chromosomal aberrations in some of the cells, although in many others fewer such aberrations were present (Figs. 1 and 2). Most of the aberrations took the form of chromosome breaks. The fact that very few two-hit aberrations were present suggests that most of the broken ends do not rejoin. Table 1 summarizes the percentage of metaphase cells showing aberrations, the mean number of chromosome and chromatid breaks per cell, and the mean number of achromatic lesions (Feulgen-negative gaps).

The location of the break points along the length of the chromosomes was far from random; indeed, nearly half of the breaks were in the region of primary constriction, thus giving rise to the production of a large number of apparently acentric fragments.

Apart from the work of Sturelid and Kihlman (*1*), we do not know of any other study in which the effect of LSD on chromosomes of plant material has been studied. Our results are not in agreement with their findings.

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References and Notes

1. S. Sturelid and B. A. Kihlman, *Hereditas* **62**, 259 (1969).
2. L. Bender and D. V. Shivsanker, *Science* **159**, 749 (1968); D. Grace, E. A. Carlson, P. Goodman, *ibid.* **161**, 694 (1968).
3. M. M. Cohen, M. J. Marinello, N. Back, *ibid.* **155**, 1417 (1967); M. M. Cohen and A. B. Mukherjee, *Nature* **219**, 1072 (1968).
4. N. E. Skakkebaek, J. Philip, O. J. Rafaelsen, *Science* **160**, 1246 (1968).
5. C. D. Darlington and L. F. La Cour, *J. Genet.* **46**, 180 (1944).

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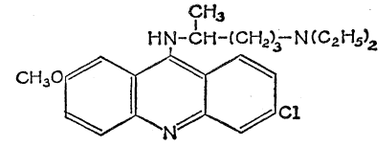
Quinacrine: Mechanisms of Antimalarial Action

Abstract. *Two new interesting modes of action of quinacrine have been discovered. The first concerns a dose-related inhibition of uptake of [8-^{3}H] adenosine into host cells of parasitized blood. Second, the drug inhibits the incorporation of tritiated adenosine triphosphate primarily into RNA but also into DNA of the erythrocyte-free malarial parasite* Plasmodium berghei.

Unequivocal evidence concerning the action of many antimalarial drugs has been difficult to establish because of the inherent difficulties in separating the parasite from its host cell (*1*). The results have been often difficult to interpret because of the effects of interplay between the red cell membrane and the parasite. Recently, we have been able to prepare biochemically active, "free" parasites that show greater ability to incorporate exogenous purines than equivalent parasitized red cells do (*2*). From the results of testing effects of the drug both on the uptake of [^{3}H]-adenosine by the parasitized cell and incorporation of [^{3}H]adenosine into the RNA and DNA of the "free" parasite system itself, it should be possible to interpret two important primary events necessary for parasite survival. We think that this is a more logical attack than separating the enzymes in question, namely, permeases and polymerases, and using purified preparations because the adenosine permease, a kinase, is mostly likely associated with the red cell membrane, and use can be made of the natural histone-DNA-RNA complex of the parasite in situ.

The methods of preparation of parasitized rat blood containing *Plasmodium berghei* have been described (*3*). The uptake of [8-^{3}H]adenosine into parasitized cells and measurement of the radioactivity associated with the cells has been described (*4*). Erythrocyte-free malarial parasites were prepared by a modified saponin-lysis technique (*4*). The free parasites from 1 ml of rat blood parasitized by *P. berghei* were suspended in a medium consisting of plasma and glucose (200 mg per 100 ml of plasma), *p*H 7.4, was added to 1 ml of quinacrine in Krebs



Quinacrine (Atabrine)

solution. [8-^{3}H]Adenosine triphosphate (ATP) (2.5 μc) was added to the mixture, and the mixture was incubated at 37°C under conditions for promoting linear incorporation of [^{3}H]ATP into the RNA and DNA of the parasites. Incubation was stopped after 20 minutes by the addition of 4 ml of 10 percent trichloroacetic acid (TCA). The acid-insoluble precipitate was washed three times with TCA, and the RNA was hydrolyzed with 1*N* KOH (20 hours at 37°C). A portion (0.5 ml) of the hydrolyzate was analyzed for radioactivity. The solids were washed twice more with TCA, and the DNA was hydrolyzed in 4 ml of 10 percent TCA

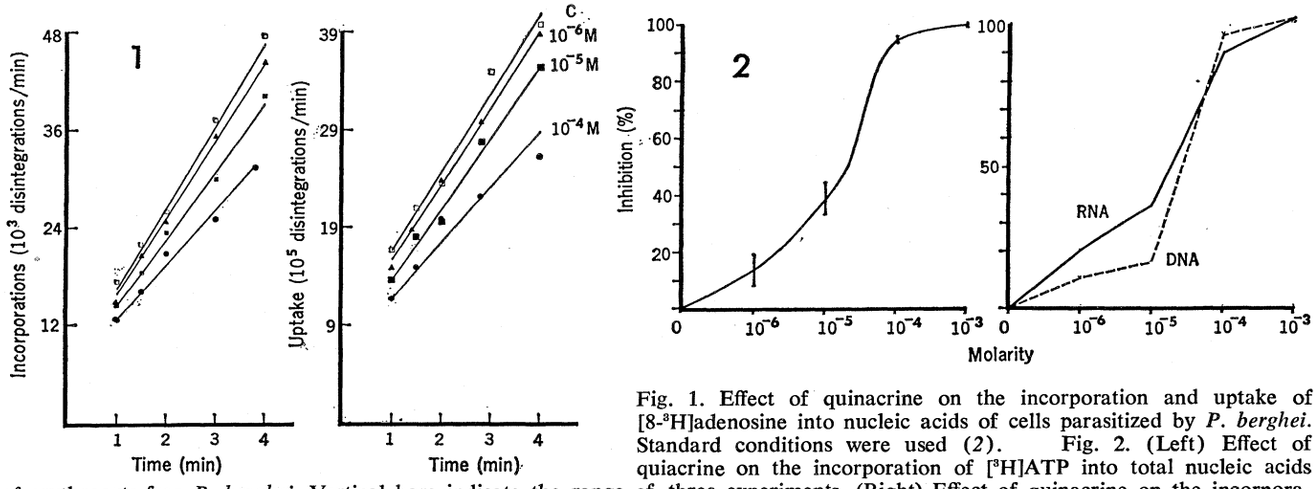


Fig. 1. Effect of quinacrine on the incorporation and uptake of [8-^{3}H]adenosine into nucleic acids of cells parasitized by *P. berghei*. Standard conditions were used (*2*). Fig. 2. (Left) Effect of quiacrine on the incorporation of [^{3}H]ATP into total nucleic acids of erythrocyte-free *P. berghei*. Vertical bars indicate the range of three experiments. (Right) Effect of quinacrine on the incorporation of [^{3}H]ATP into DNA and RNA of "free" parasites (Table 1).

Table 1. Amount of incorporation (disintegrations per minute) of [^{3}H]ATP into RNA and DNA.

Substance	Incorporation	Percent of total
Total (DNA + RNA)	319,000	
RNA	237,264	95.0
DNA	12,560	5.0
% Recovery		77.5

at 90°C for 30 minutes. The radioactivity of DNA was assessed as described for RNA.

Quinacrine has a marked effect on the uptake of [8-^{3}H]adenosine into cells parasitized by *P. berghei* at 10^{-4} and $10^{-5}M$. Results of others indicate that rat red cells contain the enzyme adenosine kinase (*5*). Our data, showing inhibition of adenosine transport by various drugs, suggest that this enzyme is membrane bound (*2*). Other observations indicate that this adenosine transport is an active, concentrating mechanism probably concerned with phosphorylation of adenosine to adenosine monophosphate (*6*). That this is reasonable is shown by the fact that once adenosine penetrates the red cell it does not wash out of these cells; and chromatography indicates that the radioactivity is associated with adenosine monophosphate (AMP). Similar observations are reported in other cell types (*5*); and acridine derivatives, such as quinacrine, inhibit adenosine transport in these cells (*7*). Owing to deficient de novo synthesis of purines, the malarial parasite is dependent on exogenously supplied purines (*4, 8*), and any inhibition of the transport system might constitute an indirect "starvation" mechanism at the membrane of the host cell. Also if transport into the parasite of phosphorylated or nonphosphorylated purine intermediates could be inhibited, this would represent an important new antimalarial mechanism. There does appear to be a relation between host red cell ATP and degree of parasitemia (*9*).

The really surprising finding, however, was the selective effect of various doses of quinacrine on the incorporation of [^{3}H]adenosine triphosphate into RNA of erythrocyte-free malarial parasites (Fig. 2) compared to the control (Table 1). The same results occurred if deoxyadenosine was used (*10*). On the basis of quinacrine's interference with the incorporation of adenosine triphosphate into DNA, several workers using polymerases from *Escherichia coli,* (*11*) have predicted the same inhibition by quinacrine in malarial parasites. However, the fact that much more incorporation occurred into RNA (10 or 20 times as much) than into DNA and that this incorporation is drug-inhibited to a greater extent shows that there is a surprising selectivity of drug action. This would possibly indicate that, in parasites, there is some basic difference in the nucleic-acid synthesizing system of parasites. There is some factual basis for this statement because ion requirement of the parasite enzyme is different—Co++ and Mn++ stimulate more than Mg++—and maximum activity occurs at *p*H 7.4, compared to *p*H 8.0 for the corresponding mammalian enzyme (*10*). Also Rifampicin (a direct inhibitor of bacterial RNA polymerase) is effective against malarial parasites in vivo (*12*).

Many of the studies with antimalarial drugs have been made with organisms other than malarial parasites (*13, 14*) and in subcellular systems rather than in more complex systems (*15*). Our data with various concentrations of quinacrine mirror the effects of the in vitro polymerase system quite well (*13*), but we think that the selective toxicity of quinacrine's action may well lie in the nucleic acid synthesizing system. Probably the interference of quinacrine's action with the RNA polymerase is indirect by way of "intercalation" in the DNA template (*16*). We have actually observed, using fluorescence microscopy, the direct result of quinacrine's intercalation. Parasitized cells were placed in the presence of quinacrine, under ultraviolet light excitation, and the parasite nuclei showed yellow-green fluorescence. This well-characterized effect has been developed both in parasitized cells with acridine orange (*17*) and in vitro with ethidium bromide (*18*). Both of these drugs are well-known intercalating compounds (*19*).

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References and Notes

1. R. L. Ladda, *Mil. Med.* **131**, 993 (1966).
2. K. Van Dyke and C. Szustkiewicz, *ibid.* **134**, 1000 (1969).
3. ———, C. H. Lantz, L. H. Saxe, *Biochem. Pharmacol.* **18**, 1417 (1969).
4. K. Van Dyke, G. Tremblay, C. Lantz, C. Szustkiewicz, *Amer. J. Trop. Med. Hyg.* **19**, 203 (1970).
5. R. P. Miech and J. N. Santos, *Physiol. Chem. Phys.* **1**, 127 (1969).
6. W. Kübler and H. J. Bretschnieder, *Pflügers Arch. Ges. Physiol.* **141**, 280 (1964).
7. C. Scholtissek, *Biochim. Biophys. Acta* **158**, 435 (1968).
8. C. J. Walsh and I. W. Sherman, *J. Protozool.* **15**, 763 (1968).
9. G. J. Brewer and C. C. Coan, *Mil. Med.* **134** Suppl. 10, 1056 (1969).
10. C. Lantz and K. Van Dyke, *Fed. Proc.* **29**, 808 (1970).
11. S. N. Cohen and K. L. Yielding, *Proc. Nat. Acad. Sci. U.S.* **54**, 52 (1965); F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, J. G. Olenik, *Mil. Med.* **131** Suppl. 9, 1071 (1966).
12. C. B. Engle, Ciba, personal communication.
13. J. Ciak and F. E. Hahn, *Science* **151**, 347 (1966).
14. ———, *ibid.* **156**, 655 (1967).
15. L. P. Wichard, C. R. Morris, J. M. Smith, D. J. Holbrook, Jr., *Mol. Pharmacol.* **4**, 630 (1968).
16. L. S. Lerman, *J. Cell. Comp. Physiol.* **64** Suppl. 1, 1 (1964).
17. G. P. Dutta, *Stain Technol.* **44**, 223 (1969).
18. K. Van Dyke and C. Szustkiewicz, *Anal. Biochem.* **23**, 109 (1968); J.-B. LePecq and C. Paoletti, *J. Mol. Biol.* **27**, 87 (1967).
19. A. Blake and A. R. Peacocke, *Biopolymers* **6**, 1225 (1968).
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Immunoassay of Plasma Low-Density Lipoproteins

Abstract. *An immunoassay was developed for determining the concentration of the protein moiety of the low-density lipoproteins of human plasma. The concentration of this protein in the plasma was variable; it was higher than normal on the average in patients with familial hyperbetalipoproteinemia (type II) and endogenous hyperlipemia (type IV) and lower than normal in patients with fat-induced (type I) and mixed (type V) hyperlipemia. Patients with endogenous hyperlipemia were separable by the immunoassay into those with normal and those with supernormal low-density lipoprotein protein concentration.*

Low-density lipoproteins (LDL) have been statistically associated with coronary heart disease (*1*). They are usually quantitated by their cholesterol content or total mass (*1–4*), although variability in the relative proportions of their several components has been recognized for some years (*5*). In light of the evidence (*5*) that the cholesterol and protein content of LDL might vary independently of each other, the concentration of LDL protein was determined in plasma of normal and hyperlipidemic subjects and compared with LDL cholesterol concentration.

Blood was drawn from 165 subjects