charge was usually very regular; an example of this appears in the initial part of the record of Fig. 2*C*, *b*. The Ib units did not reach high frequencies of firing during passive extension of the muscle. This agrees with the observation that within its physiological limits the passive tension of a muscle is only a fraction of the tension that the muscle can develop during contraction, and that the tendon organs in general do not fire at high frequencies during passive extension. On the other hand, the Ib units responded vigorously to tetanic contractions of the muscle, much like the Golgi tendon organs (Fig. 2*C*, *a* and *b*). Firing rates maintained as high as 180 impulses per second were observed for Ib DSCT units during tetanic contractions; during such contractions of the muscle the Ia DSCT units characteristically reduced their firing rate as might be expected from their origins from the primary endings of the muscle spindles. Regularity of discharge of the Ib DSCT units was not usually as marked as that of the unit illustrated in Fig. 2*C*, *b*, but neither were the Ib units usually as strikingly irregular as the Ia DSCT units.

Responses of the first- and second-order neurons of this system may be usefully compared during various degrees of static extension of the muscle. Records were made during a series of stretches short of the final length, at each of which lengths the quasi-steady-state response of the ending could be determined. A plot of the response of a Ia DSCT unit against muscle length appears in Fig. 3*A*. To match the existing information on the primary afferent fibers (*6*) the response of the unit at each length was determined as the mean frequency of firing during 0.1 second, measured 0.5 second after the end of the dynamic phase of the stretch. In Fig. 3*A* the relationship between mean firing frequency and muscle length was approximately linear over the range studied, and this is a fair general representation of our observations on 20 Ia DSCT units. Although the degree of linearity varies considerably from one unit to another, there seems to be no other simple relationship that gives a better representation of our material. In particular, there was no flattening of the curve at higher frequencies as was found by Mountcastle *et al.* (*7*) in third-order thalamic neurons mediating joint receptor information. Similar observations on another Ia DSCT unit were made during four different series of interrupted stretches (shown by different symbols in Fig. 3*B*). The results demonstrate the degree of constancy of the response of DSCT units to similar stretches and confirm the approximately straight-line relationship between response and extension. The least-squares regression line of all the observations of Fig. 3*B* considered together has a coefficient of correlation of 0.88. The slope constant of the line is 3.6 impulses per second per millimeter, with 95-percent confidence limits of 3.1 and 4.1 impulses per second per millimeter.

The slope constants of the different Ia DSCT units had rather different values. The highest slope constant observed for gastrocnemius-soleus units was 8.5 impulses per second per millimeter; for TA-EDL units, 8.7 impulses per second per millimeter. Some units were only weakly excited by stretch of the muscle, having slope constants of less than one impulse per second per millimeter; such units were weakly connected to the primary endings of the muscle that was stretched, and presumably would be activated mainly by afferent fibers from muscles denervated in our experiments. Therefore, no great value can be attached to the mean value of the slope constants of the various units studied. We can state, however, that slope constants of the order of three to five impulses per second per millimeter were frequent for both gastrocnemius-soleus and TA-EDL Ia units. These values remarkably resemble the slope constants of the corresponding sense organs determined under comparable conditions (*3*, *6*).

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DNA: Reaction with Chloroquine

Abstract. *Difference spectrophotometry shows that double-stranded DNA produces marked changes in the absorption spectrum of chloroquine; only minor changes occur with single-stranded DNA. A DNA-chloroquine complex was demonstrated to sediment in the analytical ultracentrifuge. Chloroquine strongly elevated the thermal dissociation temperature,* T_m, *of DNA. It is concluded that the drug forms a complex with DNA by ionic interaction and stabilizes the helix.*

Certain antimicrobial substances form complexes with DNA and produce biological effects by inhibiting reactions in which DNA participates. This has been demonstrated for mitomycin C (*1*), actinomycin D (*2–4*), proflavin (*5*), daunomycin, cinerubin, chromomycin A_3, and echinomycin (*6*), as well as for miracil D (*7*), and for ethidium bromide (*8*). Chloroquine (Resochin) is also known to form complexes with DNA (*9*, *10*) and to inhibit bacterial transformation (*10*) as well as DNA synthesis, RNA syn-

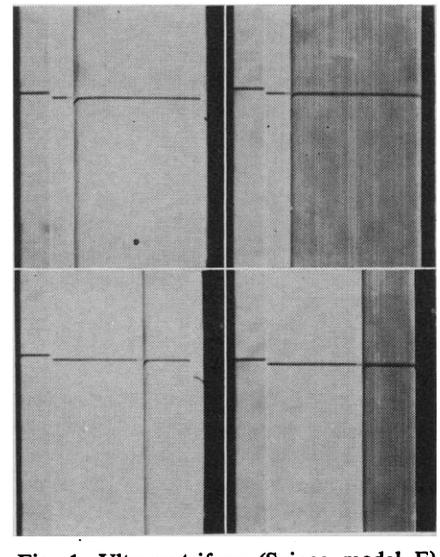


Fig. 1. Ultracentrifuge (Spinco model E) sedimentation patterns of 2 mg of calf thymus DNA (Worthington) per milliliter in the absence (left) and presence (right) of 160 μg of chloroquine per milliliter. The chloroquine used throughout these studies was a commercial preparation of the hydrochloride of the drug used for injection. The solvent was $5 \times 10^{-3}M$ tris-HCl at *p*H 7.5. The photographs were taken in light of 365-mμ wavelength, 32 minutes and 128 minutes after a speed of 59,780 rev/min had been attained. The absorbancy (A) at 365 mμ of the DNA-chloroquine mixture was 0.50 for the centrifuge cell with an optical path of 1.2 cm.

thesis, and growth in susceptible bacteria (*11*). In this report we describe the nature of the reaction of DNA with chloroquine (*12*): we have shown that the drug reacts with double-stranded DNA and stabilizes the helix.

A most general and direct method of demonstrating the existence of a complex of DNA with a colored substance is to observe the sedimentation of such a complex in the analytical ultracentrifuge. We have followed the sedimentation of DNA in the absence as well as in the presence of $5 \times 10^{-4}M$ chloroquine by photographing the schlieren pattern projected by light from a mercury lamp passing through a Corning filter No. 5840; this light source consists predominantly of the mercury lines at 365 and 366 mμ. Figure 1 shows a typical sedimentation pattern and indicates that chloroquine which is optically dense at 365 mμ sedimented with the DNA. This finding confirms directly the conclusions from less direct experiments that chloroquine forms complexes with DNA (*9*, *10*).

Figure 2 depicts the absorption spectrum of chloroquine as well as difference spectra of chloroquine at 25°C (chloroquine-DNA mixtures minus chloroquine) in the presence of either native double-stranded DNA or of DNA that had been denatured by being heated in the presence of $0.33M$ formaldehyde (*13*). Figure 2 also shows a difference spectrum of chloroquine in the presence of DNA at 98°C—that is, at a temperature at which DNA is single-stranded. It is readily apparent that DNA in the double-stranded condition produced marked changes in the absorption spectrum of chloroquine while the effects of single-stranded DNA's were weak and resembled those produced by heparin. This polysaccharide shares with nucleic acids the property of possessing one mineral anionic group per molecular weight equivalent of the order of 3×10^2 (*14*); heparin is useful as a polyanionic control substance in the study of antimicrobial compounds that specifically form complexes with DNA. Kirk, for example, found that the spectrum and biological activity of actinomycin D were depressed by double-stranded DNA but not by heparin (*2*).

The strong influence of double-stranded DNA upon the spectrum of chloroquine (Fig. 2) suggested that the drug was bound to both complementary strands of DNA. This was expected to have an effect upon the stability of the helix. Figure 3 shows that the thermal dissociation temperatures, T_m, of poly-dAT (*15*), as well as those of DNA, were increased by approximately 15°C in the presence of $10^{-5}M$ chloroquine. The observation that this increase was identical for both polymers indicates that the effect of the drug upon thermal dissociation did not depend upon the presence of either guanine or cytosine. Stollar and Levine have suggested that either guanine or cytosine

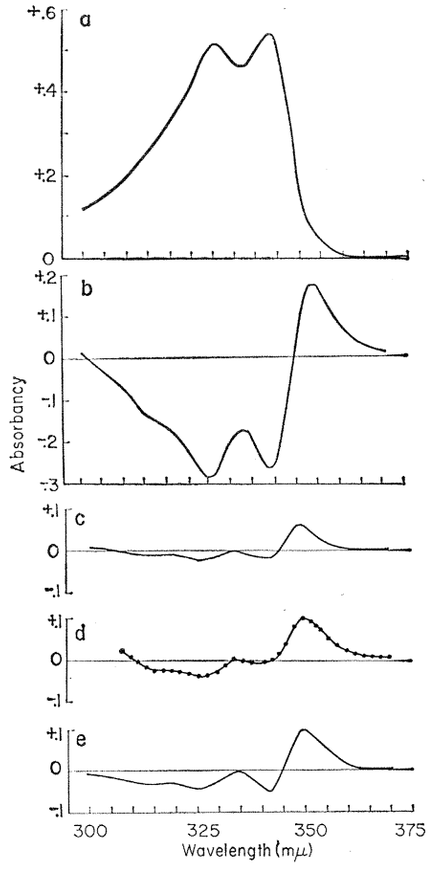


Fig. 2. Influence of DNA upon the absorption spectrum of chloroquine, $3 \times 10^{-5}M$ in $5 \times 10^{-3}M$ tris-HCl buffer at *p*H 7.4. (*a*) Absorption spectrum of chloroquine; (*b*) difference spectrum of a mixture of chloroquine and double-stranded DNA (167 μg of DNA per milliliter) minus chloroquine, recorded in a Cary 14 spectrophotometer at 25°C; (*c*) difference spectrum analogous to *b* but obtained with DNA that had been denatured by heating in the presence of $0.33M$ formaldehyde (*13*); (*d*) difference spectrum analogous to *a* but measured in a Beckman DU spectrophotometer at 98°C; (*e*) difference spectrum of a chloroquine-heparin mixture (167 μg of heparin per milliliter) recorded at 25°C.

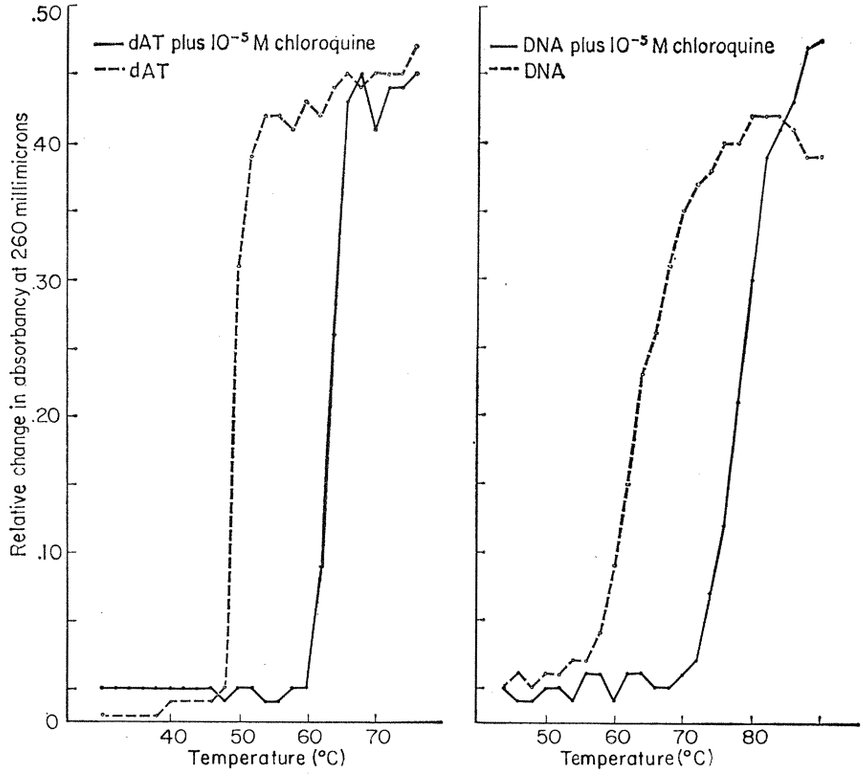
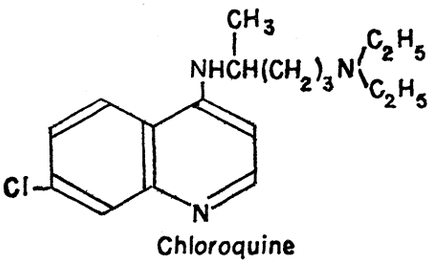


Fig. 3. Thermal dissociation of poly-dAT and of DNA in the presence and absence of chloroquine. The polymers were dissolved in $5 \times 10^{-3}M$ tris-HCl buffer at *p*H 7.5: DNA to 20 μg/ml and poly-dAT to an A^{260} of 0.375. Relative changes in absorbancies were calculated by subtracting A^{260}'s at 40°C from A^{260}'s at higher temperatures and dividing these differences by the absorbancies of DNA or poly-dAT, respectively, as measured at 40°C. This arithmetic procedure corrected for the contribution of chloroquine to the light absorption at 260 mμ.



Chloroquine

Fig. 4. Structure of chloroquine; the compound has a molecular weight of 319.9.

is specifically involved in the binding of chloroquine to DNA (*10*).

Kurnick and Radcliffe have observed an enhancement of the viscosity of DNA solutions by chloroquine (*9*); this suggests to us that the drug forms a complex with DNA in a manner which resembles the interaction of DNA with mepacrine (Atebrin) (*16*).

The two nonheterocyclic amino groups of chloroquine (Fig. 4) are separated by four carbon atoms: the drug may be considered a substituted 1,4-diaminopentane. Among primary aliphatic diamines of graded chain lengths, diaminobutane and -pentane are strongest in elevating the T_m of DNA (*17*) although concentrations of the range of $10^{-3}M$ are required to produce effects comparable to those of $10^{-5}M$ chloroquine. Spermine, on the other hand, which possesses two secondary amino groups separated by four carbon atoms, is as active as chloroquine at equivalent molar concentrations in elevating the T_m of DNA (*18*). We are proposing that nonprimary diaminobutanes stabilize the DNA helix by ionic interaction with phosphoric acid groups. The electronegatively substituted heterocyclic systems of chloroquine and mepacrine may contribute additionally to the formation of complexes with DNA.

The actions of these and other chemotherapeutic agents upon DNA may offer an opportunity to probe the DNA molecule for specific structural features that are essential for the replication of DNA or for the transcription of RNA.

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Color-Discrimination Performance of Pigeons: Effects of Reward

Abstract. *Performance of two pigeons given tasks in discriminating colors was examined on trials before and after they had occasionally received rewards for pecking when exposed to light of specific wavelengths. After a reward, the probability that the birds would respond to light stimuli that were never rewarded was higher than before the reward was given, but paradoxically the birds showed no general decline in their ability to differentiate between stimuli at wavelengths 1 millimicron apart.*

Reward plays an important role in several behavioral phenomena; responses maintained by reward cease if the reward is not made available. During discrimination tests in which responses to one stimulus are sometimes rewarded while responses to a second stimulus are never rewarded, the subject tends to respond to the rewarded stimulus with a higher probability or a faster rate. We have noted, however, that performance in discrimination is relatively poorer after a reward has been given than before; for a short time there is an increased tendency for the subject to respond to the stimulus not associated with reward, which decreases the difference in relative probability of response to the two stimuli.

Thus, in contrast with its long-range salutary effect on performance in discrimination, the short-range effect of a reward may be thought to be interference with discrimination; unrewarded stimuli elicit responses more frequently, a situation sometimes called loss of stimulus control. The exact nature of the decline in performance has not been studied.

We have analyzed much data on performance in discrimination, derived from two birds that were trained on a problem of spectral discrimination for more than 1000 hours. The birds were presented with a random sequence of monochromatic light values, closely spaced physically, in an otherwise darkened chamber. To each exposure they could respond by pecking a 2.5-cm circular key on which the light was projected for 2 seconds. Occasionally the pigeon was rewarded by the brief availability of grain if it pecked in response to certain of the wavelengths, never if it pecked in response to other wavelengths. After a peck and between trials the key was darkened for a variable period averaging 2 seconds from the end of each trial.

The two birds worked on different problems. One was presented with the ten wavelengths from 530 to 539 mμ at 1-mμ intervals and was occasionally rewarded for responding to the upper five values, 535 to 539 mμ. The second bird was rewarded only for responding to 535 mμ, the middle value of the 11 wavelengths evenly spaced between 530 and 540 mμ, inclusive. The data cover various probabilities of occurrence of the several stimuli, probabilities of reward (typically 2 or 3 times per 100 trials), and durations of rewards. Since the effects of all such manipulations proved to be virtually independent of the effects reported here, we pooled all data for the following analysis.

A LINC computer operating on-line provided all the control and recording functions. During the latter portion of the discrimination training, the schedule of stimuli and information on response were recorded and stored separately for each trial, so that the results of some 100,000 trials were available for each bird.

Analysis of the data by the computer located every trial on which a reward was available and during which a peck occurred (presumably the bird