Studies on a Deoxyribonucleic Acid–Quinine Complex

RICHARD D. ESTENSEN, ANNE K. KREY, AND FRED E. HAHN

Department of Molecular Biology, Walter Reed Army Institute of Research, Washington, D. C. 20012

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SUMMARY

Double-helical calf thymus DNA and poly dG:dC produced "marked" changes in the absorption spectrum of quinine. Single-stranded DNA, poly dA:dT, and poly dI:dC were without effect. Among the four synthetic ribonucleotide homopolymers, only poly G at high concentrations diminished the light absorption of quinine. Spectral changes caused by the complexing of quinine with DNA were reversed by thermal strand spearation, by Na^{+}, and by Mg^{++}. Results of spectrophotometric titrations of quinine with DNA were used to derive a nonlinear absorption isotherm which suggested that the drug binds to more than one class of sites in DNA. Hydrodynamic measurements indicated a decrease in the sedimentation coefficient and an increase in the viscosity of DNA in the presence of quinine. The inhibition of the DNA polymerase (from *Escherichia coli*) reaction was a function of the stabilization of the double helix at graded concentrations of quinine. We propose a partial intercalation model of the DNA-quinine complex as a structural hypothesis which is compatible with the results of our hydrodynamic studies, and cite evidence which suggests that the formation of a complex with DNA provides a molecular basis for the antiplasmodial activity of the drug.

INTRODUCTION

Quinine is perhaps the oldest chemotherapeutic drug still in use. It was adopted by Western medicine some 300 years ago as an antimalarial agent. The drug molecule is a complicated quinolinemethanol (Fig. 1) and has guided the synthesis of other quinolinemethanols (1), 8-aminoquinolines (2), and 4-aminoquinolines (3) with antimalarial properties. Quinine is selectively toxic for malarial parasites (4), and its antiplasmodial effect can be attributed to inhibition of the incorporation of building blocks into DNA, i.e., of DNA biosynthesis, in plasmodia (5, 6). Quinine has been found in our laboratory to form a molecular complex with DNA and to inhibit the DNA-dependent DNA polymerase reaction *in vitro* (7).

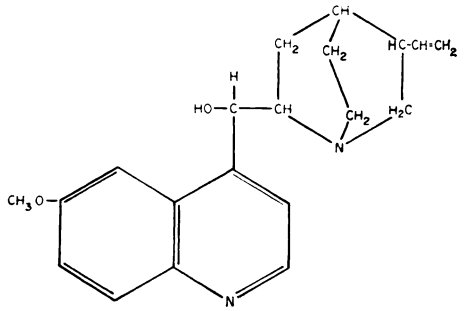

FIG. 1. *Structure of quinine*

The drug appears to belong, therefore, to that class of compounds which exert a variety of biological effects by engaging in complex formation with DNA. The present study was undertaken in order to define the conditions of binding of quinine to DNA in the hope that the results might lead to an understanding of the action of the drug.

MATERIALS AND METHODS

Quinine was used as the dihydrochloride, furnished by Vitarine Company, Inc. DNA

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of calf thymus origin, grade A, was acquired from Calbiochem. Polyribonucleotides were obtained from Miles and Company, and the polydeoxyribonucleotides were kindly furnished by Dr. Frederick Bollum. All other chemicals were standard reagent grade.

All solutions were made in 5×10^{-3} M Tris-HCl buffer at pH 7.5. Spectrophotometric measurements were carried out in either a Cary model 14 or a Gilford model 2000 absorbance recorder with a variable-temperature, multiple-sample compartment. Temperatures were controlled with a Haake constant temperature circulator and were recorded with a Gilford model 207 linear thermosensor accurate to within 0.1° of intracuvette temperature. The method described by Mandel and Marmur (8) was used to record the median strand separation temperature (T_m), except that optical density changes with increasing temperature were represented as percentages of the total increase in the optical density recorded at room temperature.

Titrations were performed by dissolving DNA (10^{-3} M) in a 10^{-5} M quinine solution and successively diluting the resultant solution with 10^{-5} M quinine. Measurements were made in a 10-cm light path cell. The optical density data derived from this titration were then plotted according to the methods employed by Müller and Crothers (9), using the expression

$$\frac{r}{m} = K_{app}(B_{app} - r)$$

where r is the ratio of bound quinine to total DNA base pairs, m is the concentration of free quinine, K_{app} is the apparent binding constant, and B_{app} is the apparent number of binding sites.

Sedimentation analyses were carried out in a Beckman model E ultracentrifuge, using ultraviolet optics, and viscosities were measured at 30° in a Beckman low-shear viscosimeter at shear stresses of less than 0.005 dyne/cm².

RESULTS

Influence of base composition of polydeoxyribonucleotides and polyribonucleotides on quinine. Native calf thymus DNA

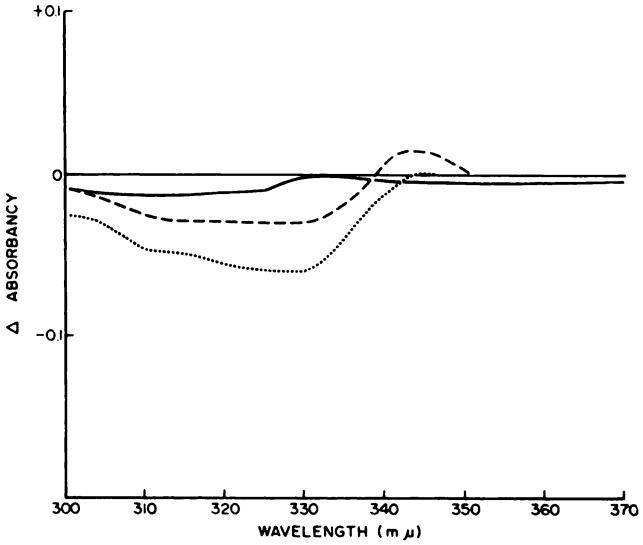

FIG. 2. *Difference spectra of polydeoxyribose compounds or calf thymus DNA with quinine (polymer-quinine mixture minus quinine)*

The quinine concentration was 2×10^{-4} M in all cases. ——, Poly dA:dT, 6×10^{-4} M, or poly dI:dC, 6×10^{-4} M; – – –, poly dG:dC, 6×10^{-4} M; · · · ·, calf thymus DNA, 6×10^{-4} M. Molar concentrations refer to phosphorus content of the polymers.

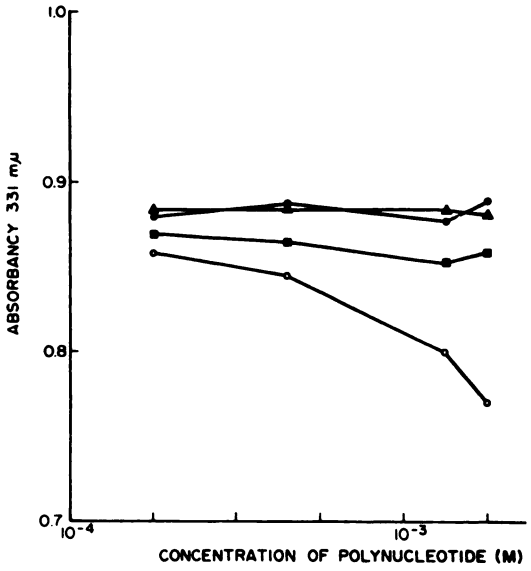

FIG. 3. *Effects of polyribonucleotides on absorption intensity of quinine at 331 mμ*

Each point represents two or more determinations. Absorption spectra were recorded for each concentration; the shape of the quinine spectrum remained unchanged. All values fell within the range of four determinations of the absorbance of quinine at 331 mμ without added polymers, except those for poly G (○) and the last two values for poly A (■); poly U (▲) and poly C (●) produced no change. Molar concentrations refer to phosphorus content of the polymers.

and poly dG:dC decreased the intensity of the absorption band of quinine at 331 mμ, as shown in the difference spectra (Fig. 2). In contrast, poly dA:dT and poly dI:dC had no effects on the absorption intensity of quinine.

Polyriboguanylic acid decreased the intensity of absorption of quinine (Fig. 3). The single-stranded polyribonucleotides, poly U and poly C, did not have any effect on the intensity of absorption of quinine at 331 mμ. Poly A at high concentrations had a slight effect on the absorption of quinine. These observations indicate that the presence of guanine in a polynucleotide was necessary to decrease the absorption of the drug.

Requirement of double-strandedness of DNA for complex formation with quinine. DNA which had been rendered single-stranded by heating and rapid cooling in either the absence or presence of 0.033 M formaldehyde (10) did not produce a hypochromic effect on the absorption of quinine, while DNA which was treated with the same concentration of formaldehyde, but not heated, gave rise to the same effect on the spectrum of quinine as native DNA (Fig. 4).

The hypochromic effect of DNA (Fig. 5) on the absorption of quinine at 331 mμ was reversed by heating the DNA-quinine complex. The increases in absorbance at 260 mμ, indicating strand separation, and absorbance at 331 mμ, indicating dissociation of a DNA-quinine complex, were similar functions of temperature. Double-stranded DNA, therefore, is essential for the spectroscopic manifestation of the binding of quinine to DNA.

Effects of Na^+ and Mg^{++} on DNA-induced hypochromicity in the absorption spectrum of quinine. Urea (6 M) abolishes the effect of DNA on the intensity of the light absorption of quinine (7); this concentration of urea does not cause strand separation of double-helical DNA. Similar observations have been reported for actinomycin D (11) and chromomycin A_3 (12). Na^+ and Mg^{++} (Figs. 6 and 7) also decreased or abolished the hypochromic effect of DNA on the absorption of quinine. We infer that interaction(s) between quinine and DNA involve both the formation of urea-sensitive bonds and electrostatic attractions between ionized groups which can be reversed by ionic competition.

Spectrophotometric titration of quinine with DNA. Spectrophotometric titration of quinine with DNA yielded an isotherm for the binding of the drug to DNA (Fig. 8). The curvature of this plot indicates the existence of more than one class of binding sites for quinine on DNA. Within the range of binding which could be examined by the spectrophotometric method, we distinguish between strongly and weakly reactive classes of binding sites. Extrapolation of the curve for small values of r (r = number of molecules of quinine bound per base pair) yielded an apparent association constant, K_{app}, of

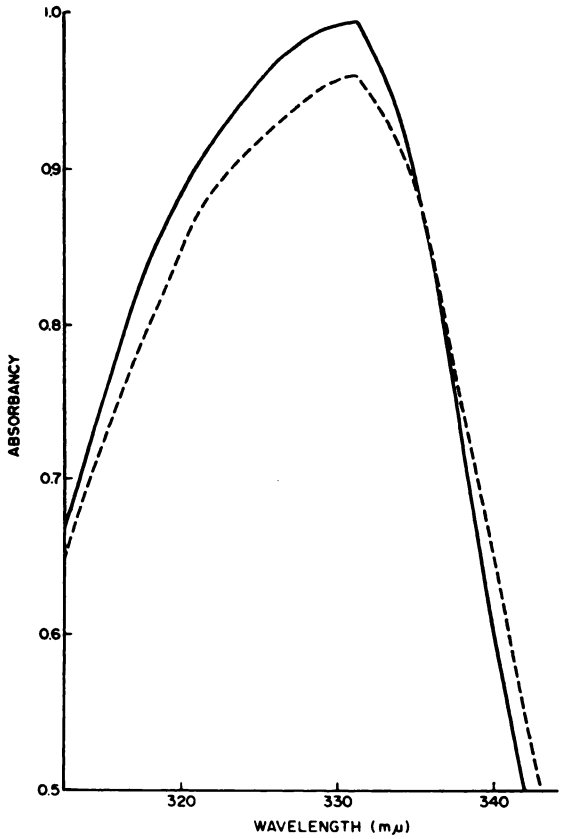

FIG. 4. *Effect of formaldehyde-treated DNA (10) and heated DNA on the absorption intensity of quinine* – – –, Quinine, 2×10^{-4} M, plus native DNA, 150 μg/ml; ——, quinine, 2×10^{-4} M, alone or in the presence of formaldehyde-treated or heated DNA, 150 μg/ml.

3.6×10^6 M^{-1}, which is large and of the same order as that of actinomycin C_3 (9), and an apparent number of binding sites, B_{app}, per base pair of 0.025, corresponding to 1 molecule of quinine per 40 base pairs. This is far less than the number of sites that would accommodate maximal intercalation, i.e., 1 drug molecule per two base pairs. Actinomycin C_3 is bound to a similarly small number of sites on calf thymus DNA (9).

Extrapolation of the isotherm for large values of r yielded, for the weaker binding process, a K_{app} value of 1.5×10^5 M^{-1} and a B_{app} value of 0.125, corresponding to 1 molecule of quinine bound per eight or nine base pairs.

Hydrodynamic measurements on the DNA-quinine complex. Lerman (13) has established two hydrodynamic criteria for intercalation of ring systems between base pairs of double-helical DNA: (a) an increase in intrinsic viscosity and (b) a decrease in the sedimentation coefficient of DNA preparations. These effects result from lengthening of the DNA molecule in proportion to the amount of cyclic compound intercalated, from a decrease in the mass per unit length of DNA when the mass of the intercalated ring system is less than that of one base pair, and from a decrease in the flexibility of DNA rods.

The intrinsic viscosity of the DNA-quinine complex was greater than that of DNA alone (Fig. 9), while the sedimentation coefficient of the complex was lower than that of DNA alone (Fig. 10).

Inhibition of the DNA-polymerase reaction as a function of the stabilization of DNA. It has previously been assumed (7)

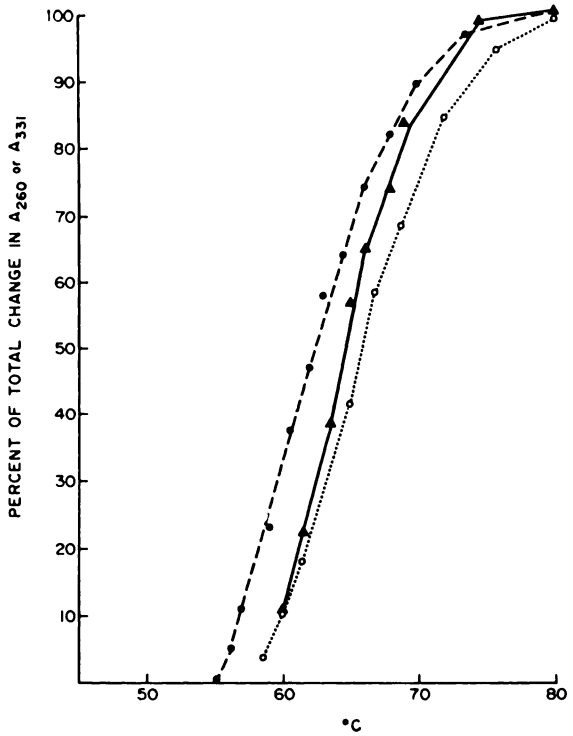

FIG. 5. *Melting curves of DNA (25 μg/ml) in the absence (●- - -●) or presence (○ · · · · ○) of 2 × 10⁻⁴ M quinine and reversal of the effect of DNA (250 μg/ml) on the absorption of quinine at 331 mμ as a function of temperature (▲——▲) and corrected for slight absorption of DNA at 331 mμ*

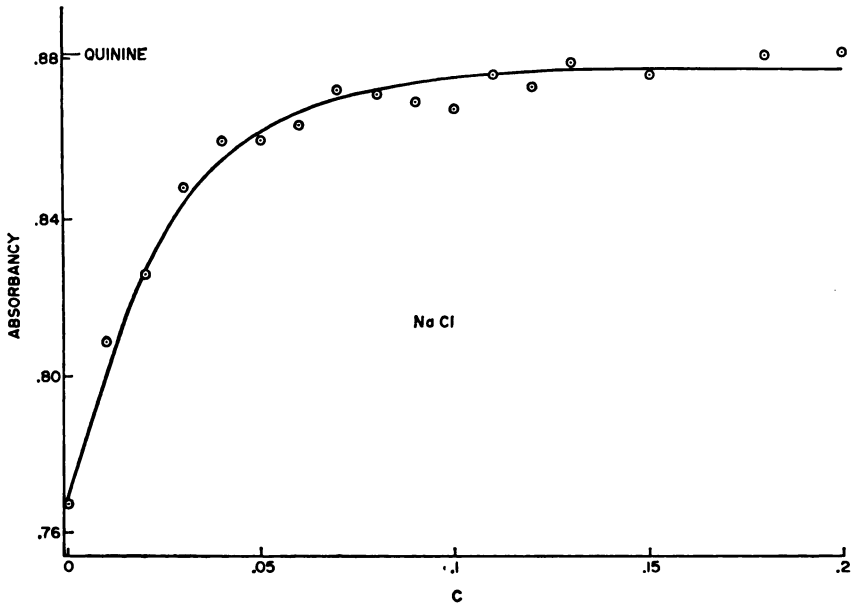

FIG. 6. *Effect of Na^+ on the absorbance at 331 mμ of a quinine-DNA mixture*

Quinine, 2 × 10⁻⁴ M; DNA, 250 μg/ml. Molar concentrations (*C*) of NaCl are indicated on the abscissa.

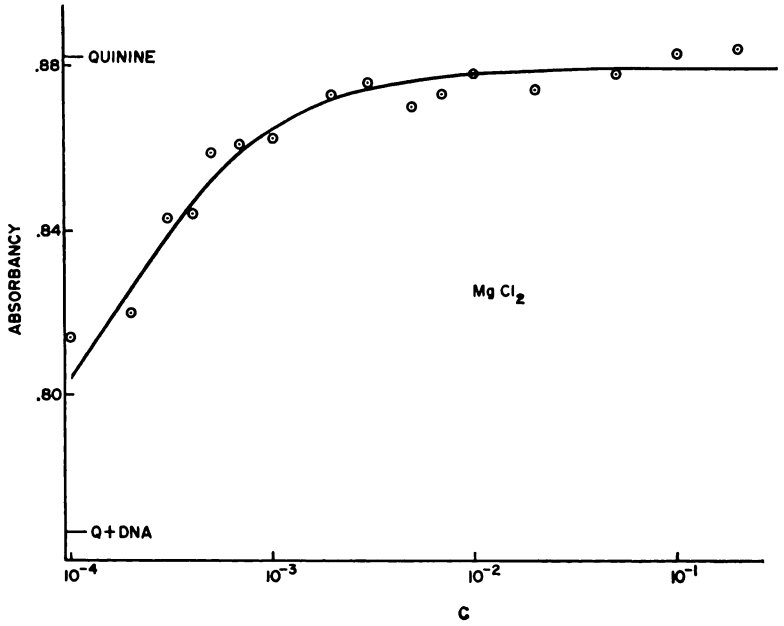

FIG. 7. *Effect of Mg^{++} on the absorbance at 331 mμ of a quinine-DNA complex*

Quinine, 2×10^{-4} M; DNA, 250 μg/ml. The ordinate represents absorbance at 331 mμ, and the abscissa, molar concentration (C) of $MgCl_2$.

that substances which interfere with strand separation of DNA should inhibit DNA replication *in vivo*, as well as the DNA polymerase reaction *in vitro*. For quinine we have tested this hypothesis by relating increases in the median strand separation temperature, T_m, of DNA to the extent of inhibition of the DNA polymerase reaction by the drug in graded concentrations between 2.25×10^{-4} and

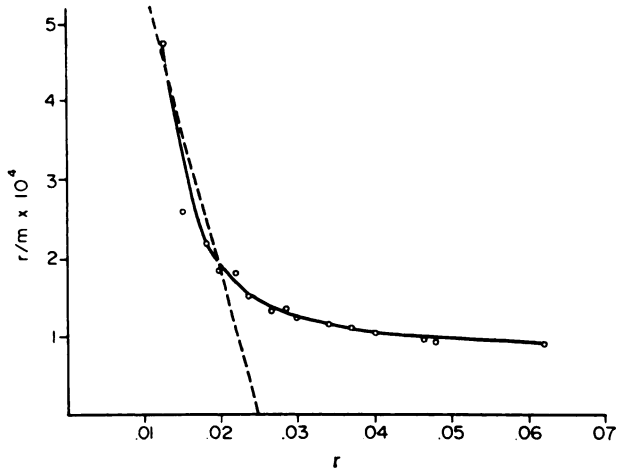

FIG. 8. *Isotherm for the binding of quinine to calf thymus DNA*

r = number of bound quinine molecules per base pair; m = concentration of free quinine. Complete binding of quinine was taken as the point determined when DNA was in 200-fold molar excess of quinine. Each point represents an average of four spectrally determined points. The dashed line represents the regression line computed from the first five points. The B_{app} was 0.025, and the K_{app} was 3.6×10^6 M^{-1} as determined from the intercepts of this regression line.

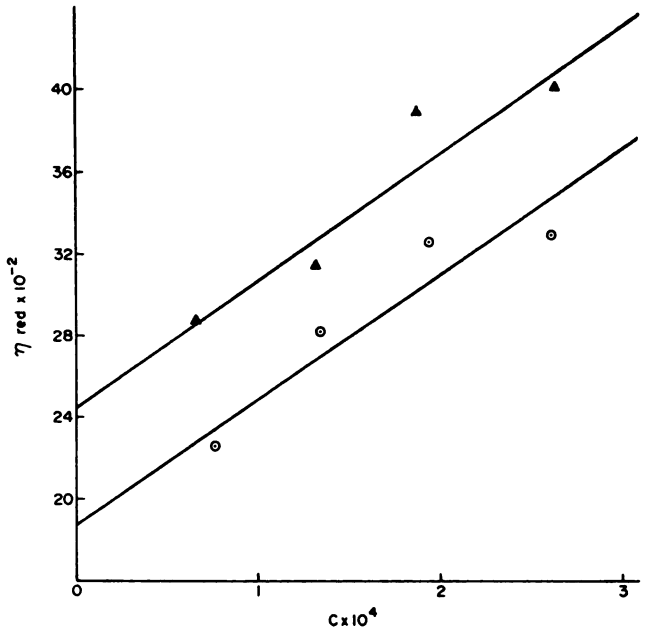

FIG. 9. *Effect of quinine on viscosity of DNA*

Numbers on the ordinate are "reduced viscosities," i.e., specific viscosities divided by DNA concentrations. △——△, DNA-quinine concentrations with a constant ratio of DNA phosphorus to quinine of 10:1; ⊙——⊙, DNA alone. The intercepts of the lines with the ordinate indicate intrinsic viscosities. Concentrations (*C*) of DNA are given in molarities of DNA phosphorus.

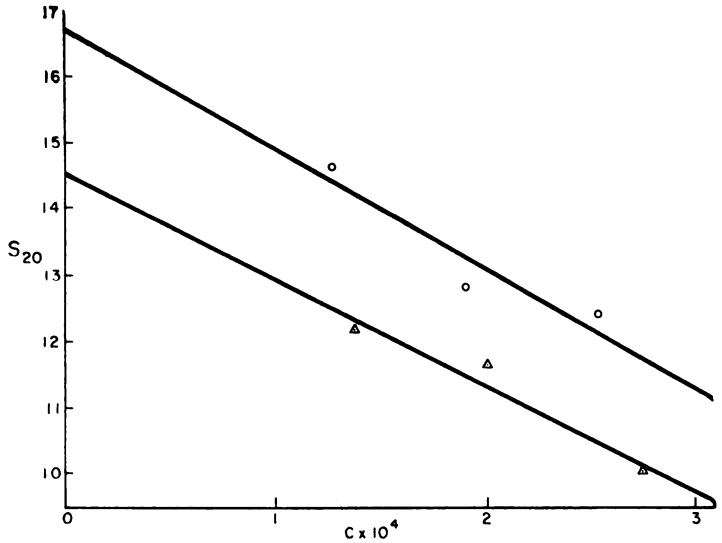

FIG. 10. *Effect of quinine on the sedimentation coefficient of DNA*

C is the molar concentration of DNA phosphorus. ○——○, DNA alone; △, DNA plus quinine in a molar ratio of 10:1.

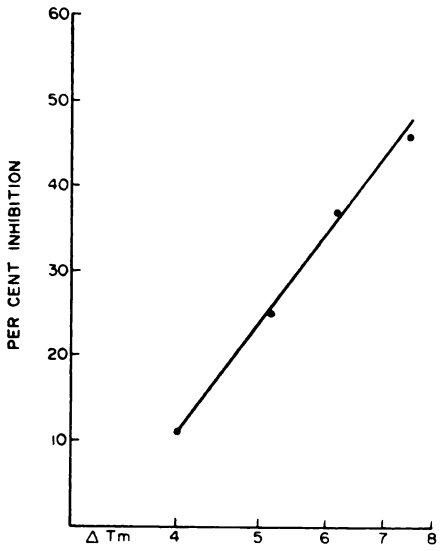

FIG. 11. *Correlation between the stabilization of DNA by quinine with thermal strand separation and inhibition of a DNA polymerase reaction*

The points represent concentrations of 2.25, 5.5, 7.75, and 10×10^{-4} M quinine, from lower left to upper right. ΔT_m is the increase in median melting temperature of DNA. Melting was measured as described in MATERIALS AND METHODS; the DNA polymerase reaction was carried out as described previously (7).

10^{-3} M. Plotting the percentage inhibition of the enzyme reaction as a function of log ΔT_m produced a diagram (Fig. 11) which indicates that the inhibition of the DNA polymerase reaction was a function of the stabilization of double-helical DNA by quinine.

Hypothetical structure of the DNA-quinine complex. A structural model of the DNA-quinine complex should be consistent with our findings of (a) a requirement for DNA to be double-helical in order to alter the absorption spectrum of quinine strongly, (b) a requirement for guanine, not satisfied by hypoxanthine, to be a constituent of those polynucleotides which decrease the intensity of the absorption spectrum of quinine, (c) a nonlinear adsorption isotherm (from spectrophotometric titrations) which does not indicate a simple stoichiometric relationship between the number of drug molecules bound and the number of guanylic acid residues per unit of DNA, (d) a reversal of the effect of DNA on the absorption spectrum of quinine by urea as well as by inorganic ions, and (e) an enhancement of viscosity and a diminution of the sedimentation coefficient of DNA by quinine. A model which fulfills these criteria is one in which the quinoline ring of this drug is intercalated between bases in double-helical DNA and the alcoholic hydroxyl group of quinine engages in the formation of a hydrogen bond, while the bulky quinuclidine moiety projects into one of the grooves of DNA and is electrostatically attracted with its tertiary aliphatic amino group to phosphates of the sugar phosphate backbones of the double helix. We have carried out model-building experiments which suggest to us that such a hypothetical structure of the DNA-quinine complex is geometrically possible.

DISCUSSION

The specificity of the effect of guanine on the absorption spectrum of quinine has previously been shown also to obtain for the spectra of chloroquine (7) and actinomycin D (14). For the latter two substances, guanine specificity has been interpreted by two alternative assumptions: (a) the 2-amino group of guanine is directly involved in the binding of ligand molecules to DNA (7, 15); (b) the marked electron donor properties of guanine, and especially of its 2-amino group, play a role in charge transfer reactions between intercalated chromophores and guanine without exclusive binding of ligands to this base (9, 16–18). With respect to the DNA-quinine complex, our present results do not support or contradict either assumption.

In addition, our results with high concentrations of poly A indicate that the ribopolymer of adenine can bind quinine. The amount of dA:dT available to us was not sufficient for further experiments to rule out some low binding affinity for the deoxyribose polymer. Our results do sug-

gest that both purines may play a role in the binding of quinine to DNA.

Since the absorption isotherm (Fig. 8) of quinine with respect to DNA was derived from spectrophotometric titration results, i.e., from a largely guanine-dependent parameter, one might have expected to find an apparent equivalence between the number of quinine molecules estimated by this method to be bound to DNA and the number of guanine residues per unit of DNA. The number of quinine molecules extrapolated to be bound by the stronger of two apparent binding processes was approximately 20 times less than expected from the guanine content of calf thymus DNA; it is evident that the contact between quinine and guanine in the double helix is restricted by conditions which are not accounted for by the proposed structural model without introducing additional and untested assumptions. Quinine resembles actinomycins (9) in the low number of binding sites on DNA, a high binding constant, and an indication that highly specific conditions must exist for strong binding.

While quinine is bound to DNA by the weaker of the two processes to the extent of 1 drug molecule per eight or nine base pairs, this still falls short of the number of guanine bases in calf thymus DNA by a factor of 5. Additional binding of quinine without spectrophotometric manifestations was discovered in equilibrium dialysis experiments, with the tentative result of 1 drug molecule being bound to four or five base pairs of DNA. Finally, at quinine concentrations above 10^{-3} M, DNA was aggregated or precipitated. This last effect may be the result of a massive occupancy of DNA phosphates by quinine base, which could occur on the periphery of the double helix without involving ring-ring interactions and, hence, spectral manifestations.

The requirement of double helicity for alterations in the spectrum of quinine and enhancement of the viscosity of DNA, as well as the diminution of its sedimentation coefficient, by quinine are consistent with the hypothesis that the quinoline ring of the drug is intercalated between bases of double helices. The search for support of this hypothesis by additional experimental criteria [flow dichroism (19), polarized fluorescence (19), radioautography (20), light scattering (21), low-angle X-ray scattering (22), and demonstration of steric hindrance to chemical reactivity of an intercalated ligand (23)] was either unpromising on statistical grounds or not possible. For this reason, the intercalation model of the DNA-quinine complex remains tentative.

The correlation between the effect of quinine on the melting temperature and inhibition of a DNA polymerase reaction lends consistency to the previous suggestion that drugs which increased the melting temperature of DNA would inhibit a DNA polymerase reaction.

We regard the formation of a DNA-quinine complex as a likely basis for the inhibition of DNA biosynthesis in plasmodia and, hence, of the antiplasmodial activity of quinine. This view might be challenged by pointing out that the concentrations of quinine necessary to inhibit the DNA polymerase reaction and to alter biophysical properties of DNA are of the order of 10^{-4} M, while those sufficient to inhibit DNA biosynthesis and schizogeny in plasmodia are two to three magnitudes lower (5, 6). Polet and Barr (6), however, have found that erythrocytes parasitized by *Plasmodium knowlesi* concentrate dihydroquinine from the suspending medium up to 200 times and thus provide a compartment of quinine concentrations which are not dissimilar to those employed in our previous (7) and current experiments.

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