Quinacrine (Atebrin): Mode of Action

Abstract. *Quinacrine at a concentration of 8 × 10^{-4} mole per liter is bactericidal for* Escherichia coli, *blocks DNA synthesis, and inhibits the syntheses of RNA and protein strongly. At a concentration of 2 × 10^{-4} mole per liter, the drug is bacteriostatic, the syntheses of protein and DNA (but not that of RNA) are partially inhibited, and the bacteria grow into giant filaments. Impairment of DNA replication is proposed as the mode of action of quinacrine.*

The antimalarial drug, quinacrine, forms a molecular complex with DNA (*1*). The acridine ring of the drug molecule is intercalated between the base pairs of double-helical DNA (*2*), and the aliphatic diamine side chain apparently bridges complementary DNA strands across the minor groove by ionic attraction to phosphate groups (*3*). Consequences of the reaction of quinacrine with DNA are (i) inhibition of the enzymatic hydrolysis of DNA (*1*), (ii) inhibition of the DNA-dependent DNA and RNA polymerase reactions (*3*, *4*), (iii) reduction of the frequency of spontaneous or induced mutations at low quinacrine concentrations (*5*), and (iv) induction of frameshift mutations at high concentrations (*6*).

Quinacrine also inhibits the growth of many bacteria, such as *Escherichia coli* (*7*). We studied the mode of action of quinacrine with this organism. When the drug was added to *E. coli* B growing exponentially in a synthetic medium with glucose as the sole source of carbon and energy, turbidimetry at 520 mμ showed that 2 × 10^{-4}*M* quinacrine reduced the growth rate by 25 percent, while 8 × 10^{-4}*M* quinacrine decreased the rate by 86 percent, measured over an interval of 1.25 duplication times. Figure 1 shows that 8 × 10^{-4}*M* quinacrine was strongly bactericidal, but at 2 × 10^{-4}*M* the drug produced bacteriostasis within one half of a duplication time. Bacteria exposed to this lower concentration of quinacrine for 18 to 24 hours grew into giant filaments (Fig. 2). Electron microscopy, performed by R. Borasky, revealed a characteristic twisted and braidlike appearance of the cytoplasmic material in the filaments.

Chemical analysis for DNA (Fig. 3) revealed that 8 × 10^{-4}*M* quinacrine inhibited bacterial DNA synthesis completely, while 2 × 10^{-4}*M* quinacrine reduced the rate of DNA synthesis by 26 percent. Syntheses of RNA and of protein (Figs. 4 and 5) were strongly inhibited at the bactericidal concentration of quinacrine; but at the bacteriostatic concentration RNA synthesis was not affected (Fig. 4), and the incorporation of C^{14}-phenylalanine into the bacterial proteins was reduced by 40 percent (Fig. 5). In view of the fact that these major biosyntheses were affected by the drug to different extents, the observed effects were not one common result of a general inhibition of the utilization of glucose as a source of energy. Such a mode of action of quinacrine has been suggested (*8*).

Quinacrine is an acridine homolog of the antimalarial drug, chloroquine. Unlike chloroquine (*9*), however, quinacrine did not produce a breakdown of bacterial ribosomes nor a change in the quantitative distribution of the major categories of bacterial nucleic acids.

Inhibition of DNA replication in *E. coli* by 8 × 10^{-4}*M* quinacrine paralleled the inhibition of the DNA polymerase reaction, catalyzed by an *E. coli* enzyme, at the identical drug concentration (*3*) and accounted for the bactericidal effect of quinacrine. The inhibition of RNA synthesis in *E. coli* likewise matched quantitatively the in-

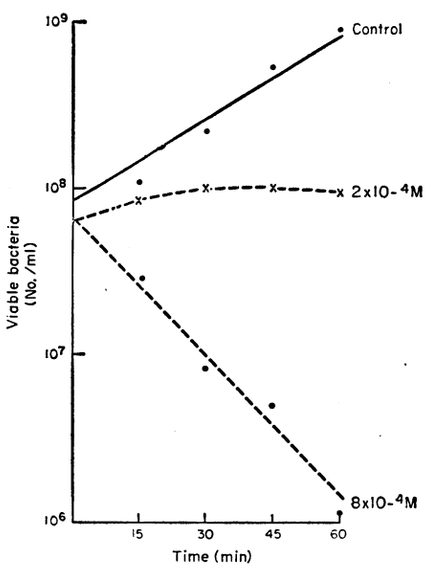

Fig. 1. Antibacterial effect of quinacrine. *Escherichia coli* strain B (ATTC #11303) was grown exponentially in a synthetic medium. Quinacrine hydrochloride was added at zero time to the concentrations indicated; no drug was supplied to the control culture. Samples were removed at intervals for serial dilution plate counting.

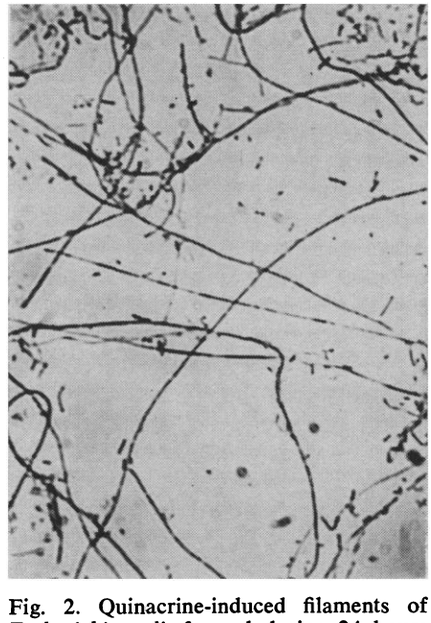

Fig. 2. Quinacrine-induced filaments of *Escherichia coli*; formed during 24 hours of growth in the presence of 2 × 10^{-4}*M* quinacrine. These test-tube cultures were not subjected to aeration or mechanical agitation during growth. Samples were heat-fixed, stained with the Gram stain, and photographed with a Leitz Aristophot.

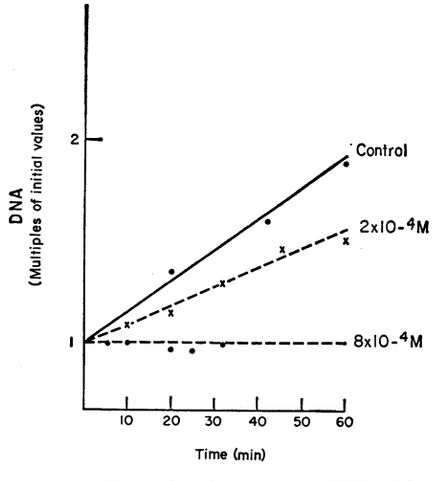

Fig. 3. Effect of quinacrine on DNA biosynthesis in *Escherichia coli*. The drug was added to exponentially growing cultures at zero time to the final concentrations indicated. No drug was supplied to the control culture. Samples of bacteria in culture were taken at intervals and sedimented, and their nucleic acids were extracted with 5 percent perchloric acid at 70°C for 60 minutes. These solutions were extracted three times with chloroform to remove quinacrine. DNA was then determined with a diphenylamine method (*9*).

hibition in vitro of the reaction of RNA polymerase from *E. coli* (*3*); the decrease in protein synthesis was probably a consequence of the decreased production of messenger RNA. The rates of growth and of DNA synthesis were reduced to the same extent by $2 \times 10^{-4}M$ quinacrine. Others have stated that $4 \times 10^{-4}M$ quinacrine permitted DNA synthesis proportional to turbidity increases in cultures of *Bacillus cereus* (*10*).

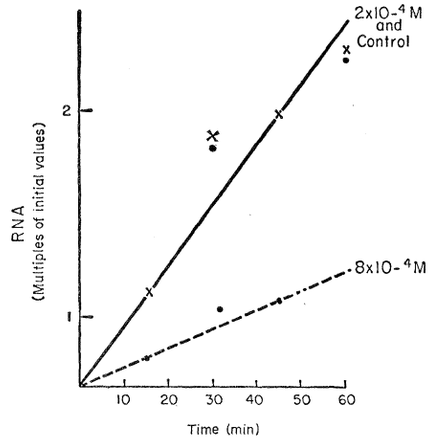

Fig. 4. Effect of quinacrine on RNA biosynthesis in *Escherichia coli*. Experimental design and procedures were the same as in Fig. 3, except that RNA was determined with an orcinol method (*9*).

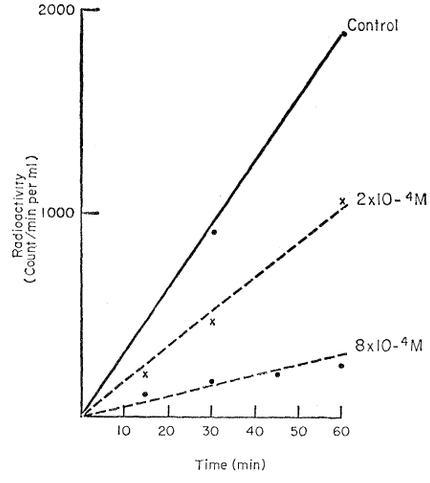

Fig. 5. Effect of quinacrine on protein biosynthesis in *Escherichia coli*. Incorporation of C^{14}-phenylalanine into bacterial proteins was measured by a membrane filter technique. Quinacrine (at indicated concentrations; none in control) and C^{14}-phenylalanine [5 μg/ml (specific activity 61 μc/μmole)] were both added to exponentially growing cultures at time zero. Samples were removed at intervals and immediately diluted with equal volumes of 10 percent trichloroacetic acid (containing 1 percent casamino acids). After 30 minutes at 2°C, the precipitates from the samples were collected on Millipore filters, washed first with 5 percent trichloroacetic acid (containing casamino acids), and then three times with chloroform to remove residual quinacrine. The filters were placed in a dioxane-based scintillation fluid, and radioactivities were counted in a Nuclear-Chicago liquid-scintillation counter.

Undiminished RNA synthesis and appreciable protein synthesis at the bacteriostatic concentration of $2 \times 10^{-4}M$ quinacrine accounted for the accumulation of large amounts of cytoplasmic material in the filaments after 24 hours (Fig. 2). This material was organized in distinct segments which were, however, not separated by cross septa. The fact that bacteria exposed to quinacrine failed to initiate events resulting in cell division may be related to the action of the drug upon DNA. By analogy, cells of *Escherichia coli* in which DNA has reacted with mitomycin C grow within shorter times to shorter filaments which exhibit structural characteristics similar to those we have observed in the filaments grown in the presence of quinacrine (*11*).

Biophysical studies on the nature of the quinacrine-DNA complex (*1–3*), inhibitions of DNA-dependent enzymatic reactions by the drug (*1, 3, 4*), as well as our study on the effects of quinacrine in whole bacterial cells are consistent with the view that the mechanism of biological action of the drug is the specific reaction of quinacrine with native, double-stranded DNA and that the resulting mode of action is an impairment of DNA replication and, at cytocidal concentrations, of RNA transcription. That quinacrine also exerts its antimalarial effect by acting on the nucleic acids of plasmodia has been postulated by Albert (*12*) and is supported by observations (*13*) that the drug inhibits the incorporation of P^{32}-orthophosphate into the nucleic acids of plasmodia.

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References and Notes

1. N. B. Kurnick and I. E. Radcliffe, *J. Lab. Clin. Med.* **60**, 669 (1962).
2. L. S. Lerman, *Proc. Nat. Acad. Sci. U.S.* **49**, 94 (1963).
3. R. L. O'Brien, J. G. Olenick, F. E. Hahn, *ibid.* **55**, 1511 (1966).
4. R. M. Hochster and V. M. Chang, *Can. J. Biochem.* **41**, 1503 (1963).
5. H. G. Johnson and M. K. Bach, *Proc. Nat. Acad. Sci. U.S.* **55**, 1453 (1966).
6. B. Ames and H. Whitfeld, Jr., personal communication.
7. C. A. Lawrence, *Proc. Soc. Exp. Biol. Med.* **52**, 90 (1943).
8. I. B. R. Bowman, T. P. Grant, W. O. Kermack, D. Ogston, *Biochem. J.* **78**, 472 (1961).
9. J. Ciak and F. E. Hahn, *Science* **151**, 347 (1966).
10. H. G. Mandel and M. L. Seligman, *Pharmacologist* **8**, 211 (1966).
11. E. Reich, A. J. Shatkin, E. L. Tatum, *Biochim. Biophys. Acta* **53**, 132 (1961).
12. A. Albert, *Selective Toxicity* (Wiley, New York, ed. 3, 1965), p. 207.
13. K. A. Schellenberg and G. R. Coatney, *Biochem. Pharmacol.* **6**, 143 (1961).

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Transverse Tubule Apertures in Mammalian Myocardial Cells: Surface Array

Abstract. *The technique of "freeze-etching" tissues for electron microscopy has permitted observation of the external apertures of the transverse tubules. The apertures appear on the cell surface in approximately parallel rows, which can be interpreted as corresponding longitudinally to the spaces between the myofibrils and transversely to the Z regions of the myofibrils.*

On the basis of his well-known experiments on the inward conduction of excitation in striated muscle, Huxley (*1*) suggested that the simplest explanation of his findings was that a transverse tubular network must exist with its lumen communicating with the extracellular space. Continuity between the lumen of the transverse tubular system [T system (*2*)] and the extracellular space was first suggested on the basis of electron-microscopic evidence in mammalian cardiac muscle (*3*), and further observations have confirmed that such a continuity exists both in mammalian myocardium (*4*) and in many other types of striated muscle (*5*).

The electron-microscopic evidence of this continuity has hitherto been obtained from sectioned material. The development of "freeze-etching" (*6*) for electron microscopy has permitted a new approach to the study of the surface relationships of the tubular systems in muscle. We have studied guinea pig cardiac (papillary) muscle using this technique (*6*). The "freeze-etching" process was carried out with a Balzers freeze-etch apparatus (*7*) which