experiments indicate that the dissociation of the enzyme lipid complex increases the activity of the enzyme permitting a more effective inhibition by the barbiturate.

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REFERENCES

1. G. G. VILLELA, O. R. AFFONSO and E. MITIDIERI, *Biochem. Pharmac.* **15**, 1894 (1966).
2. R. R. MORTON, *Nature, Lond.* **166**, 1092 (1950).
3. E. MITIDIERI, L. P. RIBEIRO, O. R. AFFONSO and G. G. VILLELA, *Biochem. biophys. Acta* **17**, 587 (1955).
4. E. MITIDIERI and O. R. AFFONSO, *Nature, Lond.* **206**, 4988 (1965).
5. G. G. VILLELA and E. MITIDIERI, *Nature, Lond.* **175**, 208 (1955).
6. O. R. AFFONSO, E. MITIDIERI, L. P. RIBEIRO and G. G. VILLELA, *Proc. Soc. exp. Biol. Med.* **90**, 527 (1955).
7. G. G. VILLELA, *Rev. Brasil. Biol.* **14**, 455 (1954).

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Induction of lambda phage by hydroxyurea

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U.V. IRRADIATION and inhibitors of DNA synthesis are able to induce phage production in lysogenic strains of bacteria. In our laboratory we are studying the induction of lysogenic *Escherichia coli* λ-28 strain by various substances under different culture conditions. According to our previous results[1] prophage detachment in a certain proportion of the population took place immediately after the addition of mitomycin C (MC), even at 2°. This step is followed by a relatively slow increase in the number of infective centers.

There are many reports that hydroxyurea (HU) is an antitumour substance[2] and that it has a specific effect on DNA synthesis[3,4] and phage synthesis.[5] In this report the effect of this substance on *E. coli* λ-28 are presented. A 16-hr-old broth culture was inoculated in a 1:10 proportion to the same medium and after incubating for 2 hr at 37° the cells were washed twice with water and suspended at a concentration of 10^6 cells/ml in minimal medium of Davis and Mingioli[6] plus 0·25% casein hydrolysate* (complete medium). In the case of shift down state, we transferred the inoculum grown in broth to the above mentioned minimal medium supplemented with 1 ml of 0·01 % casein hydrolysate/1l., that is, a state of transitory starving of the cells due to the depressed state of biosynthetic enzymes was performed. Incubation of cultures was generally made at 37°. The number of infective centers was determined by the plaque count on the indicator strain C_{600}. For measuring the number of complete phages the cells were treated with chloroform at 2° for 5 min. Incubation of the plates was carried out at 28° in the case of the count of infective centers and at 37° when determining the number of complete phages.

In complete medium 0·005 M HU induced phage production and higher concentrations (above 0·05 M) inhibited complete phage production although the count of infective centers was increased.

* Bacto casein hydrolysate (Difco).

Fig. 1 shows the time course of phage production by HU in complete medium at a concentration (0·1 M) inhibiting phage production. Contrasted with the induction brought about by MC, we observed that a substantial increase in the count of infective centers became apparent 15 min after the addition of HU. This increase is partially suppressed upon addition of 20 μg/ml chloramphenicol (CM).

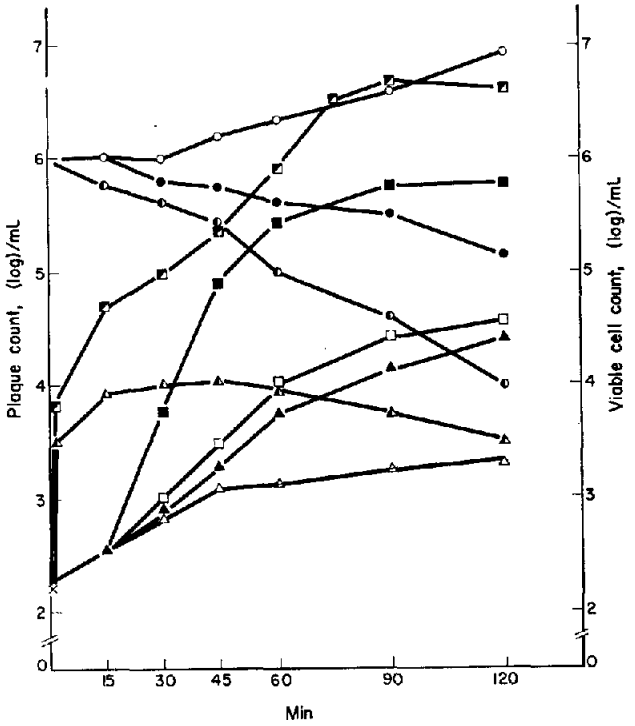

FIG. 1. Inductive effect of hydroxyurea in minimal medium supplemented with casein hydrolysate. Symbols: count of infective centers in control culture (□——□), in the presence of 0·1 M HU (■——■), 1 μg/ml MC (◩——◩), 20 μg/ml CM (△——△), 20 μg/ml CM and 0·1 M HU (▲——▲), or 20 μg/ml CM and 1 μg/ml MC (◭——◭). Viable cell count in control culture (○——○), in the presence of 0·1 M HU (●——●), or 1 μg/ml MC (◐——◐).

When induction was initiated by 0·025 M HU, the time necessary for phage production was about 60 min, while in the case of MC induction it was shorter, approximately 45 min.

The number of infective centers increased also in the shift down state by the addition of HU, but was less inhibited by CM than in complete medium (Fig. 2). HU had only a slight influence on the first, rapid phase of MC induction; however, it delayed the further increase in the number of infective centers. The maximum level reached by the use of both agents was lower than after induction with MC or HU alone.

In order to verify that the increase of the number of infective centers in the presence of HU and CM was due to prophage detachment, acriflavine was added. Acriflavine, at a concentration inhibiting the induction, added during the induced state results in a gradual decrease of the count of the infective centers, as had been found with mitomycin.[1]

The experiments described here confirm that phage induction by HU and MC are essentially different. Though both substances are apparently effective in phage induction by inhibiting DNA synthesis, the mode of action on prophage detachment differs. It seems probable that with HU the prophage detachment is not dependent on protein synthesis inasmuch as CM had a slight effect on

induction. A possible interpretation of our data, based on the hypothesis of Goldthwait and Jacob,[7] is that the intermediates which accumulate due to decreased DNA metabolism are responsible for the depression of phage production, and that there is a prolonged lag phase associated with this process.

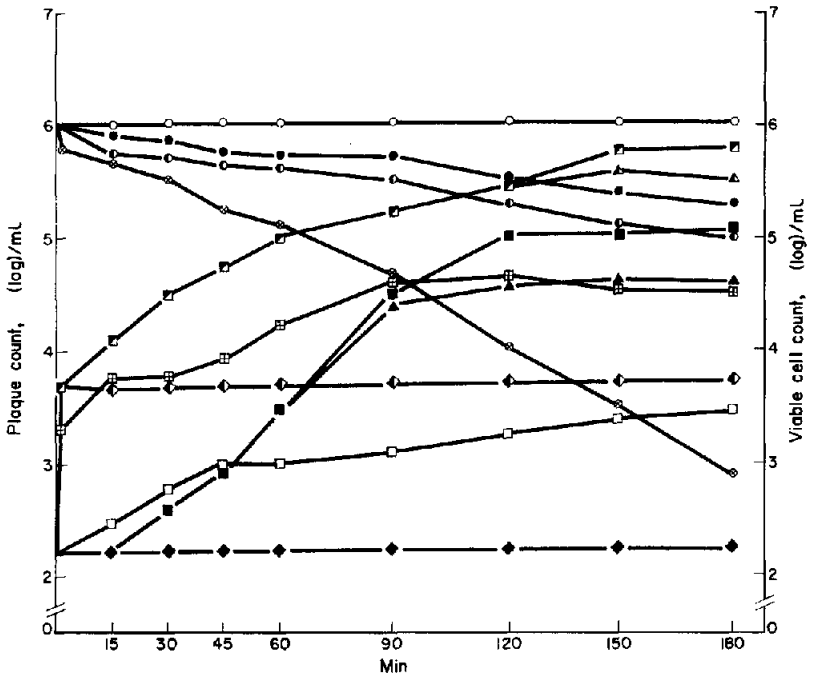

FIG. 2. Inductive effect of hydroxyurea and inhibition of mitomycin induction in the shift down state. Symbols: count of infective centers in control culture (□———□), in the presence of 0·1 M HU (■———■), 1 μg/ml MC (◩———◩), 1 μg/ml MC and 0·1 M HU (⊞———⊞), 20 μg/ml CM and 90·1 M HU (▲———▲), or 20 μg/ml CM and 1 μg/ml MC (◭———◭). Count of infective centers, incubated at 2°, in the presence of 0·1 M HU (◆———◆), or 1 μg/ml MC (◇———◇). Viable cell count in control culture (○———○), in the presence of 0·1 M HU (●———●), 1 μg/ml MC (◐———◑), or 0·1 M HU and 1 μg/ml MC (⊗———⊗).

It can be seen that HU inhibits the second phase of MC induction which takes place during the measurable period. This could be explained on the basis of the second phase of MC induction being dependent on DNA replication.

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REFERENCES

1. I. GADÓ and G. SAVTCHENKO, *Acta microbiol. Acad. Sci. hung.* **13**, 327 (1966/1967).
2. B. STERNS, K. A. LOSEE and J. BERNSTEIN, *J. mednl Chem.* **6**, 201 (1963).
3. CH. W. YOUNG and S. HODAS, *Science* **146**, 1172 (1964).
4. H. S. ROSENKRANZ and J. A. LEVY, *Biochim. biophys. Acta* **95**, 181 (1965).
5. M. C. MARGARETTEN, C. MORGAN, H. S. ROSENKRANZ and H. M. ROSE, *J. Bact.* **91**, 823 (1966).
6. B. D. DAVIS and E. S. J. MINGIOLI, *J. Bact.* **60**, 17 (1950).
7. D. GOLDTHWAIT and F. JACOB, *C.r. Séanc. Soc. Biol.* **259**, 661 (1964).