# INHIBITION OF RNA PHAGE GROWTH BY PHENETYL ALCOHOL

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Whereas several papers have been published in concern to the inhibitory effect of phenetyl alcohol (PA) on DNA synthesis (Berrah and Konetzka, 1962 ; Konetzka and Berrah, 1962), possibility that PA might act in otherwise has not been ruled out (Slepecky,1963). This communication concerns the inhibitory effect of PA on the growth of RNA phages. The phages used are β (Nonoyama, Yuki, and Ikeda, 1963) and MS-2 (kindly supplied by Dr. A. J. Clark).

PA effect on phage growth

Cells of E. coli K-12 strain W2252 (Hfr met$^-$ $\lambda^-$) were infected, in the presence of $10^{-2}$M $NaN_3$, with phage β or MS-2 at m.o.i. = 0.5. Unadsorbed phages were removed by treatment with the antiphage serum, and infected cells were kept at 37° for phage growth. To help the phage liberation, the cells were treated with lysozyme and chloroform when 60 min had passed. Gray and Tatum's medium supplemented with 0.05 % casamino acid was used.

As seen in Table 1, where PA was added to the system immediately after the antiserum treatment, the phage growth was inhibited strongly by the presence of 0.3 % PA. The concentration corresponds to that at which the growth

Table 1 PA effect on phage growth.

| Phenethylalcohol Vol. % | Relative Burst Size | |
|---|---|---|
| | β | MS-2 |
| 0 | 100 | 100 |
| 0.1 | 16.2 | 56 |
| 0.3 | 0.04 | 0.28 |
| 0.6 | 0.06 | 0.18 |
| 1.0 | 0.04 | 0.25 |

of a DNA phage was inhibited by PA (Konetzka and Berrah, 1962).

The PA effect on the RNA phage growth is not destructive but inhibitory. As a matter of fact, complete phages begin to appear after a lapse of 20 to 25 min when PA is removed from the system at 20 min after the infection (Fig.1). It must be commented, however, that the PA effect is irreversible when PA and phages are added together to the growing cells.

PA inhibits the further growth of phage when it is added during the later course of phage growth (Fig.2).

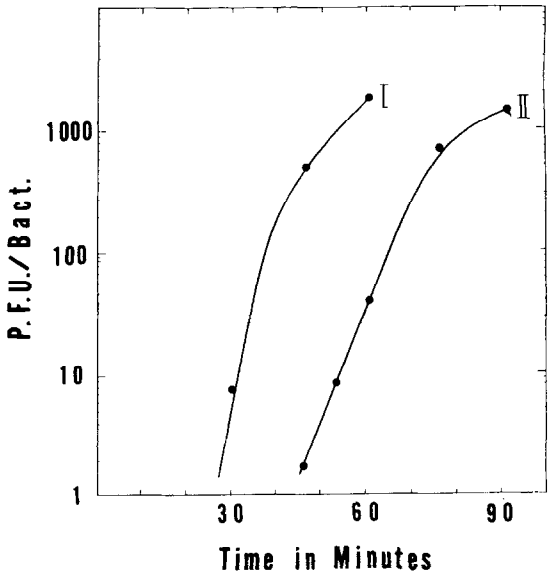


Fig. 1 Initiation of phage growth by by removal of PA.
I : without PA
II : PA was removed at 20 min.

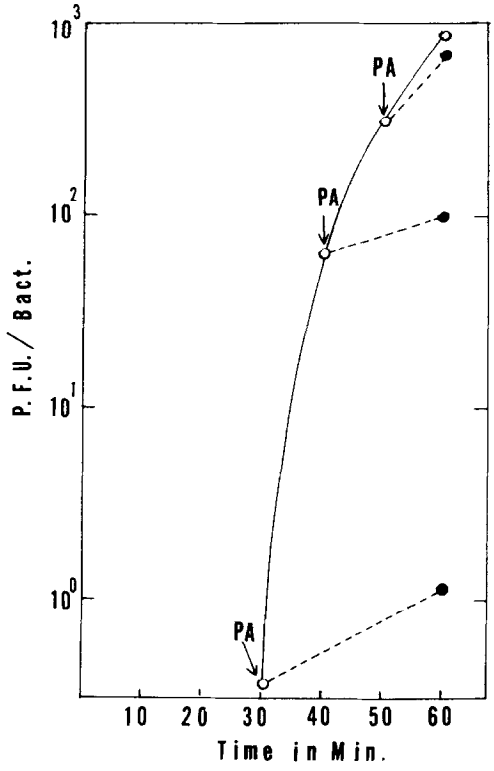


Fig. 2 Inhibition of phage growth at later period.

PA effect on viral RNA synthesis

To know whether PA could inhibit the synthesis of viral RNA or not, an experiment was carried out. Cells were infected with phage β at m.o.i. = 20 in B.P. medium (1 % Bactopeptone, 0.8 % NaCl, and 0.1 % glucose), and PA (0.3 %) and $P^{32}$(carrier free, 20 μc/ml) were added at 15 and 20 min, respectively, after the infection. When 40 min had passed, the cells were collected to suspend them in Tris-buffer (pH 7.2) containing $10^{-2}$M $Mg^{++}$ and 500 μg/ml of lysozyme. The freeze and thawing treatment was done three times, and the cells were lysed with 0.3 % SDS. A mixture of DNA and RNA was prepared from the lysate by the phenol method and precipitated with alcohol. The sample

thus prepared was charged on methylated albumin column (MAK) and eluted with NaCl solution ( a gradient from 0.67 to 1.15 was employed). In parallel, a sample was prepared from cells which had been infected without the addition of PA.

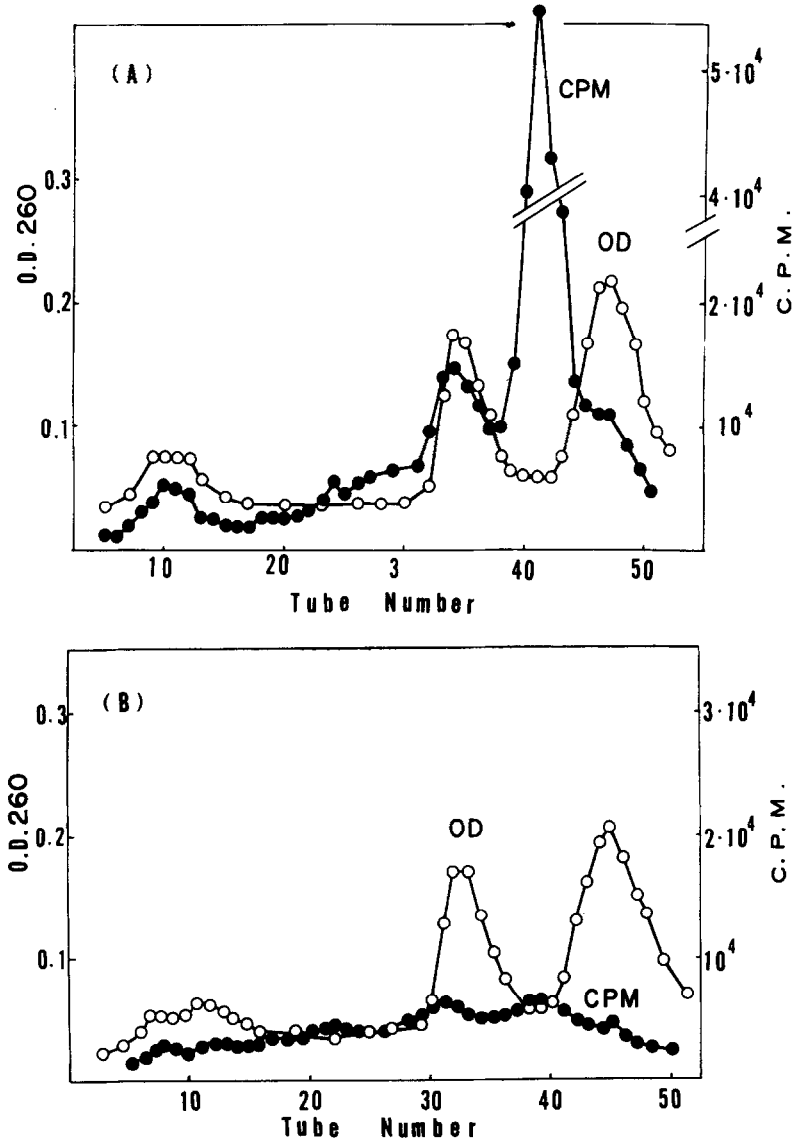


Fig. 3 Profile of viral RNA on MAK column.
A : PA untreated cells
B : PA treated cells

In Fig. 3, the open circles indicate the optical density at 260 mμ, and the peaks at tube nos. 10, 34, and 47 stand for those of DNA, 16S RNA, and 23S RNA respectively. The solid circles indicate the radioactivity. The highest peak in Fig. 3A was identified as that of viral RNA by the mixed chromatography with a standard sample. The peak at tube no. 24 seems to be a secondary product of viral RNA because a viral RNA sample which had been stocked in 0.8 M NaCl at 4° for one week gave the same peak. The nature of the RNA peak found at tube no. 33 is under investigation. As seen in Fig. 3B, these radioactive RNAs are produced in quite low amounts in the presence of 0.3 % PA.

It may be concluded on these basis that PA is inhibitory not only for the synthesis of viral DNA but also for the synthesis of viral RNA. Discussion will be made elsewhere together with additional data.

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## REFERENCES

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