CHEMICAL STUDIES ON HOST-VIRUS INTERACTIONS

II. The Chemical Simulation of the Interference Phenomenon by 5-Methyl Tryptophane

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INTRODUCTION

In the foregoing paper, it was shown that the adsorption of virus results in the inhibition of the host's multiplication in two host-virus systems (1). At the same time the rate of cellular oxygen consumption and the respiratory quotient remain at the values observed just before infection. It was noted that under the conditions of this inhibition, the conditions for the interference phenomenon in the bacteriophage-*E. coli* and in the influenza virus—chorio-allantoic membrane systems are also found to be satisfied.

It was our intent to inhibit virus multiplication by chemical means in a manner approximating the observed conditions of the interference effect. Not all mechanisms of inhibiting host multiplication suffice to prevent virus multiplication in these systems. The infection of the host, *E. coli* B, which is a prerequisite to virus reproduction, itself inhibits host multiplication. On the other hand, it might be expected that the common respiratory poisons, in so far as they cut off the energy supply of the cell would, in the process of damaging one portion of the cell, yield effects in a different aspect of its activity, namely, that essential for virus multiplication. The chemical simulation of the interference effect requires the maintenance of energy supply; it requires an inhibitory compound which does not grossly affect the rate of oxygen utilization and the R. Q. Hence the common respiratory inhibitors cannot properly be a subject of study in the fulfillment of these conditions.

A compound was available, bacteriostatic for the host under study (2) which, it was considered, might fulfill these conditions. This substance, 5-methyl tryptophane (5MT), when tested in a respirometer, at certain concentrations inhibits bacterial multiplication without appreciably altering the rate of oxygen consumption or the R. Q. A bacteriostatic concentration of 5MT did not appear to kill the strain of *E. coli* B, or the bacteriophages examined, T2 or T4, when each was separately present in the medium. However, when T2 or T4 was mixed with the host in bacteriostatic concentrations of 5-methyl tryptophane, it not only failed to multiply but the numbers of infectious centers were reduced.

EXPERIMENTAL

Materials and Methods

The preparation and assay of bacteriophage and bacteria on synthetic F medium have been described previously (5), as were the manometric estimations of oxygen consumption and respiratory quotient (1). We are indebted to Dr. W. G. Gordon and Dr. R. W. Jackson of the Eastern Regional Research Laboratory, Department of Agriculture, for the sample of 5MT employed in these studies (3). One-step growth curves were determined by the method of Delbrück and Luria (4).

The Respiration of E. coli B in Synthetic Medium (F) Containing 5-Methyl Tryptophane (5MT)

In the absence of tryptophane the bacteriostatic concentration of 5-methyl tryptophane was found to be 4 μM per liter when an inoculum of 10^6 bacteria per cc. was employed in a 22 hour test (2). The numbers of bacteria necessary for an estimation of oxygen consumption are of the order of 10^9. With such concentrations of bacteria in F medium, 5MT concentrations of 460 μM/l. were bacteriostatic for approximately 4 hours, concentrations of 46 μM/l. were bacteriostatic for only 30 minutes or less, and 4.6 μM/l. were not bacteriostatic at all.

In Fig. 1 are presented the oxygen consumption curves of 1.5 cc. of F containing 5×10^9 B, to which after 30 minutes were added 0.5 cc. of various dilutions of 5MT in F, to bring the final concentrations to 460, 46, 4.6, and 0 μM/l.

The respiratory quotient in F of *E. coli* B inhibited by 460 μM/l. was 1.04 as compared to 1.08 for freely multiplying organisms. It is not considered that this difference is significant.

The Disappearance of Bacteriophage Activity in B Systems Containing (5MT).—It was observed in several experiments that the final T2 or T4 titer in *E. coli* B systems infected in the presence of 460 μM of 5MT per liter was one-hundredth to one-thousandth that found in the absence of this substance. In concentrations of 5MT of 46 μM/l., *i.e.* a concentration inadequate to effect prolonged bacteriostasis but sufficient to act like tryptophane in facilitating the adsorption of T4 to B (2), the T2 or T4 titers were essentially the same as those of the controls.

As described previously (1), virus multiplication in the concentrated bacterial suspensions employed in these experiments does not follow the normal course of one-step growth. The relative absence of bursts and lysis is to be noted in concentrated suspensions and the amount of virus to be found by the plaque method seldom exceeds the amount of virus added. Hence, these experiments indicated only that there had been a loss of virus in systems containing 5MT.

The Stability of B, T2, and T4 in 5MT.—Incubation of B, T2 and T4 in F

medium containing 460 μM/l. of 5MT for 100 minutes at 37° resulted in no significant diminution of the titers of any of these materials when assayed on Difco nutrient agar.

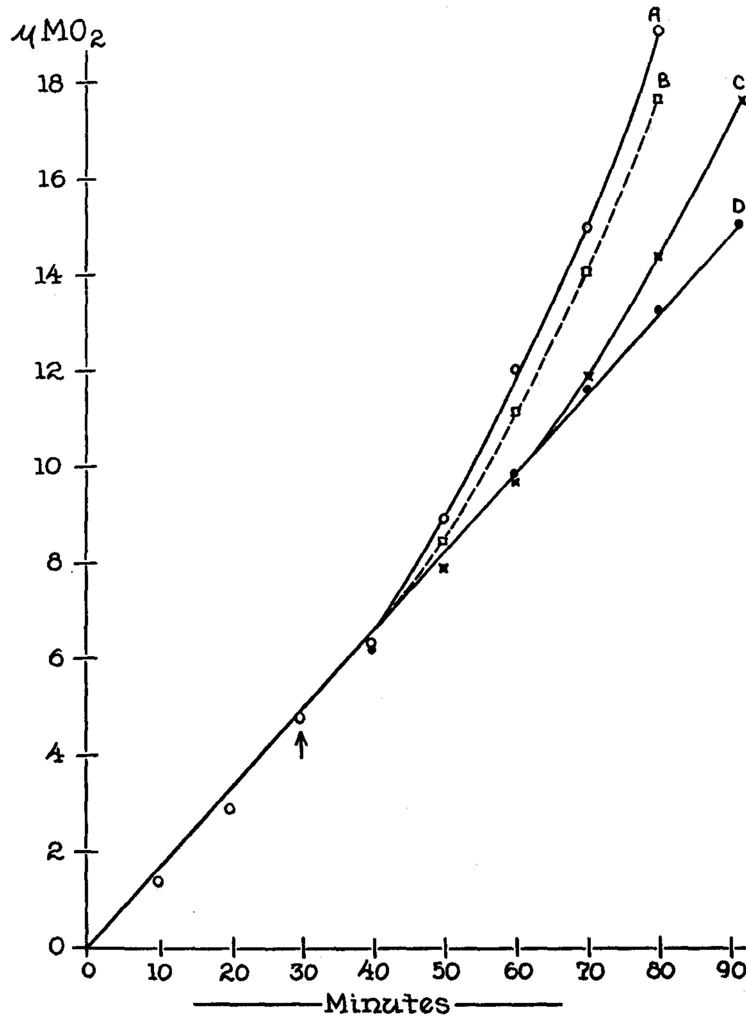

FIG. 1. The oxygen consumption curves of *E. coli* B in synthetic medium F containing various amounts of 5-methyl tryptophane. A, B, C, and D contain 0, 4.6, 46, and 460 μ M per liter of 5MT, respectively.

Attempted Growth of T4 on B in F Containing 5MT.—The one-step growth experiment was used to see whether virus T2 or T4 could grow in B in the presence of bacteriostatic concentrations of 5MT. In this type of experiment,

virus was added to comparatively concentrated bacterial suspensions (10^8/cc.) to permit rapid adsorption of virus on the host. After a short incubation period, the infected suspension was diluted 2000-fold; when new virus was liberated in a burst, it was not readily readsorbed by the host. The diluted suspension was incubated at 37° for 2 hours and aliquots were removed periodically for estimation of total infectious centers. Meanwhile, other aliquots were sedimented by a 5 minute centrifugation at 4000 R.P.M. and the supernatant fluids were assayed for free virus.

TABLE I

Schedule of One-Step Growth Experiment with Virus T4

Time		
min.		
−250	24 hr. culture of B inoculated into F medium and incubated at 37° with aeration	
−5	Assay for B. B = 6 × 10^8 per cc.	
−1	0.65 cc. B + 0.25 cc. tryptophane (try) (Tube I) or 5MT (1840 μ M/1.) (Tube II)	
0	Added 0.1 cc. T4 at 5 × 10^8 per cc. to B suspensions I and II	
5	Added 0.1 cc. mixture I and II to 10 cc. F (Tubes III and IV).	
	Removed 1 cc. of III and IV and centrifuged 5 min. at 4000 R.P.M.	
	Assayed supernatant fluids on nutrient medium:	
17	III − B + T4 + try = 4.2 × 10^4 free T4 per cc.	
18	IV − B + T4 + 5MT = 9.8 × 10^4 free T4 per cc.	
6	Added 0.1 cc. mixture III and IV to 1.4 cc. F + 0.5 cc. try or 5MT and incubated	
7	at 37°. Assayed periodically for T4 on nutrient agar.	
	B + T4 + try	B + T4 + 5MT
10	1.45 × 10^4 per cc.	1.24 × 10^4 per cc.
14	1.25	0.90
22	1.43	1.12, etc., of Fig. 2

$$\text{Burst size in absence of 5MT} = \frac{\text{Final titer} - \text{initial free titer}}{\text{Initial titer} - \text{initial free titer}} = \frac{(8.6 - 0.21) \times 10^4}{(1.40 - 0.21) \times 10^4} = 70$$

In the B-T4 systems, the initial infection and incubation occurred in F medium containing 460 μM tryptophane per liter in the control and 460 μM of 5MT in the paired sample. Thus tryptophane acted as cofactor to facilitate the adsorption of T4 (5) in the control, while 5MT acted as cofactor for T4 in the experimental sample (2). In Table I the schedules for this experiment are given in detail, since the results obtained in an experiment of this type must depend on the specific conditions employed. The results presented in Fig. 2 demonstrate the inability of B infected with T4 and incubated in F medium containing 5MT to reproduce virus assayable by the plaque method. In 2 hours, in contrast to the 70-fold increment in T4 in the tryptophane-containing tube, the disappearance of *ca.* 85 per cent of the initial infectious centers was to

be noted. It can be seen that the control curve was typical in form; a constant number of infective centers persisted for about 30 minutes, a steep growth portion and liberation of virus for about 40 minutes was followed by another

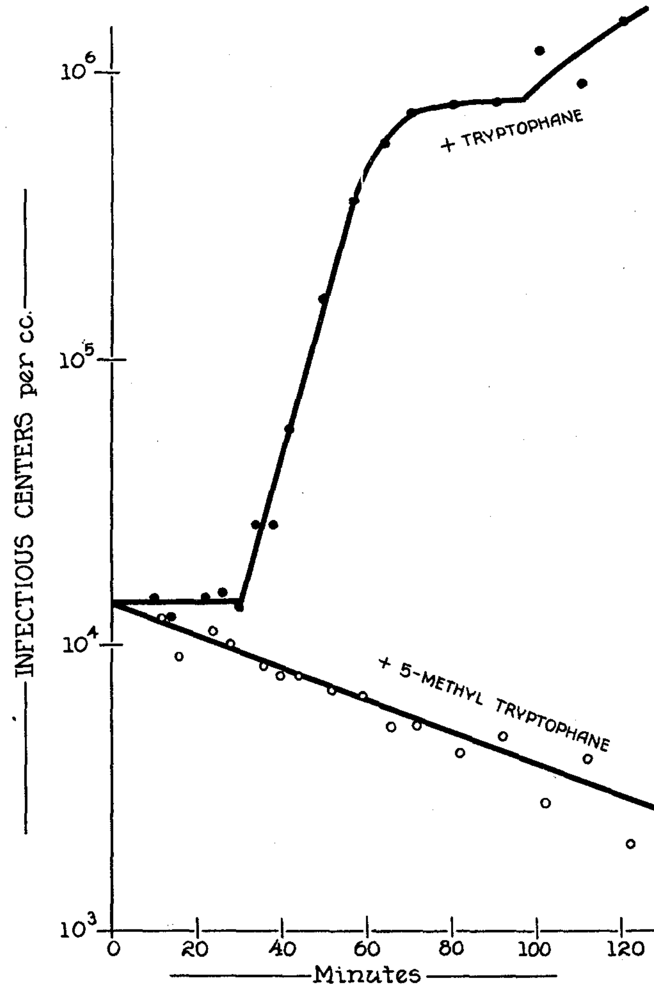

FIG. 2. The one-step growth curves of bacterial virus T4 on its host, *E. coli* B, in the synthetic medium F, containing 460 μ M per liter of tryptophane or 5-methyl tryptophane.

constant period. The observed burst size was 70. In contrast, the tube containing 5MT showed an immediate logarithmic decrease in numbers of infectious centers.

Attempted Growth of T2 on B in F Containing 5MT.—Since T2 does not require a cofactor for adsorption on *E. coli* B, trytophane was omitted from the

control tube for one-step growth of T2. Otherwise, the schedule and manipulations in this experiment were essentially as described for T4 and are presented in Table II. The results, plotted in Fig. 3, reveal that like the T4 systems, the *E. coli* B-T2 system was unable to reproduce infectious centers in the presence of 5MT. In fact, the number of infectious centers decreased at an apparently greater rate than in the B-T4 system. For some unknown reason, the assay of T2 in the growth tube containing 5MT was extremely erratic in contrast to its assay in the presence of its host under normal conditions of multiplication.

TABLE II

Schedule of One-Step Growth Experiment with Virus T2

Time		
min.		
−230	24 hr. culture of B inoculated into F medium and incubated at 37° with aeration	
− 5	Assay for B = 2.7×10^8 per cc.	
− 2	0.65 cc. B + 0.25 cc. F (Tube I) or F containing 1840 μ M 5MT per liter (Tube II)	
0	Added 0.1 cc. T2 at 5×10^8 per cc. to B suspensions I and II	
5	Added 0.1 cc. of mixtures I and II to 10 cc. F (Tubes III and IV).	
	Removed 1 cc. aliquots and centrifuged 5 min. at 4000 R.P.M.	
	Assayed supernatant fluids on synthetic medium:	
15	III − B + T2 + F = 1.05×10^5 free T2 per cc.	
16	IV − B + T2 + 5MT = 8.9×10^4 free T2 per cc.	
6	Added 0.1 cc. mixtures III and IV to 1.9 cc. F. (Alone or with 5MT)	
7	Incubated at 37°. Assayed periodically for T2 on synthetic medium:	
	B + T2	B + T2 + 5MT
10	2.45×10^4	0.95×10^4
13	2.45	3.35
21	2.36	2.8

$$\text{Burst size in absence of 5MT} - \frac{\text{Final titer} - \text{initial free titer}}{\text{Initial titer} - \text{initial free titer}} = \frac{(79.3 - 0.445) \times 10^4}{(2.58 - 0.445) \times 10^4} = 37.0$$

The normal one-step growth curve included a latent period of *ca.* 35 minutes, a burst for about 35 minutes, and another latent period. The observed burst size was 37.

DISCUSSION

It had been observed that in *E. coli*, three phenomena appeared under the conditions of the interference effect induced by two bacteriophages: the inhibition of multiplication of subsequently added virus; inhibition of the multiplication of the host; and no apparent change in the host's oxygen utilization. In general, effects produced by the viruses on the host are irreversible, that is, within experimental error, once the two combine, the host never recovers nor can it support growth of another virus.

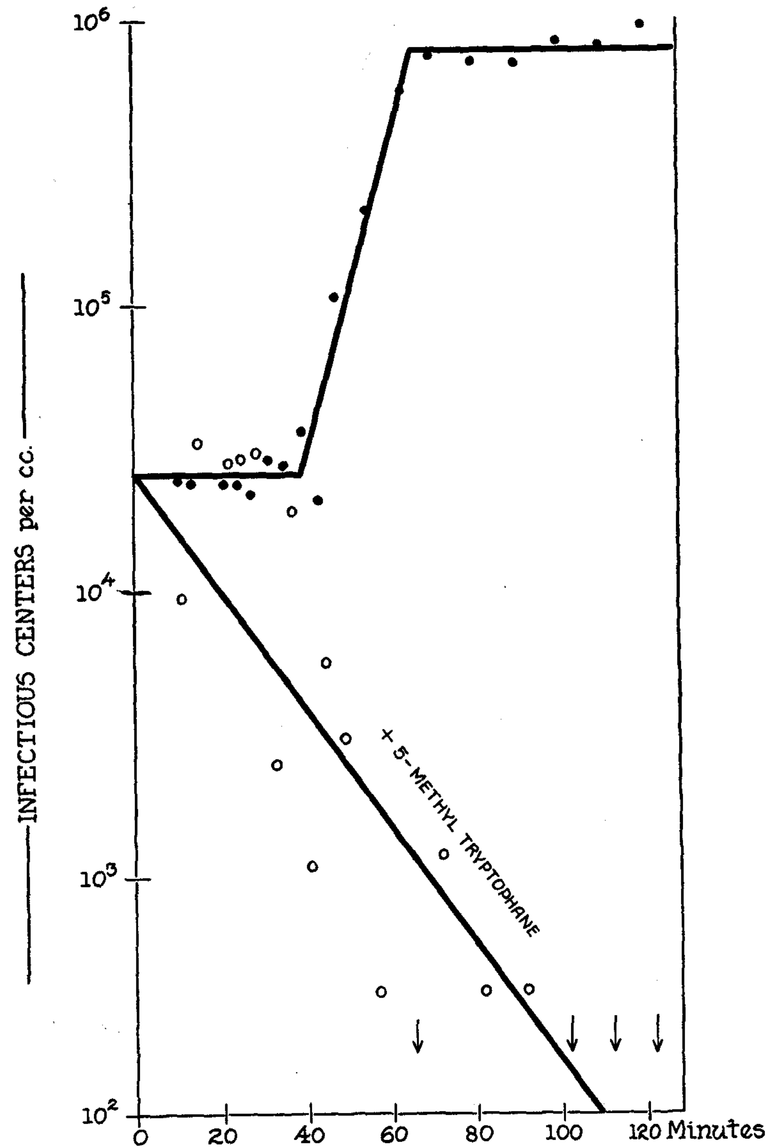

FIG. 3. The one-step growth curves of bacterial virus T2 on its host, *E. coli* B, in the synthetic medium F, or in F containing 460 μ M per liter of tryptophane. Arrows indicate fewer than 10^2 infectious centers per cc.

In sufficient concentration, the chemical analogue of tryptophane, 5-methyl tryptophane, produces all three known characteristics of the interference effect. However, the effects produced by 5MT are reversible. On removal of the

chemical from the bacteria by dilution the bacteria can recover and produce colonies which they could not have done if they had been infected by virus. Likewise, infected bacteria are unable to liberate virus in the presence of 5MT. However, virus particles which infect the bacteria in the presence of 5MT do multiply and form plaques if the preparation is diluted soon enough after infection. However, as the infected bacteria are kept in the presence of 5MT for longer times, as in the growth experiments of Figs. 2 and 3, an increasing number of them lose the ability to liberate active virus even when diluted and plated for assay. In time, then, the inhibitory effects of 5MT on the host-virus complex become irreversible. No such irreversible inhibitory effect was observed on host or virus when treated separately with 5MT.

The shape of the B-T4 one-step growth curve with 5MT indicates that infectious centers were unable to liberate free virus in the presence of 5MT. Whether this means that the infected organisms were unable to *reproduce* virus internally or to *liberate* virus in a burst is a matter for further study.

The site of action of 5MT in the interference effect is different from the site of its cofactor effect in facilitating the adsorption of T4 on its host. In the cofactor effect, the active compound appears to react primarily with the virus (6). The new combination of virus + cofactor then adsorbs on the bacterium. In contrast, the action of 5MT in the interference effect appears to be related to its bacteriostatic action. Thus 5MT interferes with virus multiplication in our experiments at a bacteriostatic concentration, rather than at the lower concentrations sufficient to facilitate adsorption (2). Furthermore, it prevents virus multiplication in T2 systems which apparently do not require a supplementary adsorption cofactor.

The indole nucleus, whether organized as 5MT, tryptophane, indole acetic acid, gliotoxin, etc., appears to be related to many phenomena of multiplication. Further intensive study of the effects of these compounds in virus and cell multiplication is certainly indicated.

SUMMARY

5-methyl tryptophane inhibited the multiplication of *E. coli* B without apparently affecting the rate of its oxygen utilization or R. Q. in a synthetic medium. *E. coli* B, under conditions of inhibition in the presence of this compound, was infected with the bacterial viruses T2 or T4. Infected organisms, in the presence of this compound, were unable to reproduce virus, assayable by the plaque method. Indeed, the number of infectious centers disappeared at a logarithmic rate in the presence of 5-methyl tryptophane, although the compound did not reduce the titers of B, T2, or T4, when the bacteria or viruses were exposed separately to the agent. In contrast to the irreversibility of the interference effects induced by viruses, the effects induced by short exposures to 5MT appear to be reversible on removal of the compound.

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