itself had no significant effect on the membrane potential, but in one strip it produced hyperpolarization of 4 mv that was significant ($P < .05$).

Dibutyryl cyclic 3′,5′-AMP (0.5 to 1.0 mmole/liter) by itself had a significant ($P < .01$) hyperpolarizing effect in only two of seven experiments conducted in the presence of a low concentration of potassium (1 mmole/liter). In three of the above experiments, although the effect of dibutyryl cyclic 3′,5′-AMP on the membrane potential was not significant, the subsequent addition of 4 m*M* theophylline hyperpolarized these preparations. In 1m*M* K^+ solution, consistent hyperpolarization could be produced by dibutyryl cyclic AMP when the latter was preceded by a half-hour treatment with theophylline or when the nucleotide and theophylline were administered simultaneously and membrane potentials were determined within 20 to 40 minutes after addition of the drugs (Table 1). In the presence of elevated concentrations of K^+ (10 mmole/liter) the same combination of cyclic nucleotide and phosphodiesterase inhibitor did not produce a significant effect on the membrane potential (Table 1).

The potentiation of the hyperpolarizing action of isoproterenol by the inhibitor of phosphodiesterase, theophylline, is compatible with a mechanism mediated by cyclic 3′,5′-AMP. The similar dependence on potassium of the electrogenic actions of the cyclic nucleotide and of isoproterenol further supports this mechanism of action. In similar experiments, adenosine monophosphate (in 1 m*M* K^+ solution) did not hyperpolarize the rabbit main pulmonary artery, indicating that the effect of the cyclic nucleotide is specific. The relatively high concentrations of cyclic nucleotide required for hyperpolarization and the potentiation of this effect by theophylline suggest that the site of hyperpolarizing action is the inner, or at least not a readily accessible, surface of the cell membrane.

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13 April 1970

Chromosomal Aberrations Induced in Barley by LSD

Abstract. *Seeds of hulled barley* (Hordeum vulgare) *were germinated and then treated with LSD. Preparations of squashed root tips stained with Feulgen revealed extensive chromosomal aberrations, most of which were chromosome breaks. Nearly half of the breaks occurred in the region of the primary constriction.*

There has been considerable interest in the possible genetic effects of LSD (lysergic acid diethylamide), and a number of conflicting reports have appeared. Most of these reports have been summarized recently by Sturelid and Kihlman (*1*), who themselves studied the effects of various doses of this drug on broad bean cells, Chinese hamster cells, and human leukocytes. Like several of the earlier authors (*2*), Sturelid and Kihlman (*1*) could find no evidence that LSD induces chromosome aberrations in their material. On the other hand, Cohen *et al.* and Skakkebaek *et al.* observed different types of chromosomal aberrations in human peripheral leukocytes (*3*) and in mice (*4*) treated with LSD.

We have studied the effect of LSD on chromosomal structure of barley. Seeds of hulled barley variety NP 113 (*Hordeum vulgare*, $2n = 14$) were germinated overnight at 25°C and then treated with an aqueous solution of LSD (Sandoz, 25 μg/ml) for either 4 or 8 hours. The treatments were followed by 4- or 8-hour recovery periods, after which the root tips were treated with 0.1 percent colchicine for 1 hour and then fixed in a mixture of ethanol and acetic acid (3 : 1). The preparations of squashed root tips were stained with Feulgen by the normal procedure. Chromosomal aberrations were scored in well-spread metaphase cells as described by Darlington and La Cour (*5*).

There was evidence of extensive

Table 1. Observations on aberrations induced with LSD, including chromosomal (B″) and chromatid (B′) breaks. The mean values are based on the scoring of total number of cells including those not showing aberrations.

Treatments			Metaphase cells scored (No.)	Aberrant cells (%)	Mean number of different chromosomal aberrations per cell		
LSD (μg/ml)	Time (hr)	Recovery (hr)			B″	B′	Achromatic gaps
0	4	0	180	0.00	0.00	0.00	0.00
25	4	4	250	37.69	2.20	.50	
25	4	8	201	44.80	3.00	.66	.74
0	8	0	290	1.63	0.08	.04	
25	8	4	131	46.34	3.05	.61	.04
25	8	8	209	56.16	4.03	.77	.10

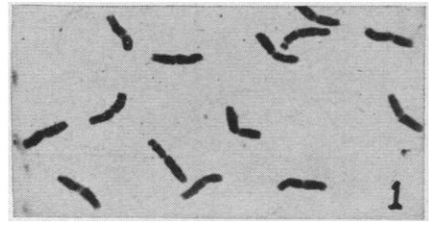
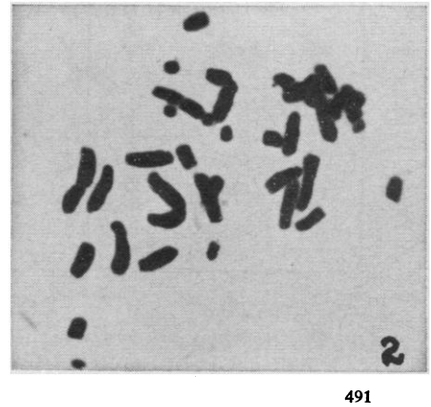


Fig. 1. Metaphase cell showing the normal complement of 14 chromosomes in the control material (× 1800). Fig. 2. Metaphase cell from root tip treated with LSD, showing extensive chromosomal damage (× 2500).



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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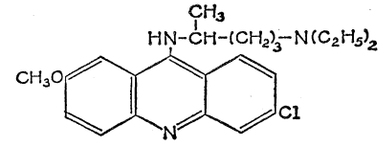
Quinacrine: Mechanisms of Antimalarial Action

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

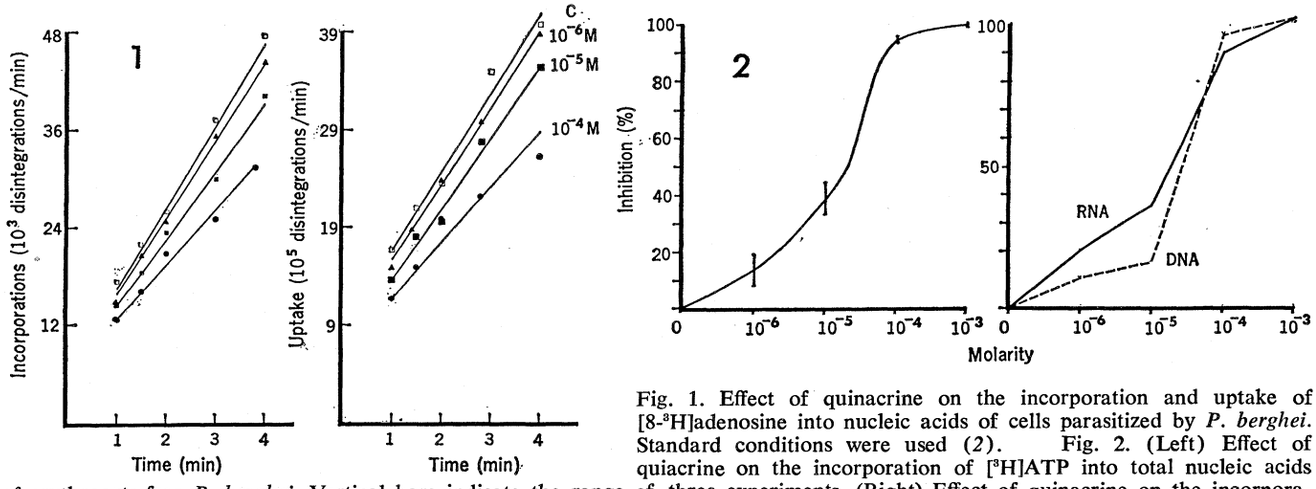


Fig. 1. Effect of quinacrine on the incorporation and uptake of [8-^{3}H]adenosine into nucleic acids of cells parasitized by *P. berghei*. Standard conditions were used (*2*). Fig. 2. (Left) Effect of quiacrine on the incorporation of [^{3}H]ATP into total nucleic acids of erythrocyte-free *P. berghei*. 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).