Quinine—Effect on *Tetrahymena pyriformis* II: Comparative Activity of the Stereoisomers, Quinidine and Quinine

K. A. CONKLIN*, P. HEU, and S. C. CHOU

Abstract ☐ Treatment of heat-synchronized *Tetrahymena* with quinine or quinidine resulted in inhibition of synchronized cell division. Syntheses of DNA, RNA, protein, and lipids were also effectively blocked by both drugs. The effects of quinine and quinidine were quantitatively the same in all instances, indicating that the intracellular action of the two drugs in *Tetrahymena* is similar.

Keyphrases ☐ Quinine, quinidine effect—*Tetrahymena pyriformis* ☐ Quinidine, quinine effect—RNA, DNA, protein, lipid synthesis ☐ Thymidine-, uracil-, amino acids-^{14}C—*Tetrahymena pyriformis* utilization

Quinine and quinidine are, respectively, the levo- and dextrorotatory stereoisomers of 6-methoxy-α-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol and exhibit similar physical and chemical properties. Biologically the drugs are similar with respect to absorption, distribution, excretion, and antimalarial activity (1), but quinidine is more effective than quinine as an antifibrillatory agent (2). Whether this difference in biological activity is due to different actions at the subcellular level has yet to be determined.

The authors investigated the action of the two drugs on the free-living protozoan *Tetrahymena pyriformis*. *Tetrahymena* is ideal for the study of drug action because: (a) this organism is easily grown in culture medium; (b) the cells can be synchronized which allows for the study of the effects of drugs on a population of cells that are all in the same growth phase; and (c) the direct action of drugs on DNA, RNA, protein, and lipid synthesis can be studied while the cells are in their normal growth environment.

EXPERIMENTAL

Cultures of *T. pyriformis*, strain GL, were grown, synchronized by heat treatment (3), harvested, and washed in Ringer's buffer as described previously (4). The following procedures were used to determine the effect of quinine and quinidine on synchronized *Tetrahymena* in Ringer's buffer.

Effect of Quinine and Quinidine on Synchronized Cell Division—At the end of the heat treatment (EHT), 5-ml. aliquots of washed cells were pipeted into 25-ml. conical flasks containing either quinine hydrochloride or quinidine sulfate and into a control flask without drug. The drug concentrations were 1.3, 2.5, and 3.8 × 10^{-4} *M*. The flasks were placed on a rotator at 28° for the duration of the experiment. Cell samples were taken at regular intervals and fixed in 0.7% formalin in Ringer's buffer; the number of cells per milliliter was determined by direct cell counts (4). The effectiveness of the drugs was determined by the observed degree of inhibition of the first (80 min. after EHT) and second (200 min. after EHT) synchronized divisions.

Effect of Quinine and Quinidine on DNA, RNA, Protein, and Lipid Syntheses after EHT—At EHT, 2-ml. aliquots of washed cells were pipeted into 25-ml. flasks containing measured amounts of quinine or quinidine plus 2.5 μc./ml. of ^{3}H-thymidine, 2.0 μc./ml. of ^{14}C-uracil, 0.25 μc./ml. of ^{14}C-amino acids, or 0.25 μc./ml. of ^{14}C-

Table I—Inhibition of Synchronized Cell Division by Quinine (Q) and Quinidine (QD)

		Concentration, moles/l. × 10^4		
		1.3	2.5	3.8
		Mean % Inhibition ± SE^a		
First	Q	15 ± 2.3	40 ± 0.6	108 ± 2.3^b
division	QD	19 ± 1.5	40 ± 2.2	101 ± 4.1^b
Second	Q	50 ± 3.2	95 ± 3.6	102 ± 3.1^b
division	QD	50 ± 3.9	102 ± 1.5^b	110 ± 4.3^b

^{a} Each value from three determinations. In no instance is p less than 0.2 for any pair of values. ^{b} Values greater than 100% inhibition are due to cell death and lysis in the treated samples.

acetate. The drugs were used at 1.3, 2.5, and 3.8 × 10^{-4} *M*, and flasks containing just the radioactive precursor served as controls. The flasks were incubated on a rotator at 28°, and incorporation of radioactive precursors was followed for 80 min. by the filter paper disk procedures of Byfield and Scherbaum for DNA, RNA, and protein (5) or Byfield *et al.* for lipids (6).

RESULTS AND DISCUSSION

Table I shows the effect of quinine and quinidine on the first and second synchronized cell divisions. The actions of the drugs at all levels tested were quantitatively the same, both drugs showing partial inhibition at 1.3 and 2.5 × 10^{-4} *M* and complete inhibition at 3.8 × 10^{-4} *M*.

To determine if arrest of cell division was due to inhibition of DNA, RNA, or protein biosyntheses, the effect of the drugs on incorporation of radioactive precursors (thymidine, uracil, and amino acids, respectively) into the macromolecules was followed. The results (Table II) show that both quinine and quinidine were effective inhibitors of all three pathways and that equal concentrations of the two drugs produced approximately the same degree of inhibition. These results indicate that both drugs have similar actions on these biosynthetic pathways and that the inhibition of cell division is not due to inhibition of one specific cellular function.

One possible explanation for inhibition of both nucleic acid and protein syntheses is that the drugs block energy metabolism in *Tetrahymena*. To investigate this possibility, the authors studied the incorporation of acetate into the acid-insoluble lipid fraction. This test has been used by others to determine the state of the energy-generating systems in *Tetrahymena* (7, 8). The results (Table II) show that both drugs were effective inhibitors of lipid synthesis, and the degree of inhibition was the same for equal concentrations of the two drugs. As stated previously for quinine (4), these results indicate that the primary action of the drugs in *Tetrahymena* may be inhibition of energy production with the action on DNA, RNA, and protein syntheses being secondary. In support of this, quinidine is known to inhibit oxygen uptake (9, 10) and the oxidation of free fatty acids (11) and carbohydrates (11, 12) in cardiac muscle, and quinine is known to inhibit pyruvate oxidation through the citric acid cycle (13), isolated respiratory enzymes (14), and oxygen uptake (15) of *Plasmodium*.

Tetrahymena has proved to be a very useful tool for studying the mechanism of action of certain drugs (4, 7, 16, 17). The results presented using this system demonstrate that, for each parameter investigated, there was no significant difference in the action of quinine or quinidine (p always greater than 0.2). These data suggest that the intracellular action of the two drugs in *Tetrahymena* is the

Table II—Inhibition of DNA, RNA, Protein, and Lipid Synthesis by Quinine (Q) and Quinidine (QD)

| | | Concentration, moles/l. $\times 10^4$ | | |
		1.3	2.5	3.8
		Mean % Inhibition $\pm$ SE^a		
DNA	Q	34 ± 3.5	69 ± 6.1	79 ± 2.6
synthesis	QD	32 ± 5.7	75 ± 2.1	78 ± 3.2
RNA	Q	33 ± 0.5	55 ± 2.4	62 ± 1.9
synthesis	QD	36 ± 2.9	56 ± 2.1	61 ± 1.9
Protein	Q	51 ± 3.9	60 ± 0.2	73 ± 1.4
synthesis	QD	50 ± 3.4	63 ± 3.8	71 ± 1.1
Lipid	Q	8 ± 1.8	14 ± 3.3	27 ± 4.1
synthesis	QD	7 ± 2.3	15 ± 1.8	35 ± 3.7

^{a} Each value from four determinations. In no instance is p less than 0.2 for any pair of values.

same. The difference in antifibrillatory action of the drugs may be due in part to differences in chemical structure which, in turn, may reflect cardiac receptor specificity.

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Physiologic Surface-Active Agents and Drug Absorption VII: Effect of Sodium Deoxycholate on Phenol Red Absorption in the Rat

STUART FELDMAN*, MICHAEL SALVINO, and MILO GIBALDI

Abstract ☐ The influence of sodium deoxycholate on the absorption of phenol red in the rat was studied using three techniques to assess absorption—*viz.*, urinary excretion data after oral administration to intact animals, loss of drug from *in situ* intestinal loops, and transfer of drug across the isolated everted intestine. Each of the methods provided evidence that the bile salt markedly enhances the absorption of phenol red by altering the permeability of the intestinal membranes. In the intact rat, these effects appear to be reversible.

Keyphrases ☐ Phenol red absorption, rat—sodium deoxycholate effect ☐ Sodium deoxycholate—effect on phenol red absorption, rat ☐ Colorimetric analysis—phenol red

The role of bile salts in the intestinal solubilization and absorption of fats and fat-soluble vitamins has been studied extensively (1–3). Recently, it has been reported that bile salts may also enhance the intestinal absorption of poorly lipid-soluble substances. Mayersohn *et al.* (4) reported a 1.5- to 1.8-fold increase in the urinary recovery of riboflavin in man when sodium deoxycholate was administered 0.5 hr. prior to the oral ingestion of the vitamin. The results suggest that the bile salt enhancement of riboflavin absorption may be due to changes in the permeability of the gastrointestinal membranes to the transport of the vitamin. Feldman and Gibaldi (5, 6) have shown that relatively low micellar concentrations of sodium taurodeoxycholate markedly increase the permeability of everted rat intestine to salicylate ion.

The purpose of the present investigation was to determine the effects of an unconjugated bile salt, sodium deoxycholate (SDC), on the absorption of a water-soluble, poorly absorbed compound, phenol red, in the rat using several experimental techniques to assess absorption.

EXPERIMENTAL

Absorption Studies in Intact Rats—Male Sprague-Dawley rats weighing between 200 and 300 g. were used in all experiments. The animals were fasted 24 hr. (with water allowed *ad libitum*) prior to gastric intubation of 1.5 ml. of a 1-mg./ml. phenol red solution in distilled water containing 100 mM sodium deoxycholate (SDC).