INFLUENCE OF URETHANE AND OF HYDROSTATIC PRESSURE ON THE GROWTH OF BACTERIOPHAGES T2, T5, T6, AND T7*

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The influence of urethane and hydrostatic pressure on the stability, in the free state in nutrient broth, of bacteriophages T1, T2, T5, and T7 (Delbrück, 1946) against *Escherichia coli* B, has been described in a previous report (Foster, Johnson, and Miller, 1949). Inactivation occurred at temperatures between 60 and 70°C., and small concentrations of urethane accelerated its rate in each case. Hydrostatic pressures up to 10,000 pounds per square inch retarded the rate with T2, T5, or T6, but accelerated that of T7, with or without urethane. At ordinary temperatures these pressures caused a slow reduction in titer of T2, but the other types were apparently unaffected.

The purpose of the present study has been to determine the effects of urethane and of hydrostatic pressure on the multiplication of the same phage types, except that T6 was included in place of T1. The influence of these factors is of interest primarily as a means of providing further evidence concerning the intermediate stages in the formation of new infective units between the time of infection and burst of the host, about which relatively little is yet known. Their action is also of general interest in connection with the mechanisms controlling biological reaction rates.

Methods

The culture medium consisted of Difco nutrient broth plus 0.5 per cent NaCl. Most of the experiments were done with singly infected cells according to the technique described in detail by Delbrück and Luria (1942). A mixture of approximately 6×10^7 phage particles per ml. and 7×10^7 bacterial cells was used for adsorption during a 3 minute period. A 1:2,000 dilution was made to stop adsorption, and the amount of free phage remaining unadsorbed in a portion of the mixture was determined by centrifuging and assaying the supernatant. Incubation in a water bath

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at 35°C. was used throughout. The normal latent periods under the conditions employed were 23, 44, 30, and 14 minutes, for bacteriophages T2, T5, T6, and T7, respectively.

Stock solutions of urethane were made up in a concentration of 1.0 M in the broth medium and sterilized by filtration. Dilutions in sterile broth were made by an amount to give the desired final drug concentration when added in a 1:1 ratio by volume to the bacterial culture just before infection or at various intervals during the latent period. At corresponding times, broth alone was added in the same ratio to the controls. When added before infection, the 1:2,000 dilution to stop adsorption was made with broth containing the appropriate concentration of urethane, and broth alone, respectively. In experiments in which it was desired to remove the drug at various intervals during the latent period, a 1:20 dilution was made with broth. This was found to eliminate, in effect, the action of urethane at all the concentrations studied. Control experiments demonstrated also that, even with the highest drug concentrations used, the extent of dilution of specimens in making the assay plates was sufficient that no influence of the amount of urethane introduced into the medium could be detected on the number of infective centers which developed.

In experiments with hydrostatic pressure, aliquot portions of an infected culture were distributed, immediately after the dilution to stop adsorption, among a series of small tubes of the type described in the previous study. The tubes, aseptically stoppered with sterile rubber plugs, were placed in small, stainless steel "bombs" and connected by individual pressure lines, each with a pressure valve, to a manifold from a hydraulic pump. Pressure could thus be applied or released on any number of the connecting bombs at the time, and specimens removed for assay at will. It was not possible, by this technique, to apply pressure before adsorption and maintain it through the 1:2,000 dilution. Moreover, although the pressure could be released at any time, it was difficult to assemble the specimens and connections on the apparatus fast enough to apply pressure on as many as 7 or 8 bombs sooner than 5 minutes after the period of infection.

An attempt was made to measure the effect of pressure on the rate of clearing during macroscopic lysis of cultures multiply infected with T7, using an apparatus similar to the one described by Johnson and Schlegal (1948), except that the full range of visible light, without a color filter, from an AH-4 mercury vapor lamp was focused through the bomb onto the photronic cell. The bacterial suspension was thoroughly aerated with oxygen just before adding the bacteriophage. The mixture could be introduced into the bomb and pressure applied within 3 to 5 minutes, and clearing usually began within 19 to 21 minutes. It was necessary to measure the rate of clearing at normal and increased pressures in successive runs. In spite of rigorous standardization of procedure, quantitative reproducibility in repeated experiments was never fully satisfactory, but certain reproducible results were observed, as described.

RESULTS

Influence of Urethane on Bacterial Multiplication

The multiplication of uninfected cells of normal bacterial cultures, prepared in precisely the same manner as those used for the growth of bacteriophage, is

measurably retarded by 0.15 M urethane, and completely blocked by 0.4 M. The latter concentration seems to have a slight killing effect, more readily apparent after 2 or 3 hours' incubation. It has no effect on the titer of free phage, however, at this temperature. The blockage is reversible, apparently after a short lag, when a 1:20 dilution with broth is made. The lag is somewhat greater when the dilution is made after as much as 60 minutes of arrested growth in a 0.4 M concentration of the drug. These relationships are illustrated in Figs. 1 and 2. An initial period of slower growth is evident during the first 60 minutes, possibly arising from the effects of dilutions at the beginning of the experiment. Most of the experiments with bacteriophage were completed within a 60 minute period, corresponding to this rate of normal growth.

Influence of Urethane Added at the Time of Infection

According to Czekalowski and Dolby (1949), 0.3 M urethane reduces the number of lytic particles of T2 from singly infected cells, with no obvious influence on the reproduction of *E. coli*, strain B. In our experiments, 0.4 M urethane, which completely inhibits the reproduction of normal cells, prevents an increase in titer of phage, when the drug is added to the bacterial culture immediately before, and remains from, the time of infection. Lower concentrations, between 0.10 and 0.15 M, do not prevent an increase in titer, but reduce the yield. Unlike proflavine (Foster, 1948), the completely inhibitory concentration of urethane is the same for each of the phage types studied. Throughout the length of the normal latent period, the number of infective centers remains constant in the presence of 0.4 M urethane, but a slow decrease in titer occurs later. If, however, the drug is effectively removed, by a 1:20 dilution in the manner described, the burst will occur. Both the time at which it occurs and the amount of the yield depend on the time of the dilution. With each phage type, dilution before a certain critical time results in a normal time of burst and normal yield, suggesting an initial stage in the multiplication process that is unaffected by this concentration of urethane. For convenience of discussion, we will refer to this stage as the "urethane-insensitive stage." It extends for about the first 7 or 8 minutes with T2, T6, or T7, but lasts for about the first 13 minutes with T5, the most slowly developing of the types studied. Moreover, if the dilution is made subsequently to the urethane-insensitive stage, the burst is delayed by a constant amount of time between the beginning of the sensitive stage and the time of dilution. Data concerning this relationship are summarized in Table I. It is as though the multiplication process is suddenly stopped by the inhibitor at a certain stage, but then proceeds at its normal rate as soon as the inhibitor is removed. It could scarcely represent the time required for the drug to penetrate the cells, both because there is no such lag in its effects on growth, and because experiments with luminous bacteria (Johnson, van Schouwenburg, and van der Burg, 1939) indicate that urethane penetrates almost instantly.

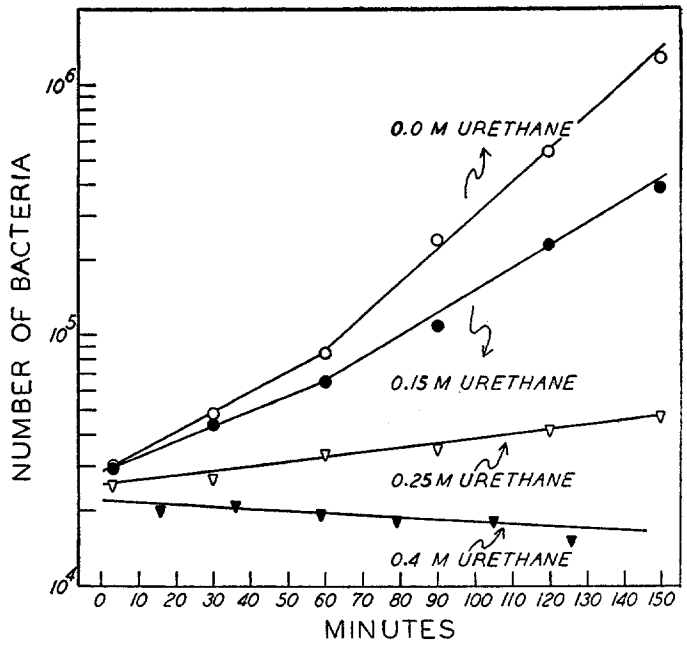

FIG. 1. Retardation in rate of multiplication of *E. coli* by different concentrations of urethane under the conditions described in the text.

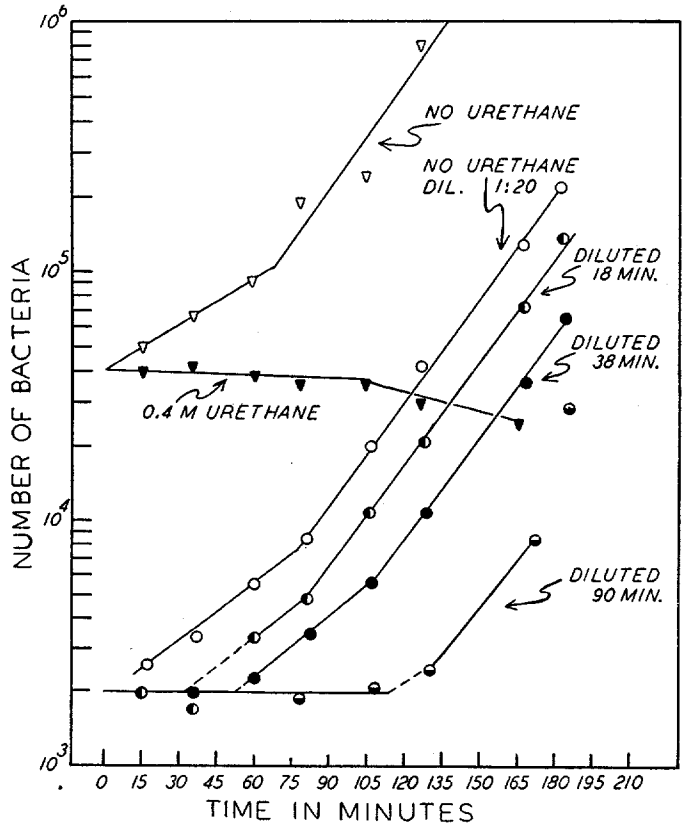

FIG. 2. Reversibility of the 0.4 M urethane inhibition of multiplication of *E. coli* on dilution with 19 volumes of sterile medium, after exposures of 18, 38, and 90 minutes to the drug. The upper curves represent plate counts of specimens taken at intervals from undiluted tubes; the lower curves from the diluted tubes.

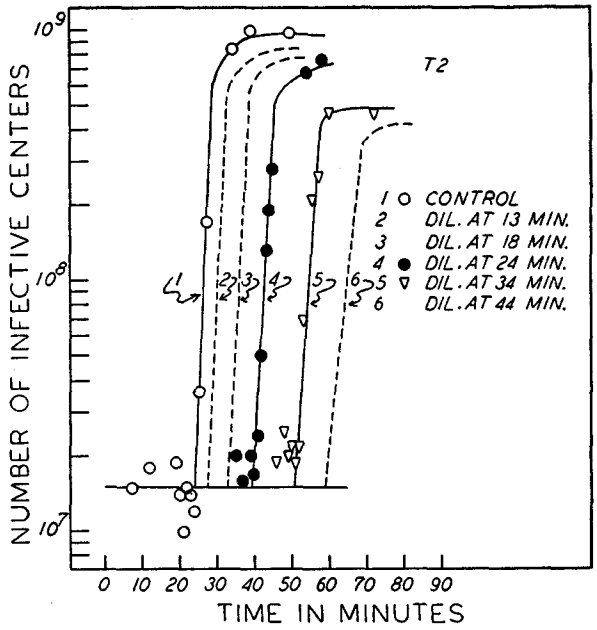

FIG. 3. Latent period and yield of T2 on dilution after increasing lengths of time of exposure to 0.4 M urethane from the time of infection. The broken lines represent data from other experiments, in which the input, adsorption, and yields were numerically different, and were multiplied by the appropriate factor to make the titer of the latent period correspond to that of the others; this does not affect the length of the latent period or relative amount of final yield.

TABLE I

Reversible Retardation of Phage Multiplication by 0.4 M Urethane

A = minutes after infection when urethane was diluted out. B = minutes after infection when titer first began to increase. (B − A) = constant time of latent period after diluting out the drug.

T2			T5			T7			T6		
(A)	(B)	(B − A)	(A)	(B)	(B − A)	(A)	(B)	(B − A)	(A)	(B)	(B − A)
Control	23			44			14			31	
8	23	15	13	44	31	24	32	8	24	49	25
13	28	15	21	50	29	44	50	6			
18	33	15	30	62	32						
24	39	15	40	70	30						
34	51	17									
44	59	15									

Figs. 3[1] and 4 illustrate the reversibility of the urethane inhibition of phage multiplication, with T2 and T5, respectively. They also show that, within the limits of experimental error, the rate of increase in titer is essentially the same in each case, even though the length of the latent period is very considerably prolonged by delaying the time of diluting out the drug. On the other hand, although the yield is only slightly, or not at all, affected when the dilution is made very shortly after the critical time of urethane sensitivity, it is progressively reduced as this time is lengthened.

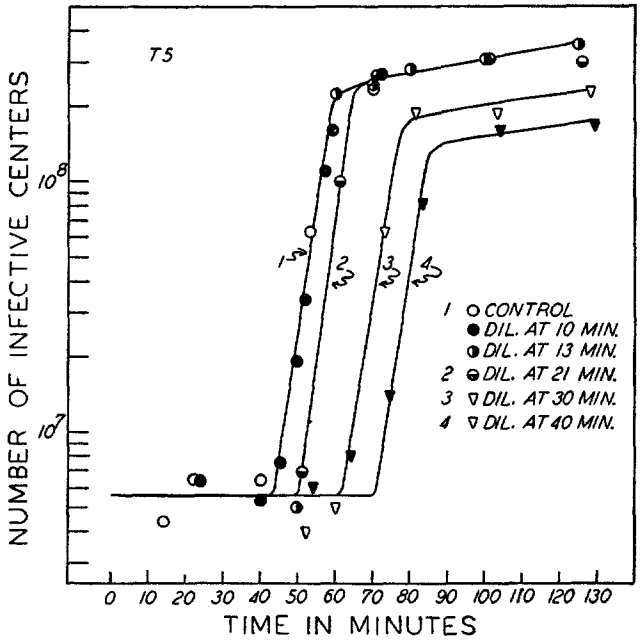

FIG. 4. Data from experiments with T5 similar to those of Fig. 3.

Addition of Urethane during the Latent Period

The length of the stage during which 0.4 M urethane apparently blocks phage multiplication was estimated by adding the drug at various intervals between the beginning of the critical time, referred to above, and the maximum yield in the control. This stage, which we will refer to as one of "complete sensitivity" to urethane was found to extend from approximately 7 or 8 minutes until 15 minutes after infection with T2 or T6, from 13 until 25 min-

[1] The logarithmic scale on the ordinate is used in this and succeeding graphs in order to make relative differences more readily apparent, especially at the low titers, but theoretical implications, from a kinetic point of view, are not intended.

utes with T5, but only from 7 until 9 minutes with T7. When added subsequently to the completely sensitive stage, there was evidence of a "partially sensitive stage" in that a rise in titer took place in the presence of the drug, and the time at which it began was not greatly affected. The yield was reduced, however, depending upon how early after the completely sensitive stage the drug was added. With very early addition, it was quite low, the yield increas-

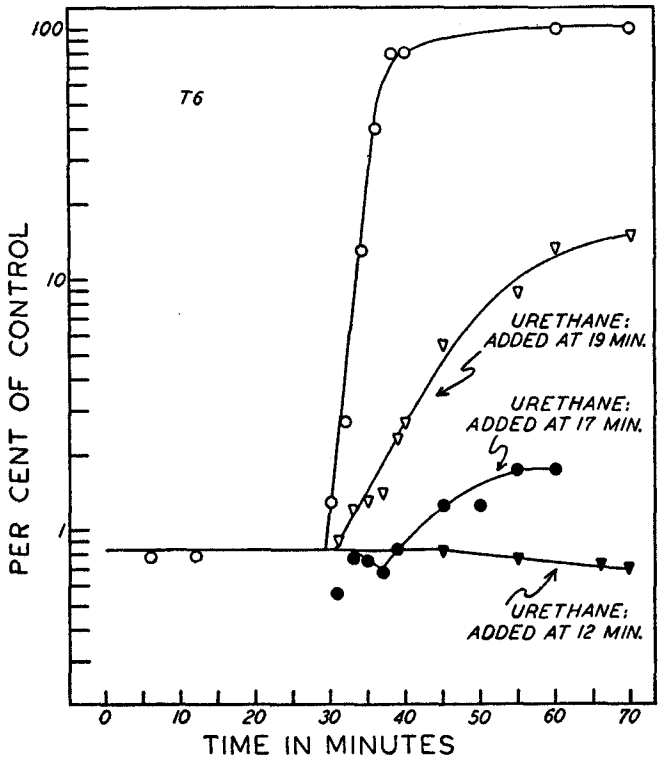

FIG. 5. Influence on the one-step growth curve of T6, of adding 0.4 M urethane at 12, 17, and 19 minutes, respectively, after infection.

ing with delay in addition time. A reduction in yield could be detected, however, when the urethane was added near the end of the rise period. These relationships are illustrated in Figs. 5, 6, and 7.

Curves resembling those of Fig. 7 have been discussed in connection with the action of proflavine (Foster, 1948). They suggest that the process of phage multiplication in different cells proceeds beyond the stage sensitive to inhibition at different times; *i.e.*, at different rates in different cells. The data of Figs. 5 and 6 support this view, as well as the established fact that the burst size among individual cells varies widely, even though the time of burst nor-

mally occurs within a relatively short period of time (Delbrück, 1945). Moreover, Doermann's experiments (1947–48) on disruption of cells during the latent period, by sonic vibration and other means, have shown that active particles of T3 and of T4, respectively, are present relatively early in the latent period, increasing in numbers up to the time of maximum yield in an ordinary control. Thus, it seems highly probable that the yield shown in Figs. 5, 6, and 7 consisted to a greater or lesser extent of phage particles that had been fully formed in some of the cells at the time that urethane was added, and there-

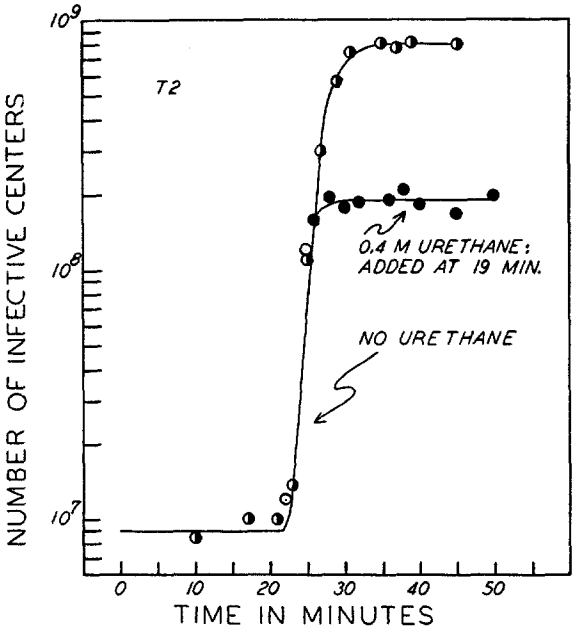

FIG. 6. Influence of adding 0.4 M urethane at 19 minutes after infection with T2.

fore, had the same insensitivity to urethane as that of free phage, even though these particles were not liberated until later, by completion of the lytic process. The partially sensitive stage, according to this view, would represent chiefly an effect of the drug on only a part of the population of infected cells.

In order to facilitate comparison between the different phage types, the time relationships of the drug's action during the respective normal latent periods are illustrated diagrammatically in Fig. 8.

In Fig. 7 it is apparent that when urethane is added near the end of the completely sensitive stage of T2, *i.e.*, at 14 minutes, the final titer is reduced to about 50 per cent of the input, indicating a loss of some of the infecting particles. This is probably an example of the phenomenon wherein lysis in

absence of phage reproduction results in the loss of even the infecting particle (Cohen, 1949). A more detailed study (Fig. 9) with T2 showed that when the

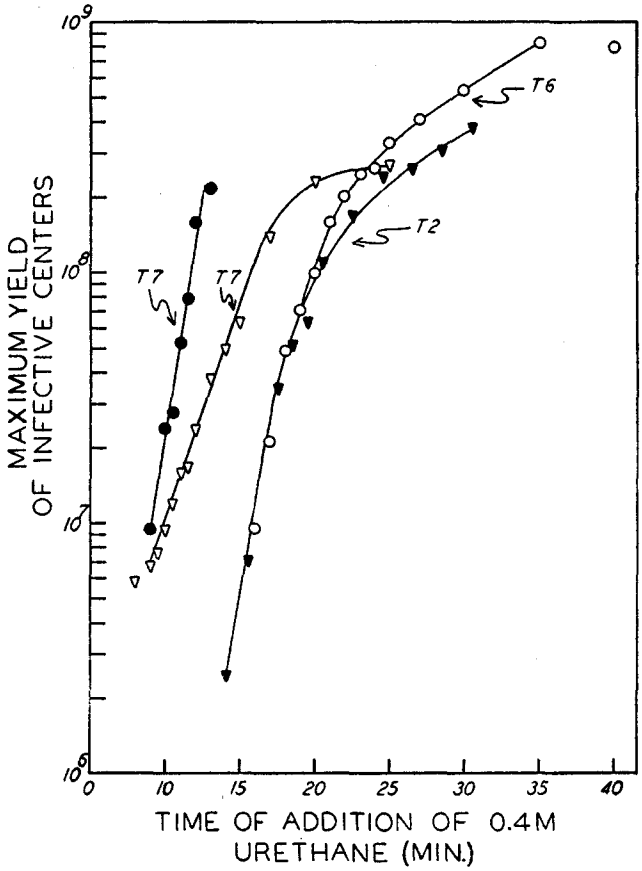

FIG. 7. Effects of 0.4 M urethane on the yield of the different phage types after addition of the drug at various intervals near the end of the respective completely urethane-sensitive periods, and later. With T2 and T7, the assays were made at the time of maximum yield in the controls, which were 3.6×10^8 for T2, and 7×10^8 and 2.5×10^8 for the two respective curves for T7. With T6, assays were made 20 minutes after the maximum yield of the control, which was 9×10^8.

drug is added at any time up to 10 minutes after infection there is no loss of original titer at the time of maximum yield in the control. During the period between 10 and 15 minutes after infection, however, there seems to be a stage in the multiplication of T2 when the addition of urethane not only blocks subsequent liberation of new particles but leads to the destruction of some of

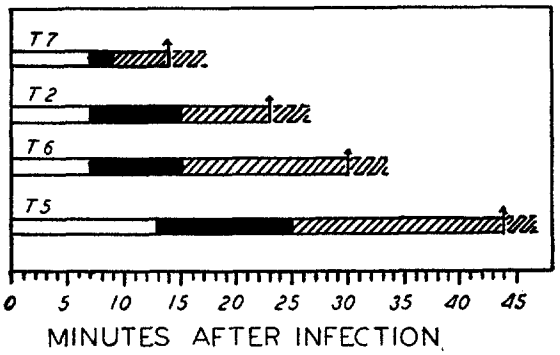

FIG. 8. Diagram to illustrate the approximate time relations of complete urethane insensitivity (clear area in first part of each bar), complete sensitivity (solid areas), and partial sensitivity (diagonally shaded areas) among the different phage types studied. The vertical arrows indicate the end of the normal latent period and beginning of the rise, whose length to the right of the arrows is not precisely specified. Yield may still be reduced by addition of urethane during the rise period.

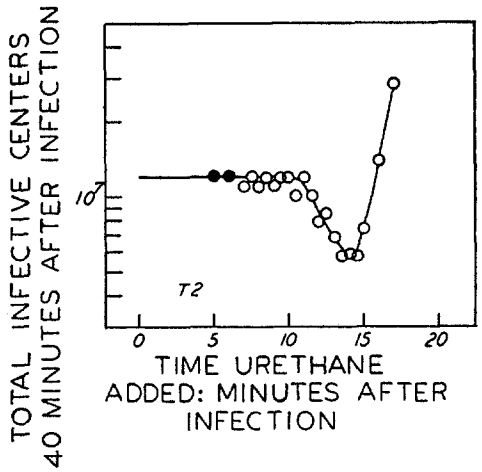

FIG. 9. Total number of infective centers, assayed at 40 to 50 minutes after infection with T2, in a series of tubes to which 0.4 M urethane was added at the times indicated.

those originally present. After about 15 minutes it appears that the process of multiplication in some of the cells has proceeded beyond such a stage, so that new particles are finally liberated.

The destructive effect illustrated in Fig. 9 depends to some extent on the continued action of urethane, as shown by the results of subsequent dilution. Thus, when the drug is added at 14 minutes and diluted out at 19 minutes,

the rise is delayed by approximately this interval of 5 minutes, but the titer increases at essentially the same rate as that of the control, and the yield is not greatly reduced (Fig. 10). When added earlier, at 11 minutes, and diluted out during the period of decreasing titer, a subsequent rise with much reduced yield takes place (Fig. 11).

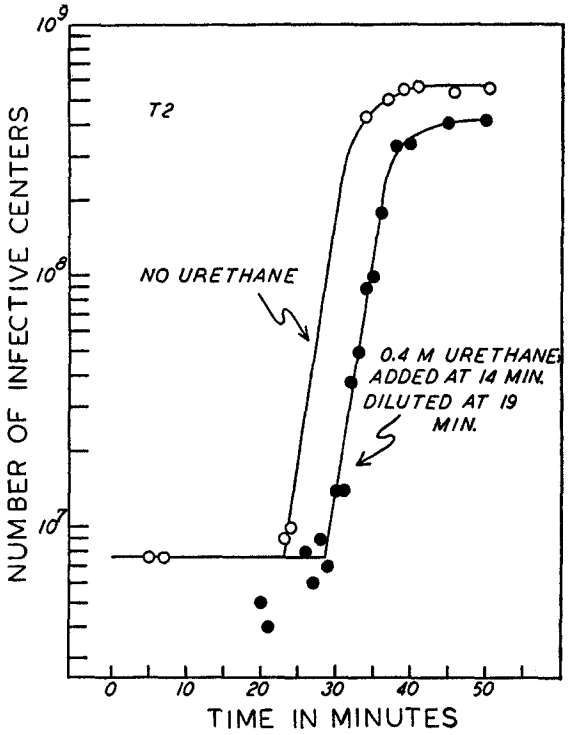

FIG. 10. Effect of adding urethane at 14 minutes, and diluting it out at 19 minutes after infection with T2.

Macroscopic Lysis

Since 0.4 M urethane does not decrease phage adsorption, nor cause a decrease in titer of free phage at the temperature of incubation, the absence of a yield could be due to either a failure of new, active particles to be produced or to a failure of the host cell to lyse, or perhaps both. Although these possibilities cannot be completely resolved through observations of macroscopic lysis, experiments with various multiplicities of infection by T7 showed that, in a concentration of 0.4 M, urethane does markedly delay clearing. A few observations were made with T6r as well as T7. The data are summarized in Table II.

With T7, the delay in clearing, in the presence of urethane from the time of infection, is inversely related to the multiplicity of infection, being generally less with increasing multiplicity. Even with a multiplicity scarcely higher than that used in the one-step experiments, however, long incubation with urethane present from the start gave evidence of some lysis. On the basis of experiments described in the preceding paragraphs, it is unlikely that such lysis liberates any active phage.

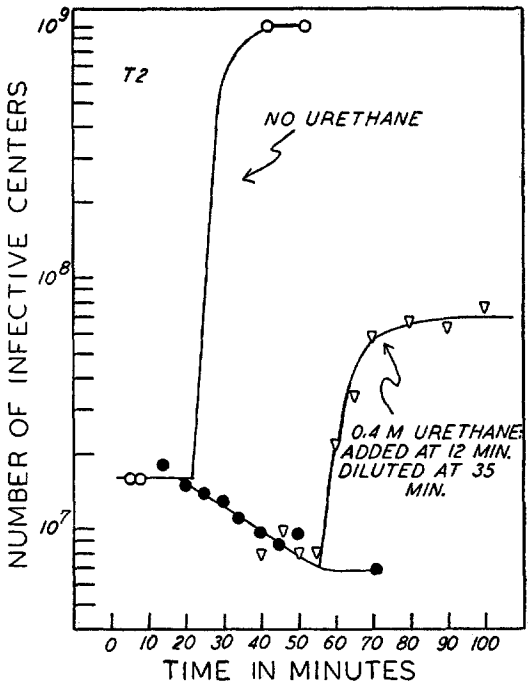

FIG. 11. Effect of adding urethane at 12 minutes and diluting it out at 35 minutes after infection with T2.

The data of Table II make it appear probable that urethane delays lysis in the one-step growth experiments. On the other hand, the rise in titer that occurs in the presence of the drug, when it is added after the stage of complete sensitivity in the latent period, indicates that, as in macroscopic lysis with higher multiplicities, a concentration of 0.4 M urethane does not altogether prevent lysis and must act also on the process of multiplication.

Discussion of the Urethane Effects

Of the three more or less distinct stages with respect to urethane sensitivity during the latent period, the third, *i.e.* the one of partial sensitivity, can be

most readily interpreted in terms of variations in the rate of formation of essentially complete virus particles in different individual cells of the population,

TABLE II

Influence of Urethane on Macroscopic Lysis at 35°C.

Relative turbidity estimated visually as 0 (clear) to 4 (quite turbid) in comparison to control, without phage or urethane, in ice bath.

Time after infection	T7													
	Multiplicity; no urethane							Multiplicity; 0.4 M urethane						
	0.8	1.6	2.4	3.2	4.0	4.8	8.0	0.8	1.6	2.4	3.2	4.0	4.8	8.0
min.														
10	4	4	4	4	4	4	4	4	4	4	4	4	4	4
20	4	4	4	4	4	3	2	4	4	4	4	4	4	4
25	4	4	4	4	3	2	1	4	4	4	4	4	4	4
30	4	4	3	2	2	2	0	4	4	4	4	4	4	4
35	3	2	1	1	0	0	0	4	4	4	4	4	4	2
40	3	2	1	1	0	0	0	4	4	4	4	4	4	2
45	2	1	0	0	0	0	0	4	4	4	4	2	2	1
50	2	0	0	0	0	0	0	4	4	4	4	2	2	1
55	0	0	0	0	0	0	0	4	4	4	4	2	1	0
60	0	0	0	0	0	0	0	4	4	4	4	1	1	0
65	0	0	0	0	0	0	0	4	4	4	2	1	1	0
85	0	0	0	0	0	0	0	4	3	3	2	1	0	0
100	0	0	0	0	0	0	0	4	2	2	2	1	1	1
	5	10	20	50				5	10	20	50			
20	4	1	2	0				4	4	4	4			
25	4	1	2	0				4	4	4	4			
35	2	0	0	0				4	2	3	1			
40	0	0	0	0				4	2	3	1			
55	0	0	0	0				0	0	1	0			
75	0	0	0	0				0	0	0	0			
	T6r, multiplicity 4													
30	4							4						
45	2							4						
60	1							4						
75	0							2						
90	0							2						

because of the reasons already listed. The fundamental significance of the first two stages, of complete insensitivity and of complete sensitivity, respectively, is problematical.

With regard to the initial stage of urethane insensitivity, lasting for as much as 50 per cent of the entire latent period of T7 about 30 per cent of that of T2

or T5, and 23 per cent of that of T6, it seems clear that the reactions relating to phage production in all the infected cells of the population are completely unaffected by the concentration of the drug which reversibly blocks both the growth of normal cells and the subsequent formation of new virus particles in infected cells. The nature of these reactions is largely a matter of speculation. The accumulated data in the literature on the concentration of urethane and similar narcotic agents required to inhibit various biological processes, both in cells and in extracts (*cf.* Winterstein, 1926; Henderson, 1930) do not permit any specific conclusion as to the type of reaction occurring during this period. Binkley[2] has recently discovered, however, that apparently protein-free ribonucleic acid preparations have powerful peptidase and other catalytic properties which are not sensitive to inhibition by narcotics such as chloroform or alcohol. It is reasonable to assume, therefore, that the urethane-insensitive stage is one predominantly of some such process of hydrolysis, perhaps involving the liberation of key units from the infecting particle, and their diffusion to sites within the protoplasm of the host where they can alter the pathway of synthetic reactions towards the production of virus, rather than host constituents.

The second, or completely sensitive stage begins at approximately the same time that, in T2 at least, the synthesis of virus desoxyribonucleic (DNA) acid begins (Cohen, 1948). Furthermore, the action of ultraviolet and x-radiation indicates that during approximately this intermediate stage, the inactivation kinetics change from a single to multiple hit type (Luria and Latarjet, 1947; Latarjet, 1948). In addition, with T2 phages, it is a period following a gradual breakdown of the normal nuclear picture, when chromatinic granulation of the cytoplasm occurs, and some of the granules undergo progressive increase in size (Murray, Gillen, and Heagy, 1950). Since it appears inescapable that the synthesis of biologically specific molecules involves a template mechanism, the hypothesis suggests itself that the templates essential for virus DNA synthesis become available or functional at this time, correlated with the multiple hit inactivation by radiation, and changes in the cytological picture of the host cell. From this hypothesis it would seem to follow that urethane does not prevent the release of the templates, but acts upon the synthetic processes in which they are concerned. Further considerations are consistent with this view, as follows.

The influence of hydrostatic pressure has indicated that the catalysis of reversible or irreversible protein denaturation by urethane sometimes involves an unfolding of the enzyme or protein from a globular to more linear configuration (Johnson, 1947; Johnson and Eyring, 1948). Pressure opposes this action

[2] Binkley, F., and Olson, C. K., paper presented at the American Chemical Society meeting in Chicago, September 6, 1950, and personal communication with Dr. Binkley.

of urethane, and also most of the thermal protein denaturations studied thus far (T7 apparently being an exception), by keeping a larger fraction of the molecules in the folded state. It has been postulated that an analogous unfolding would have to precede synthesis of globular molecules by the template mechanism, inasmuch as a molecule in the folded configuration could scarcely serve as a template for the production of a second one like it (Eyring, Johnson, and Gensler, 1946). Where such mechanisms are involved, it would be expected that urethane would interfere with synthesis because it would keep the molecules in the unfolded state, whereas pressure would interfere by keeping them in the folded state; the net result of each factor would be the same. Although other processes sensitive to urethane and to pressure, including the rates of various enzyme reactions as well as sol-gel changes (Marsland, 1942) are very likely also concerned in the total effects, the mechanisms discussed above could be the most critical ones in the influence of these factors on virus production. The fact that protein synthesis continues at a steady rate from the time of infection, whereas DNA synthesis begins 7 to 10 minutes later (T2; Cohen, 1948) shows that not all synthetic reactions are equally sensitive to urethane, and suggests that some particularly sensitive step, involving DNA, is specifically concerned. The following experiments with hydrostatic pressure lend some support to these views, for they demonstrate that pressures of the magnitude which cause reversible changes in biological systems not only interfere with virus multiplication, but also that their effects on this process resemble those of urethane in significant respects.

Influence of Pressure on One-Step Growth

With the method employed, pressure could not be applied to a series of corresponding specimens before a minimum of 5 minutes after infection. Consequently, the influence of pressure throughout the urethane-insensitive periods could not be readily determined, nor was it possible to assay the specimens immediately after release of pressure, inasmuch as a delay of a minute or more was imposed by the time consumed in opening the bomb and plating.

Experiments in which pressures of 7,000 to 9,000 p.s.i. were applied at 8 minutes after infection and released at different times after the latent period of the control showed that a rise in titer took place after a certain interval of time following release of pressure. This was true of each of the phage types T2, T5, T6, and T7, and the interval was characteristic of the type. The yield was reduced in comparison with the control, the amount of reduction being greater the longer the pressure was maintained, resembling the effects of urethane. Again resembling the effects of urethane, a decrease in original titer was observed when the pressure was maintained for considerable periods of time (Figs. 12 and 13). With T2, but not with the other types, a decrease in titer would be expected to result, in part, from the direct effect of pressure on

free phage. With T2, adsorption is particularly good, and although exact data were not obtained, it is probable that adsorbed phage constituted a considerable fraction of the total that was destroyed.

Although the time relationships of the pressure effects could not be established as closely as those of urethane, it seemed to make very little difference if pressure were applied as early as 5 minutes after infection or later, unless it

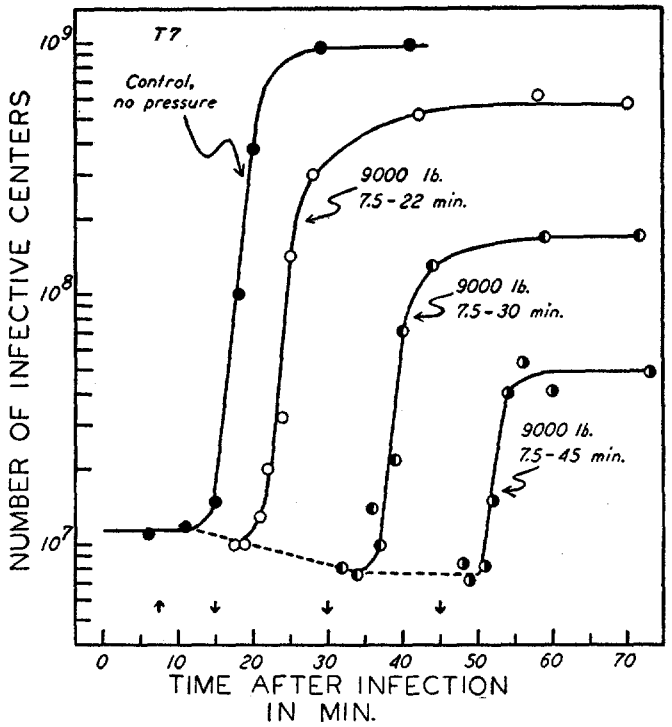

FIG. 12. Influence of applying 9,000 p.s.i. of hydrostatic pressure 7.5 minutes after infection with T7, and releasing the pressure later at the times indicated.

was not applied until a short time before the end of the normal latent period. In the latter case, large amounts of active phage were either liberated (perhaps also produced) under pressure, or explosively on release of pressure. These possible alternatives could not be distinguished. Fig. 14 illustrates the results of an experiment with T7 in which pressure was applied at 12 minutes after infection, *i.e.* about 3 minutes before the rise period, and was released after 3, 18, and 33 minutes respectively. Similar results were obtained with T2, on application of pressure at 18 minutes after infection, and release of pressure at succeeding intervals.

Effect of Pressure on Macroscopic Lysis

When applied shortly after infection, clearing was slowed and the yield reduced, but precise comparisons of rate of clearing and amount of yield could not be made with the method employed. As mentioned in the section on

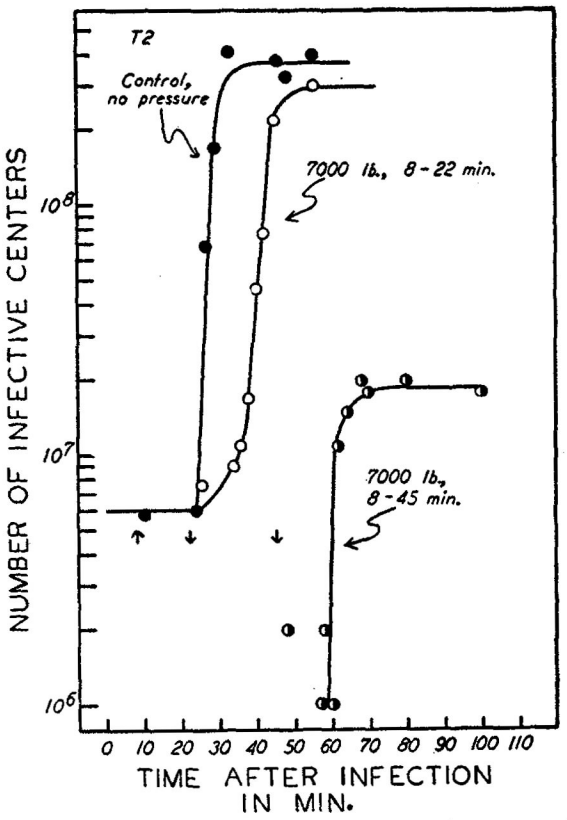

FIG. 13. Influence of applying 7,000 p.s.i. of hydrostatic pressure 8 minutes after infection with T2, and releasing the pressure at 22 and 45 minutes, respectively.

Methods, the experiments with normal and increased pressure had to be run in succession, and the bacteriophage mixtures were therefore not identical. They were made as nearly identical as feasible, either by staggering the times of inoculation and successive steps in the procedure with the culture, or by placing the growing culture in an ice bath at the time of the first run, using a portion of it later for the second run. During the course of the experiments, extending over a period of nearly a year, obscure variations occurred necessitating the use of high multiplicities of infection, of between 15 and 20 rather

than between 3 and 5, in order to obtain the beginning of lysis within the expected time of 19 to 21 minutes. The results of a large number of experiments, notwithstanding these difficulties, indicated that pressure had very little effect on the rate or amount of clearing if applied at the time clearing was just beginning, or later. When applied shortly after infection, the rate was slowed and

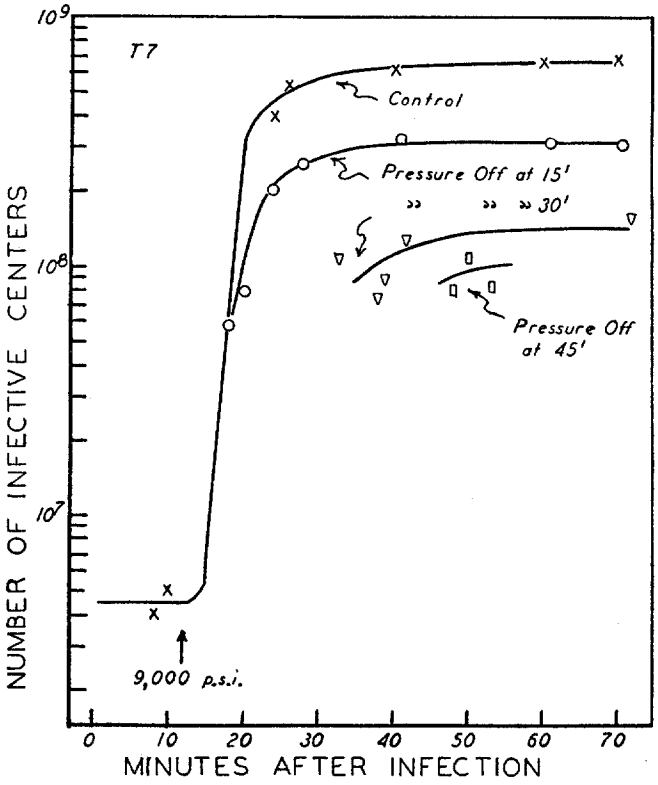

FIG. 14. Influence of applying 9,000 p.s.i. of hydrostatic pressure 12 minutes after infection with T7, and releasing the pressure at the times indicated.

the amount of clearing fell considerably short of that of a control. Since it was difficult to make a satisfactory calibration of the instrument which would accurately correlate the number of cells with galvonometer deflection during the process of lysis, and in addition, the quantitative results were somewhat variable, no general statements can be made concerning either the rate or amount of clearing, other than those above. Fig. 15 shows the results of two experiments on successive days in which the multiplicity of infection was nearly the same in each run, as determined by plate counts of the control culture at the time of infection.

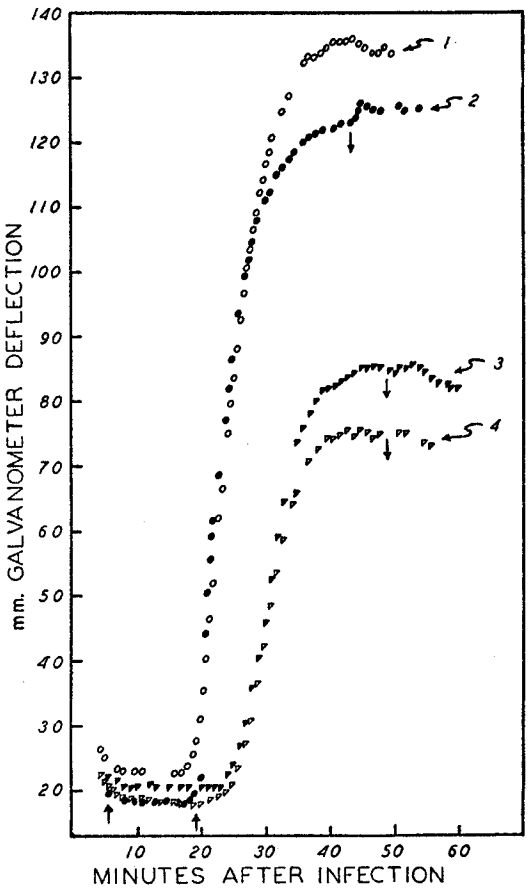

FIG. 15. Influence of 6,000 p.s.i. of hydrostatic pressure on macroscopic lysis by T7. Curve 1, control at normal pressure, multiplicity of infection, 13.1; curve 2, pressure applied at 19.5 minutes after infection, as lysis was just getting started, multiplicity of infection 12.1; curves 3 and 4, pressure applied at 5.5 to 5.6 minutes after infection, multiplicities 14.4 and 18.7, respectively. The upwardly directed arrows indicate the time of application of pressure, and the downwardly directed arrows the time of release of pressure, for the respective curves. Curves 1 and 4 represent successive runs on one day, and curves 2 and 3 on the next day. The galvanometer deflection with sterile medium in the bomb, just before the start of a run, was 182 to 185 mm.

The influence of urethane when added at the beginning of macroscopic lysis was not tested. In other respects, and possibly in this also, noteworthy similarities in the net effects of increased pressure and of urethane are apparent in the results of the foregoing experiments. Whether or not they influence primarily

the same reactions that are involved in the production and liberation of bacteriophage, as well as the precise nature of the reactions that they affect, requires further evidence.

The authors are pleased to acknowledge their indebtedness to Mrs. Robert Steele and Miss Elizabeth Flagler for valuable assistance in carrying out the experiments.

SUMMARY

In 0.5 per cent NaCl, nutrient broth at 35°C., urethane in a concentration of 0.4 M stops the reproduction of *Escherichia coli*, strain B. On dilution with 20 volumes of sterile medium, growth is resumed at its former rate after a short lag.

In the one-step growth of T2, T5, T6, or T7, in the same medium at the same temperature, 0.4 M urethane, when added at the time of infection, had no apparent effect on adsorption and caused no decrease in titer throughout the latent period of the control, but completely prevented a rise in titer. If diluted 1:20 with sterile medium prior to a certain critical time in the latent period, however, bacteriophage was liberated at the same time, and in the same amount as in the control. The initial stage of apparent insensitivity to the drug lasts from the time of infection until the approximate critical times of 7 minutes with T7, T2, or T6, or 13 minutes with T5. Under the conditions described, the normal latent periods were 14, 23, 30, and 44 minutes for T7, T2, T6, and T5, respectively.

At the critical times referred to above, there begins a stage characterized by complete sensitivity, rather than complete insensitivity, to 0.4 M urethane, in the sense that no active phage is subsequently liberated in continued presence of the drug. The length of this completely sensitive stage, as judged by addition of the drug at successive intervals during the latent period, extends from approximately 7 until 9 minutes after infection with T7, 7 until 15 minutes with T2 or T6, or 13 until 25 minutes with T5. When the urethane is added late in this stage of T2, a decrease in initial titer takes place as judged by assays made 40 minutes after infection, the maximum effect occurring when the drug is added between 14 and 15 minutes after infection. When added subsequently to the completely sensitive stage of each type, *i.e.* subsequently to 9 minutes after infection with T7, 15 minutes with T2 or T6, or 25 minutes with T5, liberation of the bacteriophage takes place in presence of the drug, but the yield is reduced, the amount of reduction being greater the sooner it is added. The yield increases as addition of the drug is delayed, but it is measurably reduced when added late in the rise period.

Macroscopic lysis with T7 is delayed by 0.4 M urethane, when present from the time of infection. The delay is less with increased multiplicities of infection. A similar delay occurs with T6r at a multiplicity of 4.

The application of hydrostatic pressures of 7,000 to 9,000 p.s.i. early in the latent period, within 5 to 8 minutes after infection, prevents a yield in each of the four phage types, and if maintained for lengthy periods of time a reduction in initial titer occurs. If released at various times shortly after the latent period, a rise in the titer occurred after a certain interval whose length was characteristic of the phage type. The yield was less the longer the release of pressure was delayed. When the pressure was first applied late in the latent period, large amounts of phage were liberated either under pressure or explosively when pressure was released to make the assays.

Hydrostatic pressure at 6,000 p.s.i. had little effect on the rate or amount of macroscopic clearing with T7 in relatively high multiplicity of infection, when applied at the start of lysis, but slowed the rate and reduced the amount of clearing when applied shortly after infection.

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