

THE NATURE OF THE ACTIVE IONIC SPECIES IN THE INHIBITORY EFFECT OF CHLOROQUINE AGAINST *ESCHERICHIA COLI*

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Chloroquine can inhibit the growth of *E.coli* and this activity is sensitive to change in pH. (Wiseman 1972). This communication reports further on the effect of pH and suggests that inhibition is due to the combined effects of the mono and di-protonated forms of chloroquine rather than to one ionic species alone as previously suggested.

The organism was *E.coli* NCTC 1093 and the medium that of Rye and Wiseman (1966) with the Tris buffer replaced by 0.1 M 3-[N-Morpholino]-propanesulphonic acid (MOPS). Growth was followed by absorbance measurements and inhibition calculated from the reduced growth rate during the secondary inhibited growth phase 120 min after the addition of chloroquine (Wiseman 1972). pH values studied were 6.4, 6.7, 7.0, 7.4 and 7.7. Partially inhibited growth curves at pH 7.7 are shown in Fig 1; results at the other pH values were similar except that higher concentrations of chloroquine were required for the same effects as the pH was decreased. Graphs of percentage inhibition against chloroquine concentration are shown in Fig 2a for all the pH values.

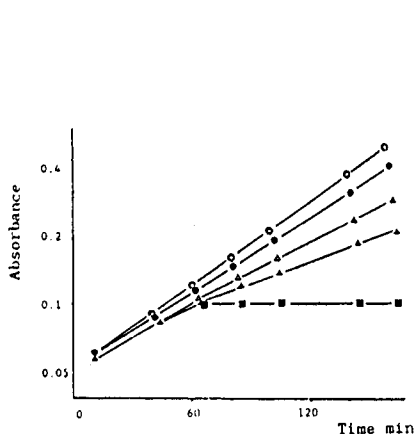


Fig 1. Changes with time in the absorbance of chloroquine treated cultures of *E.coli*.
○ 0.9 ● 0.7 △ 0.5 ▲ 0.3 ■ 0.1 mM chloroquine

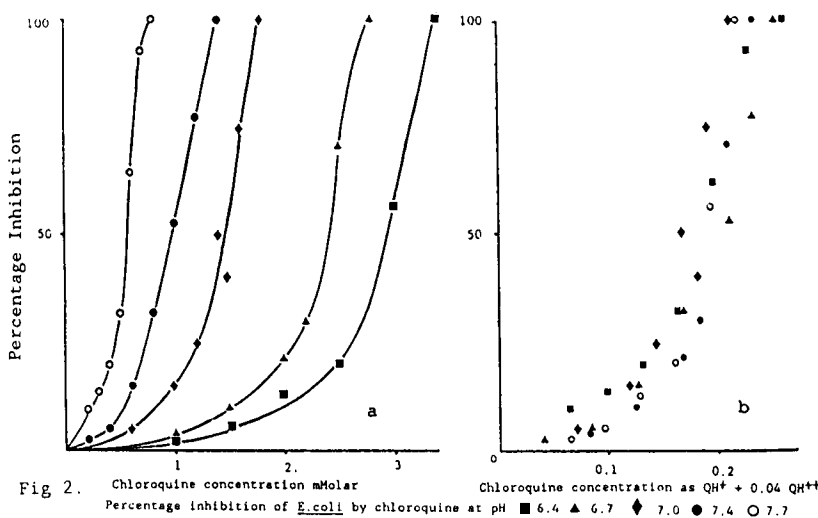


Fig 2. Chloroquine concentration mMolar
Percentage inhibition of *E.coli* by chloroquine at pH ■ 6.4 ▲ 6.7 ◆ 7.0 ● 7.4 ○ 7.7
Chloroquine concentration as $QH^+ + 0.04 QH^{++}$

Using pKa values of 8.08 and 10.16 the proportions of chloroquine present as the diprotonated and monoprotionated ions and free base were calculated for the different pH values and inhibition curves redrawn against concentrations of each ionic species or free base instead of total chloroquine. In no case were the curves for the different pH values coincident suggesting that inhibition was not due to one single species. These curves suggested no significant contribution from the low amounts of free base present and proportional inhibitory activities for the two ionised forms were calculated using simultaneous equations constructed from equi-effective concentrations at pairs of pH values. Allocating a value of 1.0 to the activity of the monoprotionated ion these calculations gave a mean value of 0.04 for the activity of the diprotonated form. Inhibition curves replotted against concentrations of the two ionic species, activity weighted using these values, are shown in Fig 2b. These results give coincident curves for all the pH values and suggest, within the pH range 6.4 to 7.7, that the inhibitory activity of chloroquine against *E.coli* can be represented as the combined effect of the mono and diprotonated forms of the compound with the monoprotionated form being about 25 times the more active.

Rye, R.M. and Wiseman, D. (1966) *J. Pharm. Pharmac.* 24: 114-118S

Wiseman, D. (1972) *Ibid.* 24: 162P