IV. GROWTH EXPERIMENTS

CHAPTER-IV

GROWTH EXPERIMENTS

Introduction:

A number of 2,3-disubstituted quinazolinone derivatives have been reported to exert bacteriostatic action. Baker et al (1958) have studied the effect of a quinazoline derivative, 2-amino, 4-hydroxy, 5,6,7,8-tetrahydroquinazoline on Streptococcus faecalis and found it to be a powerful inhibitor of growth. It inhibited the growth of S.faecalis by 50% at a concentration of 50 mμg/ml on a Flynn-folic acid medium containing 3 mμg of folic acid.

Recently, manyb other quinazoline derivatives have been synthesized and their action, as antifolate agents, studied by several group of workers (Elslager et al, 1972; Elslager and Davoll, 1974).

Because of the structural similarity of methaqualone or SRC-820 with pteridine moiety of folic acid, it was thought interesting to find out whether these two quinazolinone derivatives (SRC-820 and Methaqualone) also inhibit growth of folic acid requiring organism.

In the present study, a folic acid requiring strain of lactic acid bacterium, Streptococcus faecalis-R (ATCC-8043) [Now renamed as Streptococcus faecium var

durans (ATCC-8043), Deibel et al (1963); Diebel, (1964)] was selected and the effects of these two derivatives namely methaqualone and SRC-820 were seen on the growth of this organism.

Results:

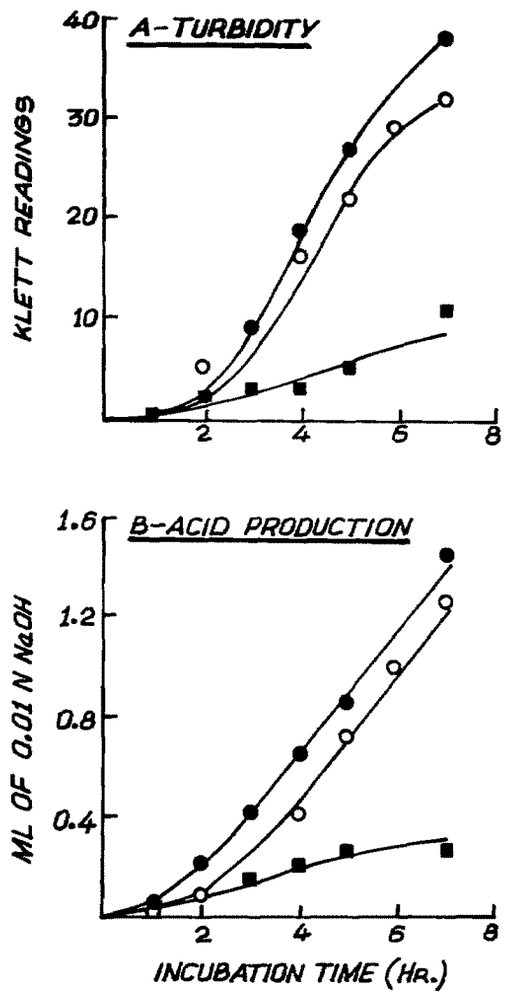
Unlike Baker's quinazoline derivative, SRC-820 when tried at the lower level of 4 X 10^{-5}M to 1.6 X 10^{-4}M, it had no inhibitory effect on the growth of S.faecalis-R. Attempts were also made to see the actions of SRC-820 at the higher levels (5 X 10^{-4}M) on the growth of this organism by means of agar-cup method. No inhibition zone in growth could be seen at this level of SRC-820. Thus, in the subsequent experiments, still higher concentrations of SRC-820 were tried.

Comparative effect of SRC-820 and methaqualone on the growth of S.faecalis.

Methaqualone and SRC-820 were found to inhibit the growth of S.faecalis-R at micromolar concentrations. SRC-820 at the concentration of 5 X 10^{-3}M, exerted inhibition by about 16% at 4 hour incubation period, whereas methaqualone showed an inhibition (in growth) by about 84% at the same level and at the same period of incubation time (Fig.3a).

Fig.3

Comparative effect of SRC-820 and methaqualone on the growth of S.faecalis.



●—● Control; ○—○ with SRC-820; 5 X 10^{-3} M

■—■ with methaqualone, 5 X 10^{-3} M

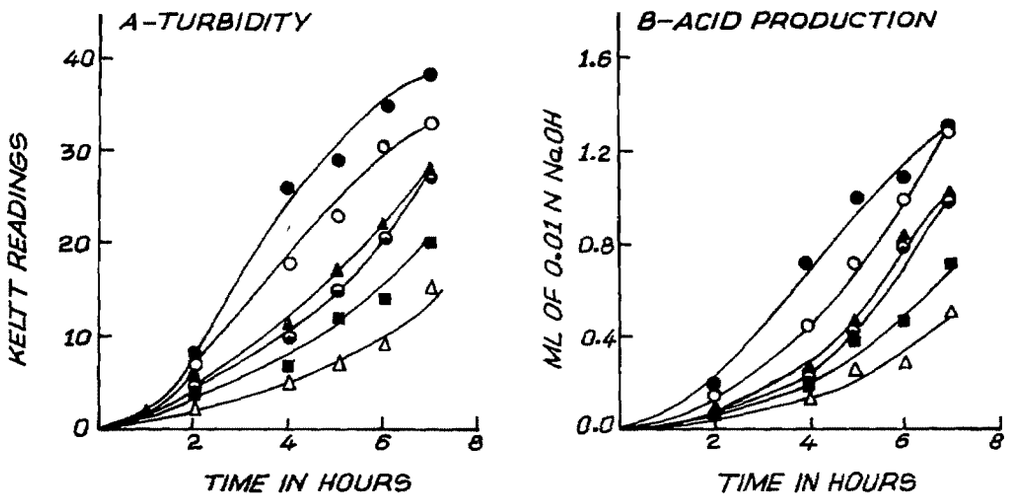
In the case of acid production, both SRC-820 and methaqualone at the levels of 5 X 10^{-3}M, showed 38% and 70% inhibition respectively at 4 hour incubation period (Fig.3b). Comparatively, methaqualone is a more potent growth inhibitor than SRC-820 and the same extent of inhibition could be achieved using 1/5 the concentration of methaqualone at 4 hour incubation period.

Effect of different concentrations of SRC-820 on the growth of S.faecalis:

Various concentrations of SRC-820 were studied from 1 X 10^{-3}M to 1.5 X 10^{-2}M on the growth of S.faecalis. With increase in concentration of SRC-820, the inhibition in growth was also found to increase (Fig.4a and 4b). The inhibition starts to establish after 2 hour of the incubation period. SRC-820 at the level of 1.5 X 10^{-2}M showed about 80% inhibition at 4 hour incubation on the basis of turbidity measurements (Fig.4a). Almost similar extent of inhibition could be seen (about 83%) at 4 hour incubation on the basis of acid production. However, as the incubation time increases, the percent inhibition was found to decrease. Very little inhibition could be seen at the concentrations of 1 X 10^{-3}M or 2 X 10^{-3}M of SRC-820 (not shown in Fig.4a and 4b).

Fig.4

Effect of different concentrations of SRC-820 on the growth of S.faecalis.



●—● Control; O—O with 3 X 10^{-3}M;
▲—▲ 8 X 10^{-3}M; O—O 1 X 10^{-2}M; ■—■ 1.5 X 10^{-2}M and
△—△ 1.8 X 10^{-2}M of SRC-820

Effect of different concentrations of methaqualone on the growth of S.faecalis:

In a similar experiment using various concentrations of methaqualone from 1 X 10^{-3}M to 5 X 10^{-3}M, it was found that methaqualone inhibits the growth of S.faecalis even at a concentration of 1 µmole/ml (1 X 10^{-3}M). With the increase in the concentration of methaqualone, the inhibition in growth was also found to increase. In case of methaqualone, inhibition established after 2 hour period of incubation.

Effect of SRC-820 on the growth of S.faecalis in the presence of added liver extract:

In these experiments, injectable preparations of liver extract (Teddington Chemical Factory, Bombay, India) containing 0.1 ml, 0.2 ml and 0.4 ml of this preparation per each 50 ml of the broth and a single concentration of SRC-820 (1 X 10^{-2}M) were used.

One ml of the liver extract was calculated to have an equivalent of 7.5 mg of folic acid and 28 ug of vitamin B_{12}.

Liver extract alone was found to stimulate the growth of S.faecalis in control experiments. The highest stimulation observed was at 3 hour incubation where in about 58% stimulation in turbidity could be seen with

0.1 ml of liver extract. With increase in the time of incubation the stimulation was found to decrease (Fig.5a). With increase in the concentration of liver extract, no further appreciable stimulation in growth could be seen in these experiments.

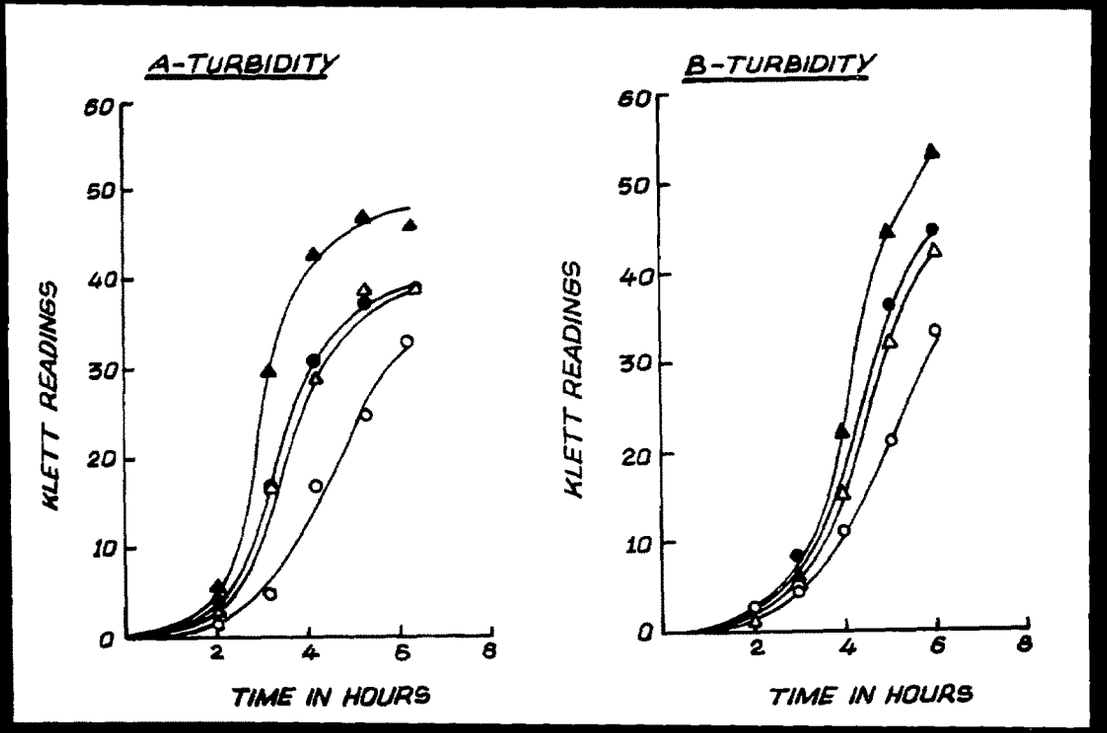
In the case of acid production, about 33% stimulation was observed with 0.1 ml of liver extract at 3 hour incubation period. Here also, as the incubation time increases, the stimulation was found to decrease (Fig.5b).

SRC-820 alone was found to inhibit the growth by about 70% at 3 hour incubation period at a concentration of 1 X 10^{-2}M as shown in Fig.5a. In the case of acid production also, 50% inhibition could be observed at 3 hour incubation period by the same amount of SRC-820 (Fig.5b). In both the cases, inhibition was found to decrease with increase in the time of incubation.

The observed inhibition by SRC-820 was found to be less in the presence of liver extract. Approximately 27% reduction in the inhibition was observed at 3 hour incubation when the liver extract was present (Fig.5a). No appreciable reversal in inhibition could be seen by this amount of liver extract, when the growth was observed by acid production (Fig.5b). On increasing the concentration of liver extract (0.2 and 0.4 ml), however, appreciable reduction in acid production was observed.

Fig.5

Effect of SRC-820 and liver extract on the growth of S.faecalis.



●—● Control; ○—○ with SRC-820, 1 X 10^{-2} M;
▲—▲ With liver extract; △—△ with SRC-820, 1 X 10^{-2} M + liver extract.

Effect of SRC-820 on the growth of S.faecalis in presence of added Cysteine:

In these experiments, lesser amounts of SRC-820 (5×10^{-3} M) was used to see the action of L-Cysteine on the inhibition produced by SRC-820.

L-Cysteine alone was found to stimulate the growth of S.faecalis in control experiments. The stimulation in growth become apparent only after the 4 hour of incubation period. About 20% stimulation in growth could be observed by L-Cysteine at a concentration of 3×10^{-3} M at 5 hour incubation period. About the same extent of (25%) stimulation was observed in the case of acid production. In both the cases, the observed stimulation was found to persist on increasing the time of incubation.

SRC-820 was found to inhibit the growth by about 42% at a concentration of 5×10^{-3} M at 5 hour incubation period and about 20% inhibition by acid production.

In the presence of L-Cysteine, a slight reduction in inhibition could be seen at 5 hour incubation period. No further reduction in inhibition could be seen when higher concentration of L-Cysteine (5×10^{-3} M) were tried.

In a similar set of experiments, various concentration of L-tryptophan upto 4×10^{-2} M were tried to see

the effect on the growth as well as on growth inhibition by SRC-820. It was observed that L-tryptophan had no effect on the growth of S. faecalis.

SUMMARY

1. SRC-820 and methaqualone (MQ) inhibit the growth of S. faecalis only at micromolar concentration. At microgram level they are ineffective. Methaqualone is more potent in this respect than SRC-820. SRC-820 and methaqualone (both at 5×10^{-3} M) inhibit the growth by about 16% and 84% respectively at the early stages of growth (4 hour). Inhibition in acid production at the same time period was 38% and 70% respectively.

2. Increasing the concentration of SRC-820 (1×10^{-3} M to 1.5×10^{-2} M) results in increase in inhibition in both growth and acid production. The inhibition could be demonstrated only after 2 hours of incubation. The highest inhibition (about 80%) is seen with SRC-820 at a concentration of 1.5×10^{-2} M and at 4 hour of incubation. After this time, inhibition decreases with increase in incubation time. Little inhibition is seen with 2×10^{-3} M of SRC-820 or less.

3. Increasing the concentration of methaqualone (1×10^{-3} M to 5×10^{-3} M) also shows an increase in inhibition of growth and acid production.

Methaqualone inhibits the growth of S.faecalis even at 1 μM/ml medium (37% inhibition). Here too, the inhibition could be demonstrated only after 2 hours of incubation.

4. Addition of liver extract (0.1 ml/50 ml of broth) to the medium results in stimulation in the growth of S.faecalis by about 58% and in acid production by about 33%.

5. The inhibition (70%) due to SRC-820 (1×10^{-2} M) can be reduced by addition of liver extract. No appreciable reduction in inhibition was seen in acid production.

6. L-Cysteine (3×10^{-3} M) stimulates the growth and acid production of S.faecalis by about 20 to 25%. In this case, the stimulation in growth becomes apparent only after 4 hours of incubation. The observed stimulation persists even with the increase in time of incubation.
The inhibition due to SRC-820 (5×10^{-3} M) gets reduced only by about 10% in presence of L-cysteine (3×10^{-3} M). No further reduction in inhibition could be seen with higher concentration of L-cysteine (5×10^{-3} M).

7. L-tryptophan upto 4×10^{-2} M has no effect on the growth as well as on the inhibition exerted by SRC-820.