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# Methylene Blue as an Inhibitor of Stone Formation

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Summary. Kinetics of growth and dissolution of calcium oxalate monohydrate were examined in the presence of small concentrations of methylene blue. The data presented show moderate retardation in growth and dissolution rates. It was also found that methylene blue decreased the decalcification rate of calcium oxalate renal calculi. The implications of these findings in the treatment of urolithiasis are discussed.

Key words: Calcium oxalate - Methylene blue - Growth - Dissolution - Inhibition.

Methylene blue has been suggested as an urolithiasis inhibitor, but the usefulness of this dye has remained a controversial issue. Boyce (2) reported that methylene blue is effective in the treatment of new stone formation in patients with renal calculi. On the other hand Wein (13) found that with oral administration of methylene blue to 27 patients with non-obstructive, radiopaque renal calculi, there were as many patients who had an increase in stone size or new stone formation as there were patients who had a decrease in stone size or stone passage. Vant Riet (12) observed that methylene blue produced marked dissolution and inhibition of calculi in rats with zinc pellet-induced vesical concretions. Other studies in rats by Chow (3) indicated that when methylene blue was added to a calculus inducing diet it produced a significant increase in calculus formation.

In vitro experiments conducted to study the effect of methylene blue on growth, aggregation and encrustation of calcium oxalate lead to different conclusions (9, 10, 11). The wide variation in experimental conditions and the lack of a well defined quantitative method to assess the physico-chemical relationship between methylene blue and stone components has led to various speculations (2, 10). The purpose of the investigation described in this paper was to:

1) Quantify the effect of methylene blue on growth and dissolution rates of calcium oxalate crystals, as well as its effect on the dissolution of renal stones.

2) Study the possible physico-chemical mechanism of the action of methylene blue and the situations where methylene blue could be of therapeutic value.

## MATERIALS AND METHODS

### Growth Rates Studies

The growth of calcium oxalate monohydrate by chemical reaction between oxalate and calcium ions was studied in 200 ml buffered normal saline containing various concentrations of methylene blue. The growth rates were determined using the particle counter[a] technique described previously (6).

In each growth experiment 5 ml of the oxalic acid solution (containing 17.9 mg of

[a] Coulter counter, Model B equipped with model M volume converter.

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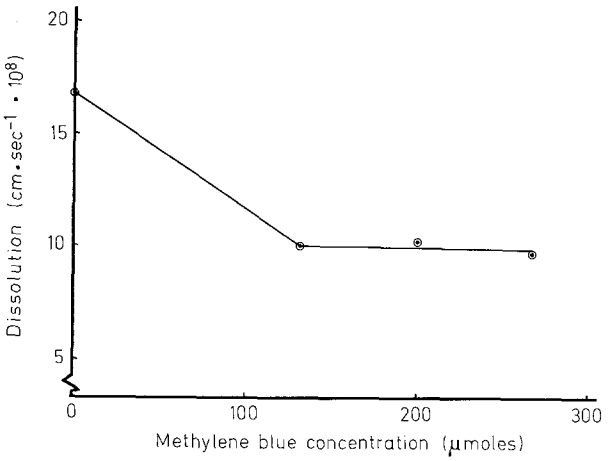


Fig. 1. Effect of methylene blue on the growth rate of calcium oxalate monohydrate crystals

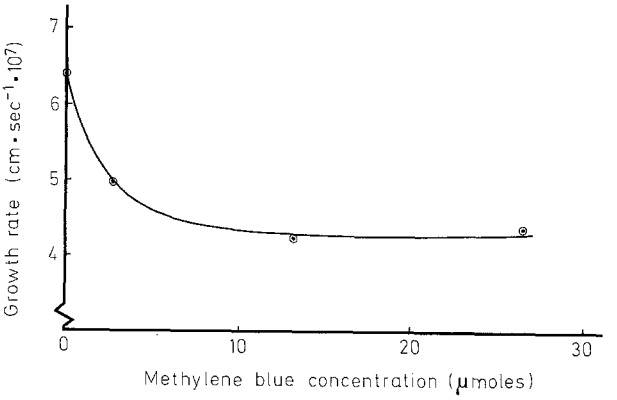


Fig. 2. Effect of methylene blue on the dissolution rate of calcium oxalate monohydrate crystals

oxalic acid dihydrate) was added to 100 ml normal saline[b]; the pH was adjusted to 6.2 and diluted to 195 ml with tromethamine buffer[c] solution (pH 6.2). Stirring at 300 rpm was maintained by a Fisher adjustable stirrer[d], and then 5 ml solution of calcium chloride dihydrate (containing 117.6 mg of calcium chloride dihydrate) was added to the agitated system.

A series of growth experiments was conducted in the presence of variable concentrations of methylene blue (1 ppm to 10 ppm). In all cases the methylene blue had been incorporated before the addition of calcium chloride solution.

Counts were taken at different intervals of time and at different threshold settings, during the growth period. The growth rate was determined in cm/sec from the change in particle size distribution curves (6).

## Dissolution Rates Studies

A suspension of calcium oxalate monohydrate crystals was added to sufficient quantity of saline solution buffered at pH 6.2 to make 200 ml. Dissolution rates in cm/sec were determined using the particle counter technique previously reported from our laboratory (1, 6). Similar experiments were conducted in the presence of variable concentrations of methylene blue (1 ppm to 100 ppm).

## Acid Decalcification of Renal Stones in the Presence of Methylene Blue

In order to study the effect of methylene blue on the dissolution rates of renal stones, we followed a technique previously reported by Linge and others (7, 8) for the determination of demineralisation rates of dental enamel.

An intact calcium oxalate stone (1.3 g) was placed at the bottom of a thermostatically controlled dissolution cell containing 200 ml of distilled water adjusted to pH 4 or 4.5. As a measure of mass transport during the dissolution process, the proton uptake was determined as a function of time using a pH-stat combi-titrator[e]. Titration was conducted with 0.0005 N nitric acid. After establishing the rate of decalcification, subsequent experiments were carried out in the presence of different concentrations of methylene blue (1 ppm to 15 ppm).

## RESULTS

The influence of methylene blue on the growth rate of calcium oxalate monohydrate crystals at pH 6.2. showed that small concentrations of the dye exerted a marked inhibition, which tended to stabilise at a concentration of 15μ moles (Fig. 1).

Figure 2 demonstrates the effect of methylene blue on the dissolution rate of calcium oxalate monohydrate crystals at the same pH. Here it is evident that methylene blue inhibited the dissolution rate and the in-

[b] Sodium chloride injection USP, Abbott Laboratories, Montreal, Canada.

[c] THAM, Fisher Scientific Co., Montreal Canada.

[d] Fisher stedi-speed, Adjustable stirrer, Model 12, Fisher Scientific Co., Montreal, Canada.

[e] Metrohm, Switzerland.

hibition process tended to stabilise at a concentration of 150μ moles.

Methylene blue exerted a marked inhibitory effect on both growth and dissolution. However there was a significant difference in methylene blue concentration in the two processes. In the dissolution inhibition, the concentration of methylene blue required to stabilise the effect was 10 times the concentration required for growth inhibition.

Figure 3 shows the effect of methylene blue concentration on the dissolution of the calcium oxalate stone at pH 4 and pH 4.5. In this case dissolution was measured in terms of mole proton uptake per minute. The results obtained indicate a similar inhibitory effect on the acid demineralisation rate of the renal stone. The inhibition was influenced by the pH of the dissolution medium. The concentration of methylene blue required to produce maximum dissolution inhibition of the calcium oxalate stone appeared to be much lower than that required for the inhibition of dissolution of calcium oxalate crystals. The limited surface area offered by the stone compared with the surface area of the crystals could explain these differences.

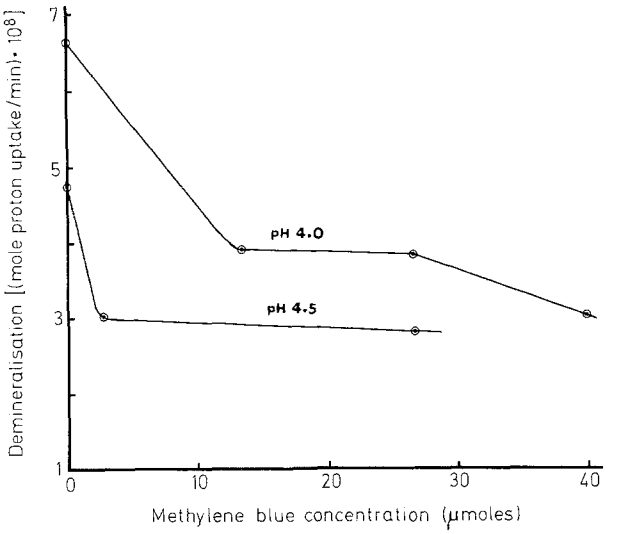


Fig. 3. Effect of methylene blue on the demineralisation rate of calcium oxalate stone

Table 1. Values of the constants K and b for the growth and dissolution of calcium oxalate monohydrate crystals in the presence of methylene blue

| | K | b |
|---|---|---|
| Growth | 0.3337 | 17.5451 |
| Dissolution | 0.4293 | 0.8627 |

## DISCUSSION

The effect of methylene blue on the growth and dissolution rates can be examined in the light of the Langmuir adsorption isotherm. If it is assumed that (a) the fractional rate reduction caused by methylene blue is proportional to the fraction of the surface covered by this inhibitor, and (b) the adsorption of the substance can be represented by the simple Langmuir adsorption isotherm, then the growth rate or the dissolution rate of calcium oxalate can be expressed as a simple function of the inhibitor concentration in the solution as:

$$R = R_o \left[1 - \left(\frac{KbC}{1 + bC}\right)\right] \qquad \text{Eq. 1}$$

Where R = growth/dissolution rate of calcium oxalate monohydrate with the inhibitor, $R_o$ = growth/dissolution rate of calcium oxalate monohydrate without inhibitor, C = concentration of the inhibitor in the bulk solution, K = fraction of the surface covered when the surface is saturated with the inhibitor, and b = Langmuir adsorption constant reflecting the affinity of the substance for the binding sites on the crystal surface.

Equation 1 was rearranged and $C/(1 - R/R_o)$ was plotted against C (Figs. 4 and 5).

From these data, a correlation with the Langmuir adsorption isotherm can be established. The values of the constants K and b were determined from the slopes and intercepts respectively (Table 1).

The K values, which are a measure of the saturation capacity, are similar but relatively low for both growth and dissolution (K is equal to unity when the surface is completely covered). For the growth studies, the value of b is 20 times higher than for the dissolution studies, which indicates an increased affinity of methylene blue for the binding sites of the crystal surface during the process of crystal growth. This difference is reflected in the results obtained in that maximum growth inhibition was obtained at a lower concentration than that required for dissolution inhibition (Figs. 1 and 2).

Although methylene blue exerts a marked inhibitory effect on the growth and dissolution of calcium oxalate monohydrate, the inhibition is much less pronounced than that ob-

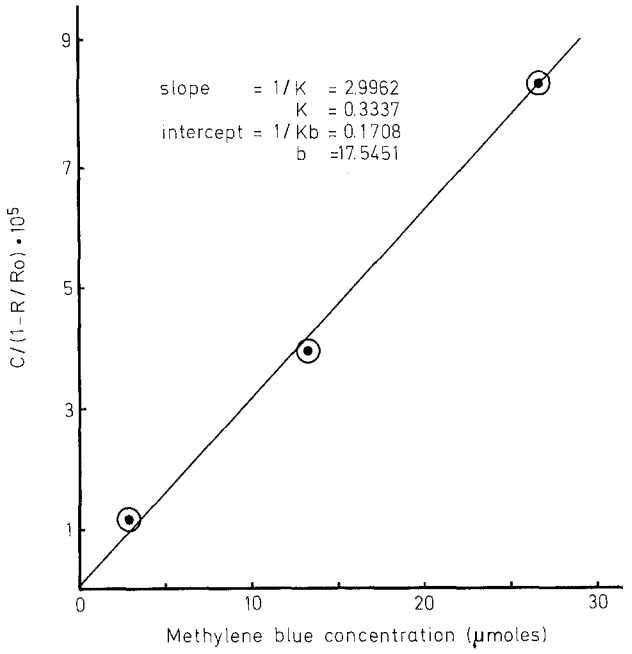


Fig. 4. Determination of adsorption constant for the growth of calcium oxalate monohydrate crystals

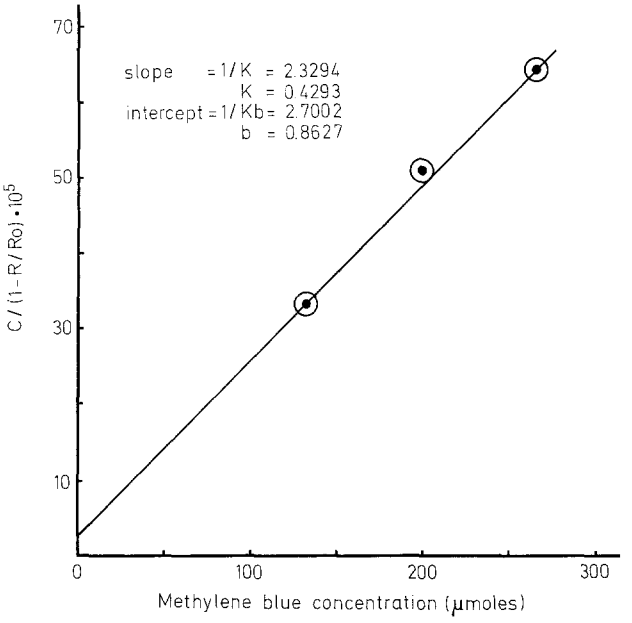


Fig. 5. Determination of adsorption constant for the dissolution of calcium oxalate monohydrate crystals

Table 2. Values of the constants K and b for the growth and dissolution of calcium oxalate monohydrate crystals in the presence of chlorophyllin (5)

| | K | b |
|---|---|---|
| Growth | 0.750 | 88.900 |
| Dissolution | 0.7029 | 1.3357 |

tained with chlorophyllin[f] (1, 4). Chlorophyllin showed a powerful inhibitory action under the same experimental conditions in both growth and dissolution. Table 2 shows the K and b values for the growth and dissolution of calcium oxalate monohydrate in the presence of chlorophyllin (5).
The K and b values, which represent surface coverage and bond strength respectively are significantly higher.

It is interesting to note that methylene blue decreased the dissolution of the calcium oxalate renal stone (Fig. 3) and the activity is in accordance with the results obtained with the calcium oxalate crystals.

These observations agree with the mechanism operating in crystal poisoning, where very small concentrations of impurities are capable of inhibiting these processes by adsorption on the primary sites of growth and dissolution of the crystal surface.

These findings raise serious doubts concerning the effectiveness of methylene blue in the treatment of urolithiasis. The dissolution inhibition results obtained with both calcium oxalate crystals and calcium oxalate renal stone suggest that the oral administration of methylene blue will either block the dissolution or enhance the growth of an existing stone in stone formers. This substantiates the results of Wein (13) who reported an increase of stone size and stone formation in some of the patients treated with methylene blue. On the basis of the growth inhibition observations, one cannot exclude completely a beneficial effect of methylene blue in preventing stone formation. However the therapeutic effectiveness of methylene blue should be carefully studied in controlled clinical trials.

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[f] Sodium-copper chlorophyllin complex, E. Merck AG., Darmstadt, Germany.

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