THE EFFECT OF CHEMICAL AND PHYSICAL AGENTS ON THE PHAGE RECEPTOR OF PHASE II SHIGELLA SONNEI

By GUY T. BARRY, Ph.D. and WALTHER F. GOEBEL, Ph.D.

(From the Laboratories of The Rockefeller Institute of Medical Research)

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The lipocarbohydrate-protein complex synthesized by Phase II *Shigella sonnei* during its growth in nutrient media is endowed with several important biological functions. Not only does this substance confer type specificity upon the microorganism and function as an endotoxin, but it also serves as the receptor for certain of the bacterial viruses to which the host itself is susceptible. Although the chemically isolated antigen will inhibit the adsorption of several of the T *coli* phages on the homologous host cell, it has been observed that different preparations exhibit considerable variation in this respect. It has been assumed that this is due to alterations in the macromolecule brought about by the chemical procedures employed in the preparation of the antigen.

In order to gain further insight into this question a study has been undertaken of the effect of mild chemical agents and physical procedures on the stability of the phage adsorption property of intact Phase II *Sh. sonnei*. In choosing this mode of attack it has been assumed (1), that the Phase II antigen lies distributed in its native state on the surface of living cells, and that any alteration in the adsorptive properties of the latter, as a result of manipulation, can be attributed to changes in the surface antigen itself.

Other investigators have shown (2, 3) that the rate of phage adsorption to susceptible bacteria is directly proportional to the concentrations of virus and microorganisms and that under optimum conditions this rate is nearly equivalent to the calculated collision frequency (4). In the present study it will be demonstrated that the rates of phage adsorption on viable Phase II *Sh. sonnei*, as opposed to those on treated bacteria, are profoundly affected by certain procedures which bring about death of the microorganism.

Materials and Methods

Bacterial Cultures.—The Phase II *Sh. sonnei* and *Escherichia coli* (strain B) cultures used for viral assays were subcultured daily on fresh nutrient agar slants. The original Phase II stock was obtained from the United States Army Medical School; the *E. coli* (strain B) was obtained from Dr. M. Adams of New York University.

The Phase II *Sh. sonnei* cultures used for adsorption rate experiments were obtained by inoculating 10 ml. of broth with 0.025 ml. of an 18 hour broth culture, followed by incubation at 37°C. with aeration for 3½ hours. The culture, still in its logarithmic phase of growth, contained between 2 and 3 $\times$ 10^8 bacteria per ml. as determined by colony count.

Bacteriophages.—The original T_3, T_4, and T_7 phage stocks were kindly supplied by Dr. M. Adams. Fresh stocks were prepared on *E. coli* (strain B).

Phage assays were performed by the agar layer method (5). The latent periods of the phages T_3, T_4, and T_7 in Phase II *Sh. sonnei* were determined by the one-step growth curve technique (6).

Antisera.—Antisera to T_3, T_4, and T_7 phages were obtained by injecting rabbits intravenously with filtered broth lysates containing at least 10^{10} plaque-forming particles per ml. The animals received an initial injection of 0.2 ml., followed by two of 0.4 ml. on alternate days. Three more injections of 0.5 ml. were given and finally two of 1.0 ml. were administered. After a rest of 1 week a test bleeding was made. If the neutralization titers of the sera was low, a second course of immunization was given. This consisted of three 1 ml. injections given on alternate days. Antisera were collected after a 1 week rest period. It was found that in the case of T_7 a third course of immunization was necessary.

Media.—Nutrient broth was employed both for the cultivation of the bacteria, and as the medium for suspending the cells used in the adsorption experiments. The medium was prepared by dissolving 8 gm. of desiccated Difco nutrient broth and 5 gm. of sodium chloride in 1 liter of distilled water. Enriched media were prepared either by adding 1 per cent casamino acids to the broth and neutralizing to pH 7.0 with 1 M sodium hydroxide, or by adding dialyzed meat extract prepared according to the method of Dole (7).

The T_3 and T_4 phages were assayed on nutrient broth-agar plates. Neopeptone plates were employed for the assay of T_7 phage, when using Phase II *Sh. sonnei* as the inoculum and were prepared as follows. To 1 pound of finely ground beef heart, from which excess fat had been removed, was added 1 liter of tap water. The mixture stood overnight at 4°C. and was then heated to 85°C. and filtered. To 1 liter of the filtrate was added 10 gm. of desiccated Difco neopeptone powder, 5 gm. of sodium chloride, 15 gm. of agar, and enough alkali to adjust the pH to 7.6.

EXPERIMENTAL

Latent Periods of T_3, T_4, and T_7 Coli-Dysentery Phages in Phase II Sh. sonnei.—Before the adsorption rate of the phages on viable Phase II *Sh. sonnei* could be determined it was first necessary to know the latent period (i.e. the time interval between the initial infection and the release of new phage) of each individual virus in this microorganism. It is obvious that without this knowledge adsorption rate measurements might be carried beyond the latent period when new phage would be released. The newly released phage would give an increased number of plaque counts which in turn would indicate an apparent low adsorption of virus during the later stages of the adsorption experiment. The latent period of T_3, T_4, and T_7 in Phase II *Sh. sonnei* was therefore determined. A typical experiment is as follows:—

A tube containing 10 ml. of broth was seeded with 0.025 ml. of an 18 hour culture of Phase II *Sh. sonnei* and the tube incubated at 37°C. with aeration for $2\frac{1}{2}$ hours. The bacterial concentration at this time was 4.15×10^7 B/ml. as determined by colony count.

To 1.8 ml. of the bacterial suspension was added 0.2 ml. of T_3 phage containing 1.66×10^8 P/ml. After 6 minutes at 37°C., 0.1 ml. of the adsorption mixture was added to 0.9 ml. of a T_3 antiserum (diluted 1:800). After standing $4\frac{1}{2}$ minutes a 0.1 ml. sample was removed from the serum tube and diluted to 10^{-1} in broth. Two further dilutions to 10^{-2} and to 3×10^{-1} were also made in broth. The latter were designated as the First Growth Tube (F. G. T.)

and Second Growth Tube (S. G. T.), respectively. All dilutions were made at 37°C. At the time intervals recorded in Fig. 1, 0.1 ml. samples were plated in duplicate from the F. G. T. and S. G. T. using Phase II *Sh. sonnei* as the host cell. The plates were incubated for 8 hours at 37°C.; plaque counts were made and recorded.

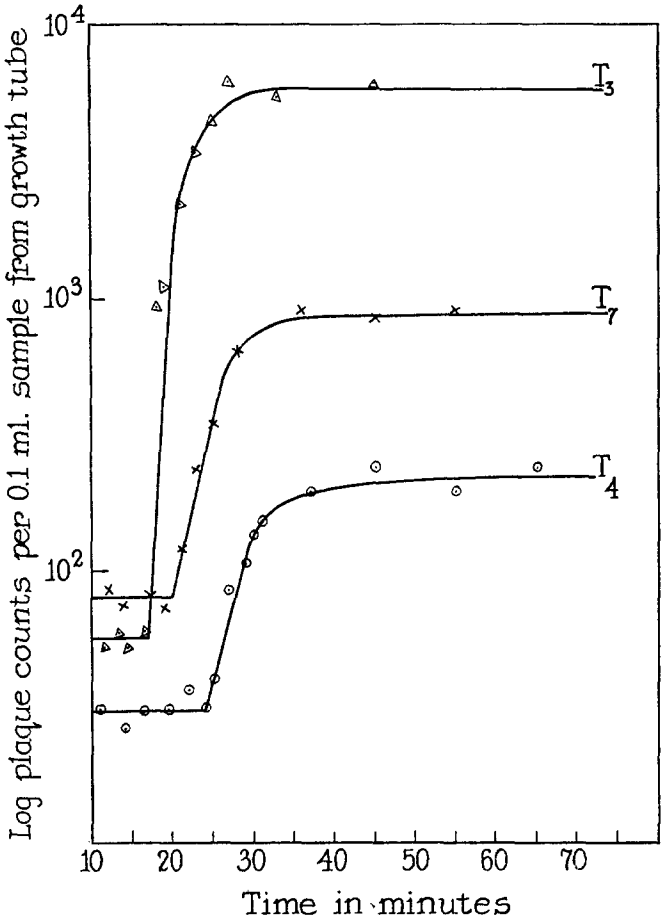

FIG. 1. One step growth curves of T_3, T_4, and T_7 *coli*-dysentery phages when grown in Phase II *Shigella sonnei*.

A separate serum control tube containing 0.9 ml. of T_3 antiserum was diluted with 0.1 ml. T_3 phage containing a 10^{-1} dilution of the input phage. After 4½ minutes an aliquot of this tube was diluted to 10^{-3} in broth and 0.1 ml. was plated in duplicate. No plaques appeared on these plates, thus indicating that neutralization of free phage had taken place. A separate phage assay was made from the adsorption mixture tube. The results of three separate experiments, in which the latent periods of T_3, T_4, and T_7 were determined, are presented graphically in Fig. 1 in which the number of phage particles released is plotted against time.

Since all unadsorbed virus was neutralized by the antiserum the mean of the plaque counts obtained before the rise represented the number of viable organisms initially infected. From the data plotted in Fig. 1 the average burst size per bacterium was calculated by dividing the number of phage particles finally released by the number of infected bacteria. The step size, or yield, was calculated by dividing the final phage titer by the input titer. The duration of both the latent period and the rise period (*i.e.* the time over which new phage is released) was obtained from the graph.

TABLE I

Comparison of the Growth Characteristics of T_3, T_4, and T_7 Coli-Dysentery Phages When Grown in Phase II Shigella sonnei

Phage	B/p	Latent period	Average burst size	Step size	Rise period
		min.			*min.*
T_3	2.2	17 ± 0.5	104	35.8	13
T_4	2.1	24.5 ± 0.5	7	2.2	12–13
T_7	2.2	20 ± 0.5	11.5	6.0	14–15

TABLE II

Latent Periods of T_3, T_4, and T_7 Coli-Dysentery Phages When Grown in E. coli (Strain B) and in Phase II Shigella sonnei

Phage	Time		Increase in latent period
	Phase II *Sh. sonnei*	*E. coli* B	
	min.	*min.*	*min.*
T_3	17 ± 0.5	13	4
T_4	24.5 ± 0.5	23.5	1
T_7	20 ± 0.5	13	7

A summary of the results obtained from three separate one-step growth experiments with T_3, T_4, and T_7 phages is presented in Table I. In each instance the B/p ratios were essentially identical. From these data it can be seen that the latent period of each virus in Phase II *Sh. sonnei* differs. In addition, the average burst size per bacterium and step sizes show wide variations. The rise periods, on the other hand, are essentially the same.

Comparison of Latent Periods of T_3, T_4, and T_7 Coli-Dysentery Phages in Phase II Sh. sonnei and in E. coli (Strain B).—Because Phase II *Sh. sonnei* has a longer generation time (22 minutes) when grown in nutrient broth than does *E. coli* (strain B) (19 minutes) it was of interest to compare the latent periods of the T_3, T_4 and T_7 viruses in these two microorganisms.

From the data summarized in Table II it is apparent that the latent periods

of all three viruses are longer when Phase II *Sh. sonnei* served as the host. The latent period of T_7 in the latter organism is some 50 per cent longer than in *E. coli* (strain B) whereas that of the T_3 and T_4 phages is greater by 30 and 5 per cent respectively. There does not appear to be any simple correlation between differences in the generation times of the two microorganisms and differences in the latent periods of the three viruses.

One-Step Growth Experiments of T_4 and T_7 Coli-Dysentery Phages at Different B/p Ratios.—In order to determine whether larger yields of virus could be obtained when T_4 and T_7 were grown in Phase II *Sh. sonnei*, one-step growth experiments were carried out under conditions of single infection and

TABLE III

One-Step Growth Curves of T_4 and T_7 Coli-Dysentery Phages When Grown in Phase II Shigella sonnei at Different B/p Ratios

Virus	B/p	Average burst size	Step size
T_4	9.5	1–2	1.0+
	3.7	1–2	1.0+
	2.1	7	2.2
	0.72	34	2.6
	0.42	52	2.4
	0.22	22	1.6
	0.094	1–2	1.0+
T_7	1.4	10	2.6
	2.2	17	6.3
	4.3	35	17.4
	4.7	28	13.0

of multiple infection where the B/p ratio was varied over a considerable range. In the case of T_4 the ratio of microorganisms to virus was varied over a hundredfold range, and over a three-fold range for T_7.

In Table III are recorded the results of such experiments. Here it can be seen that, when low multiple infection conditions prevailed, the growth of T_4 was optimum, although the step size remained low and was never greater than 2.6 under the most favorable B/p ratios. It is of interest that these observations are analogous to those obtained by Price (8) with a *Staphylococcus muscae* phage.

The experiments carried out with T_7 phage and Phase II *Sh. sonnei* showed that the best yields of phage were obtained at high B/p ratios under conditions of single infection. From the value of the step sizes it did not appear advantagenous to vary the B/p ratios beyond the limits chosen.

One-Step Growth Experiments with T_4 and T_7 Coli-Dysentery Phages in Differ-

ent Media.—It will be recalled (Table I) that T_3 was the only virus which grew well in Phase II *Sh. sonnei* when nutrient broth served as the medium. To determine whether better growth conditions could be found for the T_4 and T_7 phages, the yield of virus from Phase II *Sh. sonnei* was studied in different media, using the one-step growth technique. The results of these studies are presented in Table IV.

It is apparent, from the data summarized in the table, that the medium containing broth to which casamino acids had been added greatly increased the yield of phage as compared to that obtained in broth alone. The addition of individual amino acids to nutrient broth revealed that glutamic acid appeared to be an important growth accessory substance, for it gave rise to a

TABLE IV

One-Step Growth Curves of T_4 and T_7 Coli-Dysentery Phages When Grown in Phase II Shigella sonnei in Various Media

Phage	Medium	B/p	Average burst size	Step size
T_4	1 per cent casamino acids	1.7	16.2	2.5
	Broth + 1 per cent casamino Acids	2.2	175	64.1
	Broth + 1 mg./cc. Glutamic acid	4.1	43	11.1
T_7	Broth + 1 per cent meat Extract (7)	1.4	—	2.8
	Broth + 1 per cent casamino Acids	4.3	43	21.9
	Neopeptone broth	4.7	73	22.6

marked increase in yield of the T_4 phage. Fowler and Cohen (9) have also reported that T_2 phage, which is closely related to T_4, likewise requires glutamic acid when grown in *E. coli* (strain B) in a synthetic medium. In conclusion it should be noted that the latent periods of both the T_4 and T_7 phages were unaffected by variations in growth conditions despite the fact that the yields of viruses vary over a wide range.

Adsorption Rates of T_3, T_4, and T_7 Phages to Viable Phase II Sh. sonnei.—The adsorption rate of phage to bacteria follows a pseudomonomolecular reaction rate which can be expressed by the equation (2–4).

$$\frac{-dp}{dt} = KPB \tag{1}$$

where p = concentration of virus remaining free at time t, in the presence of the bacterial concentration B. The value K is defined as the adsorption rate constant in millilitres per minute.

The integration of equation (1) yields the form

$$K = \frac{\ln P_0/P}{t(B)} \tag{2}$$

It has been demonstrated (3) that the adsorption rate of phage is a function of the host's physiological state, and that optimum adsorption conditions are attained with bacteria only during their logarithmic phase of growth. In the experiments about to be reported phage adsorption rates were therefore determined, using bacteria in their logarithmic growth phase and under conditions of single infection. Phage concentrations of 10^7 P/ml. and bacterial concentrations of 2 to 3 $\times$ 10^8 B/ml. were employed. A typical experiment is described below:

15 ml. of broth was seeded with 0.08 ml. of an 18 hour broth culture of Phase II *Sh. sonnei* and the tube incubated at 37°C. with aeration for 3 hours. The bacterial concentration at the expiration of this period was 2.09 $\times$ 10^8 B/ml. as determined by colony count.

To 1.8 ml. of the bacterial culture at 37°C. was added 0.2 ml. of T_3 phage containing 10^8 P/ml. At 1, 2, 3, 5, 7, and 9 minute intervals, 0.1 ml. aliquots were withdrawn from the adsorption tube and added to separate 0.9 ml. portions of T_3 antiserum (diluted 1:100). After 4 minutes aliquots (0.1 ml.) were taken from each of the serum tubes. The serum tube containing the sample withdrawn at 1 minute was diluted to 10^{-2} in broth; all others were diluted to 2 $\times$ 10^{-2}. Duplicate 0.1 ml. samples from each of the dilution tubes were then plated. The plates were incubated for 8 hours at 37°C. and plaque counts made and recorded. A separate serum tube containing 0.9 ml. of T_3 antiserum (diluted 1:100), to which was added 0.1 ml. of a 10^{-1} dilution of the input phage, served as control. After 4 minutes a 0.1 ml. aliquot was diluted to 10^{-2} in broth and 0.1 ml. plated in duplicate. No plaques appeared on these plates indicating that free phage had been neutralized. An assay made from the adsorption mixture tube showed that the input virus titer was 1.08 $\times$ 10^8 P/ml.

In Fig. 2 are recorded the results of this experiment and those in which the T_4 and T_7 viruses were employed. Since all unadsorbed phage was neutralized by the homologous antiserum, the plaque counts represented the number of infected bacteria. Because the experiments were performed under conditions of single infection the unadsorbed virus was represented by the difference between the input phage and infected bacteria.

The log per cent of phage survivors was plotted against time as indicated in Fig. 2. By substituting the experimental values obtained for the bacterial concentration, time, and log per cent of phage survivors in Equation 2 the adsorption rate constant K is obtained. As an example the calculation of this constant for the T_3 virus in the experiment just described is as follows:—

$$K = \frac{2.303 \log P_0/P}{t(B)} = \frac{2.303 \log 100/50}{9.4 \times 1.88 \times 10^8} = 3.9 \times 10^{-10} \text{ ml./min.}$$

In a similar manner the adsorption rate constants were determined for Phages T_4 and T_7 and found to be 5.6 and 8.9 $\times$ 10^{-10} ml./min. respectively. The K values for viable cells indicated in Table V reveal that the T_7 phage has

the most rapid adsorption rate whereas T_3 and T_4 are adsorbed somewhat more slowly.

Adsorption Rates of T_3, T_4, and T_7 Phages on Phase II Sh. sonnei Killed by Various Methods.—It was previously pointed out that different preparations

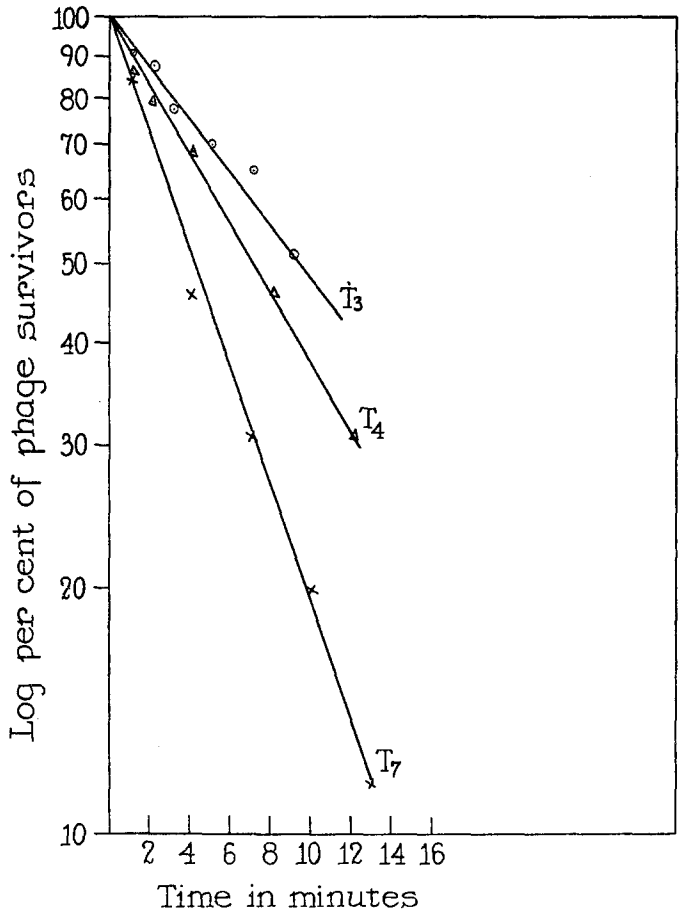

FIG. 2. Adsorption rate of T_3, T_4, and T_7 *coli*-dysentery phages on viable Phase II *Shigella sonnei*.

of the chemically purified somatic antigen obtained from Phase II *Sh. sonnei* showed variations in their ability to inhibit phage, in particular T_3. It was believed that these discrepancies arose either from the effects of the agents used for killing the microorganisms, or from the subsequent manipulations employed in the purification of the antigen. The effect of various physical and chemical agents on the adsorptive properties of Phase II *Sh. sonnei* was therefore studied.

(*a*) *Heat.*—The rate of adsorption of the three phages T_3, T_4 and T_7 on heat-killed organisms was first determined.

Aliquots of the cultures used for determining the adsorption rates of the three viruses on viable organisms were heated at 100°C. for 2 minutes. To 1.8 ml. of the heated culture was added 0.2 ml. of phage of the same concentration as that used in determining the rate of adsorption on viable cells. At the intervals indicated in Fig. 3, 0.1 ml. samples were withdrawn from the adsorption mixture and diluted to 5×10^{-3} in broth. Duplicate 0.1 ml. platings were made. The plates were incubated at 37°C. for 8 hours in the case of T_3 and T_7 and plaque counts made. When determinations of the T_4 virus were made, the plates were incubated for 18 hours. As in the previous experiments on viable organisms, the log percentage of unadsorbed phage was plotted against time and the results are shown in Fig. 3. The rates of adsorption were calculated as before and are recorded in Table V.

It should be pointed out that because of the poor adsorption of T_3 on the dead cells the determination of the adsorption rate constant K is not as precise as might be desired. The average of several determinations gave the value $K = 2 \times 10^{-12}$ ml./min. Despite the inaccuracy of the determinations it is apparent that the rate of adsorption of T_3 phage on heat-killed Phase II *Sh. sonnei* was some 200-fold less than the value obtained with viable cells. A comparison of the data presented in Figs. 2 and 3 and in Table V reveals that the rate of adsorption of T_4 to the dead cells has also been altered. The T_4 phage adsorbs on heat-treated organisms some ten times more slowly than on viable cells, whereas the T_7 phage adsorbed at approximately the same rate both on heat-killed and on viable Phase II bacteria.

TABLE V

Adsorption Rate Constants in Milliliters per Minute of T_3, T_4, and T_7 Coli-Dysentery Phages on Phase II Shigella sonnei*

Phage	Live bacteria	Bacteria killed with				
		Heat	Phenol	Formol	Ethylene oxide	Ultraviolet light
	K	K	K	K	K	K
T_3	4.3×10^{-10}	2.0×10^{-12}	Very slow	Very slow	Very slow	2.8×10^{-10}
T_4	5.6×10^{-10}	4.5×10^{-11}	2.2×10^{-10}	2.5×10^{-10}	6.6×10^{-11}	5.3×10^{-10}
T_7	8.9×10^{-10}	6.3×10^{-10}	8.8×10^{-10}	7.3×10^{-10}	5.4×10^{-10}	4.5×10^{-10}

* The values of K are the mean of two or more determinations.

(*b*) *Chemical Agents.*—The adsorption rates of T_3, T_4 and T_7 phages on bacteria killed by dilute phenol and formol were next determined.

To 30 ml. of a culture of Phase II *Sh. sonnei* containing 2×10^8 B/ml. was added 0.6 ml. of a mixture of equal parts of liquid (88 per cent) phenol, alcohol, and water, or 0.3 ml. of 37 per cent formol. After standing for 30 minutes the killed bacteria were separated by centrifugation, washed 3 times with 10 ml. portions of broth, and finally resuspended in 30 ml. of fresh broth.

The adsorption rates were determined as previously described and the data are assembled in Table V. At the concentrations of virus and bacteria employed, the adsorption of T_3 was too slow to permit an accurate determination of the

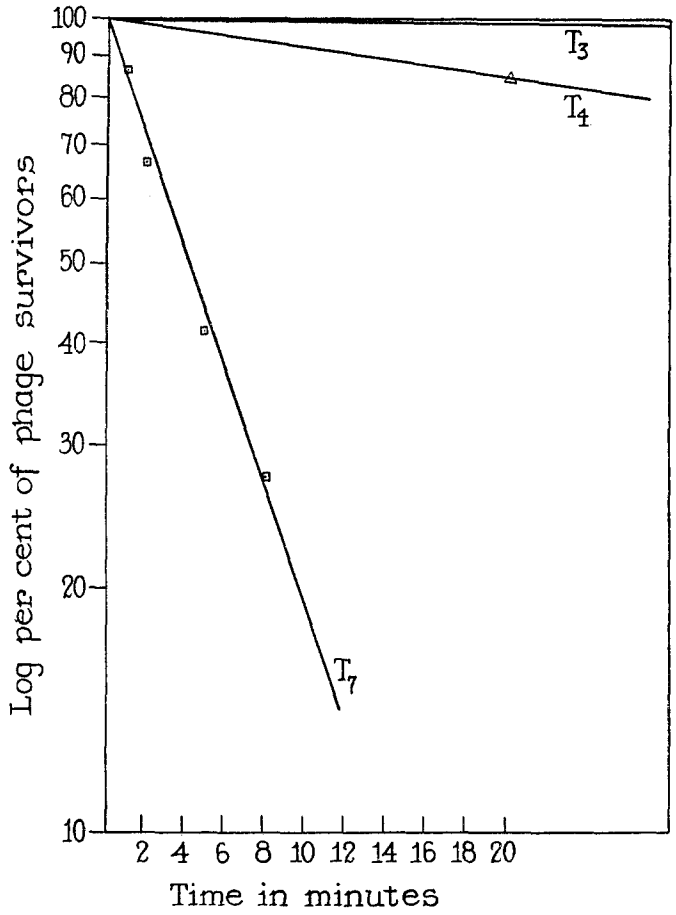

FIG. 3. Adsorption rate of T_3, T_4, and T_7 *coli*-dysentery phages on heat-killed Phase II *Shigella sonnei*.

adsorption rate constant. The adsorption rate of T_4, on the other hand, was reduced approximately by half, whereas that of T_7 was little affected by treatment with the chemical agents.

Ethylene oxide has been used by Wilson (10) for the sterilization of bacterial cultures. This substance possesses certain advantages over the usual bactericidal agents in that it is volatile at 37°C. and can be readily eliminated. Ethylene oxide is a highly reactive reagent and it is questionable whether this substance is suitable for killing microorganisms from which labile constituents

are to be isolated. Nevertheless, an experiment was undertaken to learn whether the adsorption properties of the Phase II *Sh. sonnei* were impaired by this reagent.

To 30 ml. of a culture of Phase II *Sh. sonnei* grown to a concentration of 2.5×10^8 B/ml. and cooled to 4°C. was added 0.15 ml. of ethylene oxide. The mixture was shaken and left in the cold room for 1 hour at 4°C., then warmed to 37°C. for 24 hours to remove excess ethylene oxide (10).

Adsorption rate determinations performed on the killed bacteria showed (Table V) that the T_7 phage was adsorbed on the dead cells at a rapid rate, while the T_4 phage was adsorbed at a rate below that obtained with live cells. The T_3 phage, on the other hand, was adsorbed very slowly.

Several additional bactericidal agents were employed in an attempt to find a suitable method for killing the cells which would permit uniformly rapid adsorption of the three phages. Among the chemical substances tried were 70 per cent ethanol, chloroform, thymol, and sodium periodate.

In general, cells killed with these reagents never permitted the T_3 phage to be adsorbed at a measurable rate. In addition the sonic[1] disintegration of the bacteria for 30 minutes likewise destroyed the receptor substance so effectively that no inactivation of any of the three phages could be detected. It was also found that successive freezing and thawing of the bacteria reduced the viable cell count to a low value and that the dead bacteria did not adsorb T_3 appreciably; however, the T_7 phage was still rapidly adsorbed. Osmotic shock brought about by suspending the bacteria in 7 M urea or in 4 M sodium chloride, followed several hours later by dilution with distilled water, yielded cells which failed to adsorb T_3; the T_7 phage, on the other hand, was adsorbed at a rapid rate. Table VI indicates the type of adsorption obtained with cells killed by these various procedures.

Ultraviolet Light.—Ultraviolet light has been extensively used for killing bacteria and viruses without incurring an apparent gross destruction of their labile components; it was desired therefore to learn whether the receptors of Phase II *Sh. sonnei* were destroyed by irradiation.

15 ml. of a culture of Phase II *Sh. sonnei* containing 2.5×10^8 B/ml. was placed in a 6 inch Petri dish covered with a quartz plate. The dish was irradiated by a Hanovia[2] ultraviolet lamp (radiation energy, 102 ergs per cm.^{2} per sec.) for 15 minutes at a distance of 25 cm. During irradiation the dish was gently shaken. Under these conditions the viable cell count was reduced to less than 0.001 per cent.

Determinations of the adsorption rates of T_3, T_4, and T_7 phages (Table V) on the irradiated bacteria showed that in each instance the virus was adsorbed

[1] Apparatus manufactured by the Raytheon Manufacturing Co., Waltham, Massachusetts.

[2] Hanovia quartz mercury resonance radiation lamp manufactured by Hanovia Chemical Co., Newark, New Jersey.

by the treated microorganisms but at rates below those observed for viable cells. It will be observed that of all the methods employed, short irradiation with ultraviolet light was the only one which did not appreciably destroy the receptor for the T_3 bacteriophage. It was found that excessive irradiation (30 minutes) destroyed the ability of the dead cells to adsorb the T_3 virus. Anderson (11) obtained similar results with T_2 and T_7 while investigating the adsorption of these viruses to irradiated *E. coli* (strain B).

TABLE VI

Effects of Various Agents on the Adsorption Properties of Phase II Shigella sonnei for T_3, T_4, and T_7 Coli-Dysentery Phages

Agent	Adsorption		
	T_3	T_4	T_7
1 per cent phenol	P	G	G
1 per cent formol	P	G	G
1 per cent ethylene oxide	P	F	G
70 per cent ethanol	P	—	—
0.005 M sodium periodate	P	—	G
0.01 per cent thymol	P	—	—
Chloroform	P	—	—
Heat 100°C., 2 min	P	F	G
Sonic vibration: 10 min	P	—	F
30 min	P	—	P
Freezing and thawing	P	—	G
Ultraviolet irradiation	G	G	G
Osmotic shock: saturated sodium chloride	P		G
7 M urea	P	—	G

P = poor; F = fair; G = good.

DISCUSSION

From the results which have been presented in this report there can be little doubt that the substance distributed on the surface of Phase II *Sh. sonnei* with which the T_3, T_4, and T_7 phages combine is sensitive to a number of physical and chemical agents. It is possible, furthermore, to impair the ability of the intact microorganism to combine with the T_3 phage without materially affecting this function in so far as T_4 and T_7 are concerned. In a recent communication Puck (4) has emphasized the important role which electrolyte concentration plays in the adsorption of phages to *E. coli* (strain B). From the data presented here it is evident, that the specific chemical nature of the surface substances present on Phase II *Sh. sonnei* likewise plays a very important role in the adsorption process. Most certainly, the ready appearance of virus-resistant bacterial mutants in mixtures of

phage and susceptible cells suggests that chemical changes in the nature of the bacterial surface substance are factors directly concerned with the adsorption of phage. The alteration, by artificial means, of cells capable of absorbing virus to cells which no longer adsorb is by no means unique. Both Hirst (12) and Burnett (13) have demonstrated that it is possible to destroy selectively the virus adsorption centers of erythrocytes by the periodate ion and by certain enzymes. These observations have led Burnett (14) to suggest that red cells exhibit a virus adsorption gradient for mumps, Newcastle, and influenza viruses. Hirst (15) further suggested that the surface of erythrocytes possesses a series of different receptors for these viruses A, B, C, etc. The experimental data obtained from our studies on the adsorption rates of the T_3, T_4, and T_7 phages on Phase II *Sh. sonnei* indicate that microorganisms killed in various ways can also exhibit a gradient of affinity for these three viruses. It has been seen that the adsorption rates of the three viruses follow a definite pattern, that of $T_7 > T_4 > T_3$. Regardless of the methods employed for killing the dysentery bacillus, the order of the adsorption gradient has consistently remained the same. It must not be thought that because it has been possible, by chemical or physical treatment of the cells to reduce the adsorption rate of one of the T viruses without affecting too greatly that of the others, it is necessary to assume that Phase II *Sh. sonnei* possesses a multiplicity of virus receptors. It is suggested that the receptor, as it occurs in its native state on the surface of living Sonne bacilli, is but a single substance having groups which are essential for the adsorption of these phages. The impairment of some of these groups diminishes the ability of the receptor molecule to adsorb the T_3 phage without greatly affecting this function in so far as T_4 and T_7 are concerned.

It will be recalled that a substance was indeed obtained from Phase II *Sh. sonnei* which showed electrophoretic homogeneity and which had the ability to inhibit *in vitro* the T_3, T_4, and T_7 viruses (1). This substance, the type-specific somatic antigen, was isolated from formol-killed bacilli. The purified complex inhibited in high dilution the T_4 and T_7 viruses although it was less effective against T_3. The fact that an effective inhibitor could be obtained from chemically treated bacteria suggests that the type-specific antigen may be distributed throughout the cell and only that portion which lies on the surface is inactivated by the agents employed.

SUMMARY

A study has been made of the effect of bactericidal agents on the phage adsorption properties of Phase II *Sh. sonnei*. The ability of this microorganism to adsorb T_3, T_4, and T_7 phages can be altered by treating the bacteria with chemical and physical agents.

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