on membrane filters (Millipore). Filters were dissolved in a dioxane-based scintillation fluid and radioactivities counted in a Nuclear-Chicago liquid scintillation counter.

Results.—Base specificity of drug binding to DNA: The absorption spectrum of quinacrine was altered to similar extents by DNA, poly dGdC, poly dAdT, and poly dIdC as shown in Figure 1. Evidently, the interaction between quinacrine and DNA does not depend on the presence of base-specific binding sites in DNA.

In contrast, Stollar and Levine[7] have inferred from stoichiometric considerations based on equilibrium dialysis studies that the binding of chloroquine to DNA involved either guanine or cytosine. Furthermore, the relative hypochromicity of chloroquine bound to apyrimidinic DNA is similar to that produced upon binding to complete DNA, while the effect of apurinic DNA upon the chloroquine spectrum is much smaller.[15] Therefore, the two sets of findings point to guanine as being specifically involved in the interaction between DNA and chloroquine. Figure 2 shows that guanine, indeed, is required to produce a significant change in the absorption spectrum of chloroquine. Small changes caused by poly dAdT or poly dIdC were of the same order as those produced by heparin[3] and, hence, are considered nonspecific. The failure of poly dIdC to effect a significant change in the chloroquine spectrum by comparison to the marked change caused by dGdC,

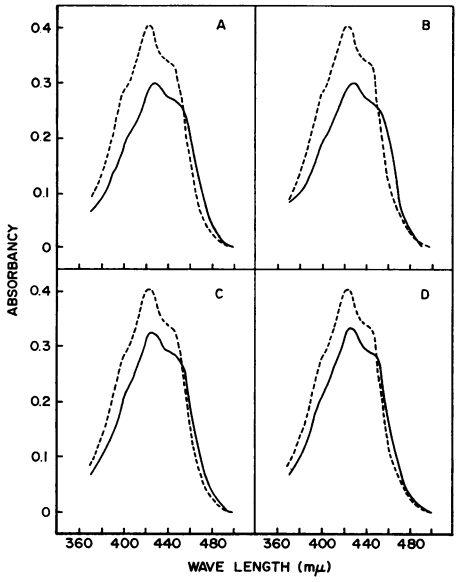

FIG. 1.— Lack of base specificity for the interaction of quinacrine and DNA. Quinacrine spectrum in the absence (- - - -) and the presence (———) of (*A*) calf thymus DNA, (*B*) poly dGdC, (*C*) poly dAdT, (*D*) poly dIdC. Solutions contained 5×10^{-5} *M* quinacrine, 0.005 *M* Tris buffer, pH 7.5, and, when included, 1.6×10^{-4} *M* polynucleotide phosphorus. Absorption spectra were determined with a Cary 14 recording spectrophotometer.

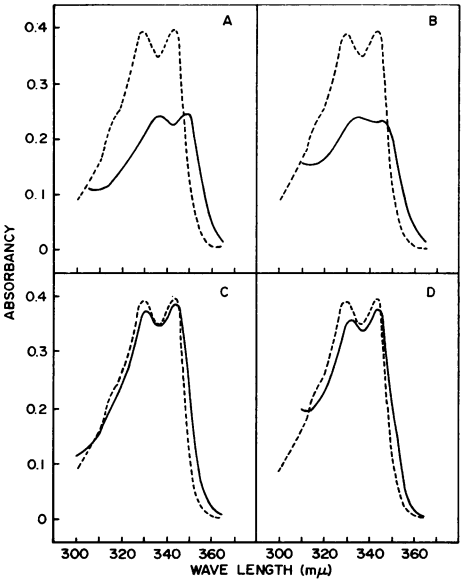

FIG. 2.—Guanine requirement for interaction of chloroquine and DNA. Absorption spectra of chloroquine in the absence (- - - -) and presence (———) of the same polynucleotides, respectively, as in Fig. 1. Solutions contained 2×10^{-5} *M* chloroquine, 0.005 *M* Tris buffer, pH 7.5, and when included, 4×10^{-4} *M* polynucleotide phosphorus.

narrows the requirement for a spectrophotometrically manifest interaction of chloroquine with DNA and DNA-like polymers to an absolute need for the 2-amino group of guanine. Actinomycin D[18] and chromomycin A_3[19] have a similar guanine requirement.

Stability of DNA-drug complexes in urea: The guanine-specific complexes of DNA with actinomycin D and chromomycin A_3 are unstable in urea.[19, 20] Apparently, these antibiotics react with DNA through the formation of hydrogen bonds. In contrast, the spectrophotometrically determined interaction of DNA with chloroquine as well as with quinacrine is stable in 6 *M* urea, as shown in Figure 3, but is reversed by ionic cosolutes.[14, 21]

Difference spectra of quinine (DNA-quinine complex minus quinine), Figure 4, show a large DNA-induced change which was abolished in 6 *M* urea. We infer that, unlike the DNA-chloroquine and DNA-quinacrine complexes, the complex of DNA with quinine is formed by hydrogen bonding. Because of the limited supplies of synthetic deoxyribopolynucleotides, studies on base-specificity of quinine binding to DNA could not be carried out.

Inhibition of DNA-primed polymerase reactions by antimalarial drugs: The influence of quinine, quinacrine, and chloroquine on the DNA-primed DNA and RNA polymerase reactions was studied with results shown in Figure 5. The molar concentrations necessary to produce 50 per cent inhibition of the DNA polymerase

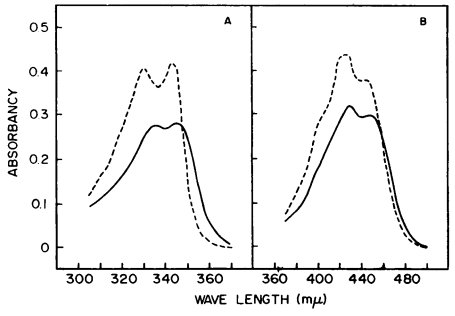

FIG. 3.—Stability of the DNA-chloroquine and DNA-quinacrine complexes in urea. The absorption spectra of chloroquine (*A*) and quinacrine (*B*) in the presence (———) and absence (- - - -) of calf thymus DNA. Solutions in *A* contained 2×10^{-5} *M* chloroquine, 0.005 *M* Tris, pH 7.5, 6 *M* urea, and, when indicated, 4×10^{-4} *M* DNA phosphorus. Solutions in *B* contained 5×10^{-5} *M* quinacrine, 0.005 *M* Tris, pH 7.5, 6 *M* urea, and, when indicated, 5×10^{-4} *M* DNA phosphorus.

reaction increased in the sequence quinacrine:chloroquine:quinine. With all three compounds, the RNA polymerase reaction was less sensitive to inhibition. Our results with chloroquine agree with those of Cohen and Yielding.[13]

It should be noted that we have standardized the assay conditions for the two polymerase reactions so that they are identical with respect to hydrogen ion, DNA, substrate, and inorganic ion concentrations. For this reason, the extents of inhibition of the two reactions can be directly compared. Such experimental precautions

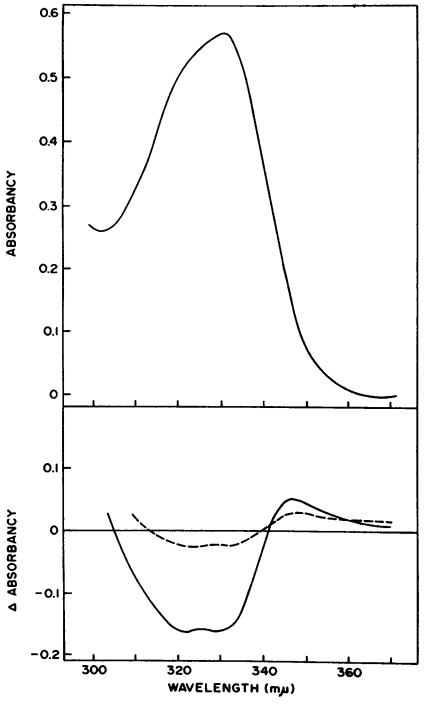

FIG. 4.—Formation of a DNA-quinine complex and its dissolution by urea. The upper portion of the figure shows the absorption spectrum of quinine. The lower portion shows the difference spectra of quinine produced by DNA in the absence (———) and presence (- - - -) of urea. Concentrations were: 10^{-4} *M* quinine, 0.001 *M* DNA phosphorus, 6 *M* urea, 0.005 *M* Tris, pH 7.5.

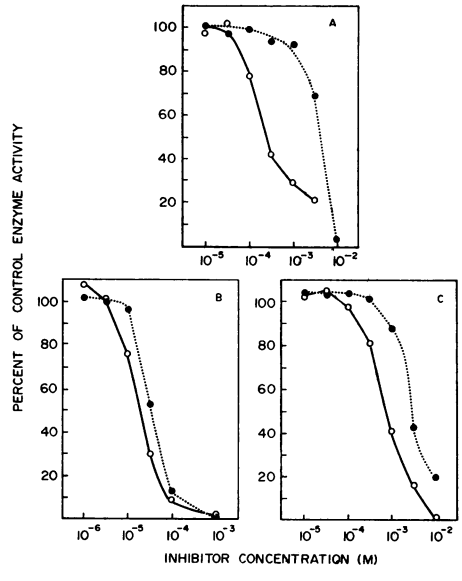

FIG. 5.—Inhibition of RNA polymerase (●- - -●) and DNA polymerase (○———○) by chloroquine (*A*), quinacrine (*B*), and quinine (*C*). Reaction conditions were: pH 8.0 in 0.005 *M* Tris buffer, 0.005 *M* $MgCl_2$, 10^{-4} *M* deoxy- or ribonucleotides, and 60 μg DNA/ml. DNA polymerase assays were performed in 0.3 ml with 400 μg of enzyme. RNA polymerase assays were performed in 0.25 ml containing 50 μg enzyme, 10 μg of pyruvate kinase (Calbiochem), and 0.004 *M* phosphoenolpyruvate.

have not always been observed by others who have compared the effect of inhibitors of both enzymatic processes.

Effects of spermine on DNA-primed polymerase reactions: A structural relationship between spermine and the nonprimary diamine side chain of quinacrine and chloroquine has been noted.[3] Figure 6 depicts the responses of the DNA and RNA polymerase reactions to spermine. The potency of spermine as an inhibitor of the DNA polymerase reaction was intermediate between those of quinacrine and chloroquine. In contrast, the RNA polymerase reaction was progressively stimulated by graded concentrations of spermine to 10^{-3} *M;* above this concentration, the reaction was inhibited. A similar observation has been reported by Krakow.[22]

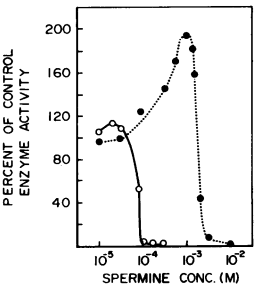

FIG. 6.—Effect of spermine on DNA polymerase (O——O) and RNA polymerase (●- - -●) reactions. Conditions same as noted in Fig. 5.

Discussion.—The DNA-complexing substances that we have studied can be regarded as molecular probes for those structural properties of DNA that are of importance to DNA replication and RNA transcription, i.e., to key processes of biological information transfer. Replication of DNA requires the separation of the two companion strands of the parental double-helical molecule.[23, 24] Substances which interfere with strand separation should, therefore, inhibit DNA replication *in vivo.* We propose that inhibitions of DNA biosynthesis by quinacrine[10, 12] and chloroquine[10, 11] are the result of stabilization of double-stranded DNA.

Evidently, this reasoning can also be applied to the DNA polymerase reaction *in vitro.* DNA, when covalently cross-linked by mitomycin C, is essentially inactive as a primer;[25] quinacrine, chloroquine, and spermine, forming ionic complexes with DNA and stabilizing the double helix,[3, 5, 6] are effective inhibitors of the DNA polymerase reaction (Figs. 5 and 6), and actinomycin D which stabilizes the double helix only at approximately 100 times its cytostatic concentration[26] inhibits DNA polymerase only at that same high concentration.[27] Quinine which interacts with DNA by hydrogen bonding also stabilizes the double helix: 10^{-3} *M* quinine elevated the median denaturation temperature of calf thymus DNA by 8.0° [21] and inhibited the DNA polymerase reaction by 60 per cent (Fig. 5).

The structural basis of the transcription of RNA and of its inhibition by substances that complex with DNA is hypothetical. Mitomycin C cross-linked DNA permits RNA biosynthesis to proceed *in vivo*[28] and is an effective primer for RNA polymerase *in vitro;*[29] evidently, complete strand separation is not required for transcription. A hypothesis, however, which attempts to explain the transcription of RNA from a native DNA template assumes the occurrence of transient and regional strand separations in order that the purine and pyrimidine recognition sites that are buried inside the double helix may be presented consecutively to the ribonucleotide assembling process; a sequential "swinging out" of the bases in double-helical DNA has been suggested as one such mechanism of information transfer.[30]

Inhibitions of RNA transcription *in vivo* and of the RNA polymerase reaction *in vitro* might, therefore, be produced not only by substances that are thought to occlude the minor groove of DNA and sterically hinder the polymerization process such as actinomycin D[31] but also by intercalation of planar cyclic compounds be-

tween base pairs in DNA and restraining of the bases in their intrahelical positions. Evidence of an intercalation of the acridine ring of quinacrine has been presented by Lerman,[32] and biophysical studies from our laboratory suggest that the 7-chloroquinoline ring of chloroquine is also intercalated between DNA bases.[33] Intercalated heterocyclic rings may contribute to the stability of heterocyclic bases in their stacked configuration by London-van der Waals forces arising from fluctuating induced dipoles among similar ring systems. While such forces *per se* may not be sufficient to cause inhibitions of RNA transcription, the quinoline and acridine rings of chloroquine and quinacrine are additionally bound *in situ* by stronger interactions between the aliphatic diamino side chain of both drugs with phosphoric acid groups of DNA. The guanine requirement for the interaction of chloroquine and DNA may reflect a specific attraction between the 7-chloro substituent of the intercalated quinoline ring and the 2-amino group of guanine.[34] DNA when complexed with quinacrine or chloroquine may be incapable of replication because the double helix is stabilized and may be deficient as a primer of RNA transcription because its component bases are constrained.

Summary.—The formation of the complex of DNA and chloroquine requires the 2-amino group of guanine in DNA; the formation of a similar complex with quinacrine does not depend upon the presence of base-specific binding sites. Quinine interacts with DNA by hydrogen bonding. The three drugs and spermine inhibit the DNA-primed DNA polymerase reaction. The RNA polymerase reaction is affected to a lesser extent by the antimalarial compounds and is either stimulated or inhibited by spermine depending on the concentration of this polyamine.

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*INITIATION OF E. COLI PROTEINS**

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Recent experiments and theoretical arguments suggest that formylmethionyl sRNA is employed as an initiator of protein synthesis.[1-6] For example, R17 and f2 bacteriophage coat protein was found to have the amino terminal sequence

formyl met ala ser $AspNH_2$ phe thr...

when synthesized in a cell-free extract programed with viral RNA.[1, 2] The known amino-terminal sequence of R17 and f2 coat protein from intact phage (ala ser $AspNH_2$ phe thr...) must be generated by specific cleavage of the formylmethionyl residue from the nascent polypeptide chain. These studies also indicated that other phage proteins synthesized in the *in vitro* system were initiated with formylmethionine.[1] Thus we have an example of an amino acid incorporating system directed by a polycistronic messenger in which the different proteins are initiated by the same mechanism to the extent that the same initiator, formylmethionine, is used. These observations provided a basis for believing that formylmethionine could be the unique initiator of protein synthesis. A necessary consequence of having a unique initiator, as in the bacteriophage system, is the existence of an enzymatic apparatus for the removal of the formylmethionyl residue exposing other known amino-terminal amino acids. The finding by Clark and Marcker[3] and by Nakamoto and Kolakofsky[4] that proteins synthesized under the direction of synthetic messengers such as poly UAG and poly UG are initiated with formylmethionine also supports the unique role of that sRNA species as the initiator of protein synthesis.

One would like to extend the above results to see whether *E. coli* proteins are initiated in a similar manner. To answer this question we have studied *in vitro* protein synthesis programed with endogenous *E. coli* messenger RNA. A number of models can be envisioned which employ formylmethionyl sRNA as the initiator of *E. coli* protein synthesis. For example, let us discuss two models. As stated above, the *in vitro* amino terminal sequence of R17 coat protein, which may be