STUDIES ON BACTERIOPHAGE

II. Inhibition of Lysis of Escherichia coli B by the Somatic Antigen of Phase II Shigella sonnei

By WALTHER F. GOEBEL, Ph.D.

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

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The characterization of substances which inhibit the multiplication of pneumonia virus of mice and mumps virus (1) and the identification of those cellular constituents with which certain bacterial viruses combine immediately prior to infection of the host and subsequent viral multiplication (2) have been subjects of investigation in these laboratories during the past several years. In undertaking the latter problem the T series of bacteriophages and the host cells *E. coli* B (3) and *Sh. sonnei* Phase I and II (4) were selected for study, first, because the viruses themselves have been so well characterized, and, second, because the antigenic structures of the two Sonne bacilli have been extensively investigated (5).

Distributed on the cell surface of smooth variants of dysentery bacilli is a protein-lipocarbohydrate complex; it is this substance which differentiates one serological type from the other, conferring upon the microorganism its type specificity (6). Although *E. coli* B is susceptible to each of seven of the T series of viruses employed the *Sh. sonnei* are not; the Phase I microorganism is attacked and lysed only by the T_2 virus and T_6, whereas the Phase II bacillus is susceptible not only to these two viruses, but to the T_3, T_4, and T_7 as well. In the case of the Phase II bacillus there are grounds to believe that it is the surface antigen with which the T_3, T_4, and T_7 viruses combine at the instant of infection, for it has been found that *in vitro* this material will inactivate all these three viruses and render them incapable of affecting young, actively growing bacilli. In the presence of appropriate cofactors, though not in their absence (7), this effect is produced by exceedingly small amounts of the protein-lipocarbo-hydrate complex. In contrast, the T_2 and T_6 viruses, which likewise attack Phase II *Sh. sonnei*, are in no way affected by the agent, and for this reason it is assumed that these viruses attach themselves to receptor sites on the surface of the Phase II organism wholly different from the specific protein-lipocarbohydrate complex.

In the following account it will be shown that *E. coli* B possesses an antigen serologically related to the protein-lipocarbohydrate complex of Phase II *Sh.*

sonnei, and that lysis of *E. coli* B by three of the T viruses, T_3, T_4, and T_7, can be inhibited by the somatic antigen of the Phase II dysentery bacillus. It will also be seen that at least one of these viruses is not a homogeneous population, but is comprised of particles having different affinities for the specific inhibitor and, in addition, contains particles some of which are capable of infecting both Phase II *Sh. sonnei* and *E. coli* B, and others only the latter.

Materials and Methods

Somatic Antigens of Phase I and II Sh. sonnei.—The Phase I and II somatic antigens of *Sh. sonnei* used in these experiments were obtained by methods previously described (5). Sterile solutions of the antigens were prepared as follows: 10 mg. of substance was placed in a sterile test tube. The latter was flamed for most of its length and after cooling the antigen was wetted with 0.5 cc. of 70 per cent alcohol. After standing for several hours 3.15 cc. of sterile 0.1 M Na_2HPO_4 was added, and the tube warmed to 50°C. until solution of the antigen was complete. 1.35 cc. of 0.1 M K_2HPO_4 was now added and the solution was ready for use.

Bacteriophages.—The bacteriophages were obtained from Dr. Mark Adams of New York University as was the strain of *E. coli* B. Freshly prepared stocks of the phages were employed and were obtained by infecting actively growing aerated cultures of *E. coli* B in nutrient broth with the particular virus under investigation. All experiments described in this communication were carried out in the latter medium except those with the T_7 virus in which a meat infusion–neopeptone medium was used. Fresh meat infusion appears to contain a cofactor of unknown nature which greatly enhances absorption of T_7 on Phase II *Sh. sonnei*. Viral assays were made by the technique of Hershey (8).

Antisera.—Antibacterial sera were obtained from groups of rabbits injected with *E. coli* B and *Sh. sonnei* Phase II respectively. In addition, antisera to the chemically purified Phase II somatic antigen were likewise prepared as previously described (5). In all instances injections of the immunizing agent were given for 6 consecutive days followed by a rest period of 1 week and then a second series of injections was made. Bleedings were taken 8 days later. It has been observed in this laboratory that the sera of some normal rabbits even in high dilution agglutinate *E. coli* B (9). Before immunizing the animals it is essential to test them for the presence of such agglutinins, and to discard those animals which show positive reactions. Since cultures of Phase I *Sh. sonnei* contain approximately 1 per cent of Phase II bacilli which are present because of spontaneous mutation (6), the sera of animals immunized with Phase I organisms usually show a low agglutinating titer for Phase II bacilli. In order to have monovalent sera the Phase I sera were absorbed with heated or formal-killed suspensions of Phase II organisms.

Agglutination and Precipitin Reactions.—In conducting the agglutination reactions it was found desirable to use in all instances as the agglutinogen 4 hour cultures of living organisms. The bacteria were washed once in nutrient broth before using. Since cultures of *E. coli* B have a tendency to agglutinate spontaneously when diluted in saline, dilutions of the vaccines and sera were made in nutrient broth. The test mixtures were incubated at 37°C. for 2 hours, and the reactions read after the tubes had stood for 18 hours at 5°C. Precipitin reactions were carried out in the conventional manner. When quantitative precipitin reactions were performed, the phototurbidimetric technique described by Libby (10) was employed.

Inhibition Tests.—Viral inhibition tests were performed as follows: The virus under investigation was diluted so as to contain approximately 2×10^8 particles per cc. Into each of four tubes was placed 0.5 cc. of a solution of inhibitor[1] at the appropriate concentration. The first

[1] It has recently been noted that different preparations of the specific antigen of Phase II *Sh. sonnei* exhibit variation in their ability to inhibit phage. The reasons for this are still ob-

tube in the series contained 0.5 cc. of inhibitor (2 mg. per cc.) which had been dissolved in 0.1 M phosphate buffer at pH 7.2; all subsequent dilutions (in increments of 10) were made in nutrient broth. A fifth tube served as a control, and contained 0.5 cc. of broth. 0.5 cc. of the virus dilution was added to each of the five tubes and the latter were incubated at 37°C. for 24 hours. 0.1 cc. of each inhibitor-virus mixture was plated, and plaque counts were made after 12 hours of incubation. In several instances a second control was included in the experiment in which virus was diluted not with broth, but with 0.1 M phosphate buffer (comparable to the first inhibition tube). The diluent made no difference, however, in the final plaque count. This test will also be referred to later as a test for the "resistance pattern" of a given virus.

EXPERIMENTAL

Agglutination Reactions of E. coli B and Phase I and II Sh. sonnei in Homologous and in Heterologous Antisera.—In order to study the serological rela-

TABLE I

Agglutination Reactions of Phase I and II Sh. sonnei in E. coli B Antiserum

Microorganism tested	Final dilution of antiserum						
	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12,800
E. coli B	4	4	4	4	3	2	0
Sh. sonnei Phase I	0	0	0	0	0	0	0
" " Phase II	4	4	4	3	2	0	0

4 = complete agglutination, clear supernate.
0 = no agglutination.

tionship between *E. coli* B and Phase I and II *Sh. sonnei*, agglutination reactions of the three microorganisms in *E. coli* B antiserum were first carried out. These tests are recorded in Table I. It can be seen that Phase II *Sh. sonnei* is agglutinated by *E. coli* B antiserum in high dilution, although the Phase I organism is not. This fact indicates that the Phase II bacillus possesses an antigen related to that on the surface of the colon bacillus. Further evidence in support of this is presented in Table II where it is seen that *E. coli* B agglutinates in the sera of rabbits immunized with Phase II bacilli, as well as in an antiserum prepared by injecting animals with the purified and electrophoretically homogeneous somatic antigen obtained from the Phase II dysentery organism. The latter serum presumably contains antibodies directed against only the specific antigen of the Phase II bacillus and not toward other surface components of the microorganism. In view of the fact that *E. coli* B is agglu-

scure but the observation is being actively followed up. A single highly active preparation was used throughout the work reported here. Preparations of the Phase II somatic antigen, when kept in the dry state for periods as long as 15 months, have shown no diminution in their ability to inhibit susceptible phages.

tinated by this antiserum, it seems reasonable to conclude that the colon bacillus possesses a surface antigen closely related to that present in Phase II *Sh. sonnei.*

Still further evidence is derived from the fact that solutions of the Phase II somatic antigen precipitate in *E. coli* B antiserum (Table III). To be sure, the reaction is not as vigorous as that in homologous antiserum, yet precipitation occurs with reasonably high dilutions of the Phase II antigen. It is unfor-

TABLE II

Agglutination Reactions of E. coli B in Phase II Sh. sonnei Antibacterial and Somatic Antigen Antisera

Serum tested	Microorganism tested	Final dilution of antiserum						
		1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400
Phase II antibacterial	Phase II	4	4	4	4	3	2	1
	E. coli B	4	4	4	3	2	1	0
Phase II antisomatic antigen	Phase II	4	4	4	4	3	2	0
	E. coli B	3	3	2	1	0	0	0

TABLE III

Precipitin Reactions of Phase II Antigen in Homologous and in E. coli B Antibacterial Immune Sera

Serum tested	Final dilution of test antigen			
	1:10,000	1:50,000	1:250,000	1:1,250,000
Phase II antisomatic antigen	++++	+++±	++	+
E. coli B antibacterial	++±	++	+	0

++++ = disk-like precipitate, clear supernate.

tunate that a reciprocal relationship could not be determined, because the surface antigen of *E. coli* B has not yet been isolated.

Agglutination Reactions of E. coli B and Sh. sonnei Phase II in Phase II Antiserum after Absorption.—In order to ascertain whether the antigens of *E. coli* B and Phase II *Sh. sonnei* are identical or merely similar, absorption experiments were performed as follows:—

3 cc. of a pooled serum of rabbits immunized with the chemically purified somatic antigen of the Phase II organism was diluted with 9 cc. of saline. To this was added the packed cells from the 3 cc. of a phenol-killed suspension of *E. coli* B containing 2×10^{11} bacilli per cc. After 2 hours incubation at 37°C. and 18 hours in the ice box, the bacilli were separated by centrifugation. The process was repeated a second and finally a third time. The serum was then tested for its ability to agglutinate both Phase II bacilli and *E. coli* B.

Since the immune serum originally contained antibodies directed only toward

the somatic antigen of the Phase II organism, absorption with *E. coli* B should remove agglutinins for both microorganisms if the antigens of Phase II and *E. coli* B are identical. If they are not, but are merely similar, then absorption with the heterologous organism should remove only those antibodies reactive with *E. coli* B, and leave those still specifically reactive with the homologous Phase II microorganism.

It is apparent from Table IV that absorption of the immune serum with *E. coli* B removes only agglutinins for the latter microorganism and leaves those still specifically reactive with the Phase II bacillus. Absorption of the serum with the heterologous microorganism has apparently diminished but slightly its agglutinative power for the homologous Phase II bacillus. Quantitative nephelometric precipitin measurements of the serum antibody reveal, however (Fig. 1) that some 30 per cent of the total antibody present in the original serum,

TABLE IV

Agglutination Reactions of Phase II Sh. sonnei and E. coli B in Phase II Antigen Antiserum after Absorption with E. coli B

Phase II antigen antiserum	Microorganism tested	Final dilution of serum						
		1:100	1:200	1:400	1:800	1:1500	1:3200	1:6400
Unabsorbed	Phase II	4	4	4	4	3	2	0
	E. coli B	3	3	2	1	0	0	0
Absorbed with *E. coli* B	Phase II	4	4	4	4	3	1	0
	E. coli B	0	0	0	0	0	0	0

and reactive with the homologous Phase II somatic antigen, was removed after absorption with *E. coli* B.

To varying quantities of a dilution (0.2 cc. of serum per cc.) of the original and absorbed sera, and at constant volume (1 cc.), was added 1 cc. of a solution containing 50 micrograms of Phase II antigen. The turbidity which developed was measured, and the results plotted as indicated in the accompanying figure.

The results of these experiments further substantiate the evidence already presented that the somatic antigen of Phase II *Sh. sonnei* is related to, but not identical with, that found on the cell surface of *E. coli* B. In this respect the two antigens are analogous to the capsular polysaccharides of Types III and VIII pneumococcus (11).

Inhibition of Lysis of E. coli B by the Somatic Antigen of Phase II Sh. sonnei.— It was previously pointed out that both Phase I and Phase II *Sh. sonnei* are susceptible to certain of the T bacteriophages and that lysis of the Phase II bacillus by T_3, T_4, and T_7 is inhibited by small amounts of the homologous bacterial antigen (2). It seemed of interest, particularly in view of the immunological similarity exhibited by *E. coli* B and Phase II *Sh. sonnei*, to determine

whether lysis of the colon bacillus by the T phages could be inhibited by the dysentery antigens.

The viral inhibition tests were performed as outlined above. In each instance varying quantities of the inhibitor were incubated with the virus under investigation for 24 hours before plating. Platings were made simultaneously on Phase II *Sh. sonnei* and on *E. coli* B. The plates were incubated 12 hours at 37°C. before counting the plaques.

The results of these experiments are summarized in Table V where it can be seen that lysis of *E. coli* B by T_3, T_4, and T_7 was indeed inhibited by the Phase II antigen, and that lysis of the organism by T_1, T_2, T_5, and T_6 was in no way affected. Although data concerning the Phase I antigen are not included in

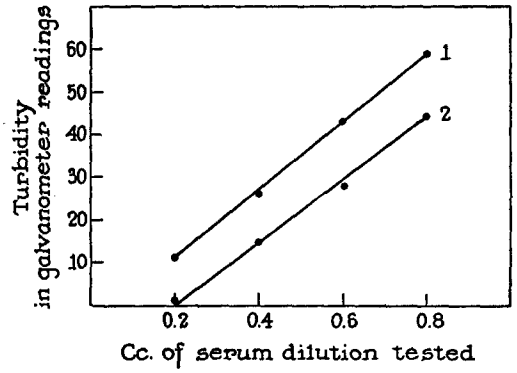

FIG. 1. Quantitative precipitin reaction of Phase II antigen in homologous antiserum before and after absorption with *E. coli* B.

1, serum before absorption with *E. coli* B.
2, " after " " " " " .

the table it should be stated that this substance caused no significant inhibition of any of the viruses. The significance of these results will be discussed later.

Susceptible and Resistant Variants of T_3.—It has been observed on occasions that certain stocks of T_3, T_4, and T_7 viruses, when stored at 5° C. over long periods of time, gradually lose their infectivity titers. In some instances these losses amount to as much as 3 log units. This change in titer is accompanied by a diminution in the capacity of the virus to be inhibited by the Phase II somatic antigen. Thus, a stock of T_3 virus which had been kept for 8 months had dropped in titer from 1.2×10^{10} to 5×10^7 particles per cc. When the resistance pattern of the original virus was determined it was found to be fully susceptible to the action of the inhibitor. Whereas 10^3, 10^2, and 10^1 micrograms of inhibitor inactivated in each instance 100, 99, and 65 per cent of the original virus, the aged virus showed 26, 7, and 0 per cent inactivation when tested with these same quantities of inhibitor. Similar observations were made on stocks of T_4 and T_7, though the decrease in susceptibility was not as striking.

In all instances the viruses could be restored to their original sensitivity by one or more passages on *E. coli* B in nutrient broth.

Since T_3 appears to be the least susceptible to the action of the inhibitor, this virus was chosen for study in order to ascertain whether it might not contain particles resistant to the action of the agent. That this proved to be the case will be seen from the following.

To 1 cc. of the T_3 stock containing 8×10^{10} particles per cc. was added 1 cc. of a solution containing 5 mg. of the Phase II somatic antigen. A control tube was likewise prepared containing 1 cc. of virus diluted with an equal volume of the same buffer as that used in preparing

TABLE V

Inhibition of Lysis of Phase II Sh. sonnei and E. coli B by the Somatic Antigen of Sh.sonnei Phase II

Plating microorganism	Virus tested	γ of inhibitor*			
		5×10^3	10^3	10^2	10^0
Sh. sonnei Phase II	T_1	0	0	0	0
	T_2	0	0	0	0
	T_3	99	89	34	11
	T_4	100	100	97	74
	T_5	0	0	0	0
	T_6	0	0	0	0
	T_7	92	87	44	9
E. coli B	T_1	0	0	0	0
	T_2	0	0	0	0
	T_3	91	78	22	6
	T_4	100	99	96	65
	T_5	0	0	0	0
	T_6	0	0	0	0
	T_7	94	90	50	6

* The figures represent per cent of virus inactivated.

the antigen solution. Both tubes were maintained at 37°C. and the virus concentrations determined in each at varying time intervals, using Phase II *Sh. sonnei* as the host cell. At the termination of the experiment the virus remaining in the tube containing the inhibitor was separated from the latter by centrifugation at 20,000 *g* for 1 hour. The virus was resuspended in 10 cc. of broth, again sedimented, and the washing repeated. The residual virus was now taken up in 2 cc. of broth and assayed (5×10^6). The resistance pattern of this virus, referred to in the accompanying table as T_3R_1, was then determined. The material was next divided into two equal portions. To one was added an equal volume of the inhibitor sólution used above; the other, which served as a control, was diluted with an equal volume of buffer. Again the decrease in virus concentration was followed at the indicated time intervals, and the resistance pattern of the remaining virus (T_3R_2) likewise determined.

The results of these experiments which are presented graphically in Fig. 2 and in Table VI reveal several salient facts. First, it should be pointed out

that the rate of inactivation of the T_3 virus is relatively slow. This is in contrast to the observation made on T_4 which is rapidly inactivated by the Phase II antigen (2). Second, it will be observed that by no means all of the virus

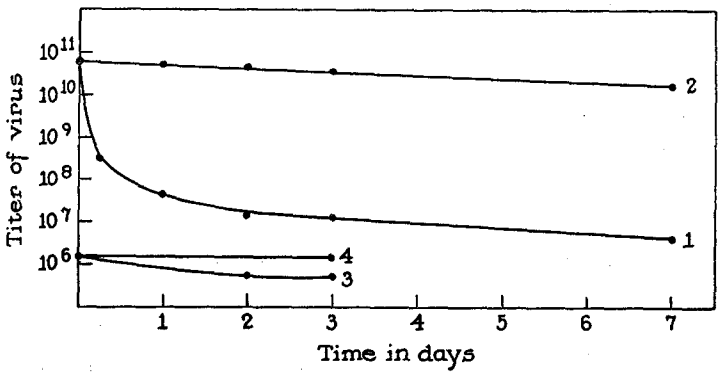

FIG. 2. Rate of inactivation of T_3 by the somatic antigen of Phase II *Sh. sonnei*.

1, T_3 on standing in presence of 2.5 mg. per cc. of Phase II antigen.

2, virus without inhibitor (control).

3, remaining T_3 virus in presence of fresh inhibitor.

4, virus without inhibitor (control).

TABLE VI

Resistance Pattern of T_3 and of the Residual Virus Remaining after Inactivation by Phase II Antigen

γ of inhibitor	Percentage virus inactivated		
	T_3	T_3R_1	T_3R_2
10^3	98	55	0
10^2	89	25	0
10^1	34	0	0
10^0	11	0	0
Ratio $\frac{\textit{E. coli}\ \text{B}}{\text{Phase II}}$	1.12		40.0

T_3 = original virus stock before treatment with specific inhibitor.
T_3R_1 = residual virus after first treatment with specific inhibitor.
T_3R_2 = residual virus after second treatment with specific inhibitor.

was inactivated by the inhibitor. In a 72 hour period the titer of the virus had dropped from 8×10^{10} to a constant value of 1×10^7. When the resistance pattern of this residual virus (T_3R_1) was determined (Table VI) approximately half of the viral particles were still found to be susceptible to the action of the inhibitor. The addition of new inhibitor to the sedimented and washed virus

brought about a decrease in titer of about one-half log unit. This residual virus (T_3R_2) was now entirely resistant to the action of the inhibitor as can be seen in Table VI.

Thus it appears that T_3 consists of a population of at least two types of viral particles, those which combine with the specific inhibitor and are rendered incapable of infecting the host, and those which are unaffected even by high concentrations of the inhibitor and retain their ability to infect the Phase II microorganism. These two types of virus will be referred to as susceptible and resistant. They appear to coexist in normal stocks of T_3 in the ratio of approximately 10^4:1, respectively. It will be shown later that in stocks of T_3 still another variant exists which is incapable of infecting Phase II *Sh. sonnei*.

Is Resistance a Stable Characteristic?—If the property of resistance exhibited by the residual virus described above is transmissible to new generations of viral particles, it should be possible to demonstrate this in the virus of plaques arising from resistant particles.

0.1 cc. of a dilution of the resistant virus T_3R_2 containing 2×10^2 particles per cc. was plated with Phase II *Sh. sonnei*. After 12 hours' incubation, ten of the plaques, which appeared on the plate and varying in size from 2 to 0.5 mm., were removed by means of a sterile glass eye-dropper of appropriate diameter and having a fine sharpened edge. The plaques, together with the agar beneath them, were each transferred to 5 cc. of sterile broth at 0°C. After standing for 15 minutes the tubes were warmed to 37°C. for 7 minutes, again cooled to 0°C., and filtered in the cold to sterilize. The ten virus preparations were next assayed, then an aliquot diluted so as to contain 2×10^3 particles per cc. An equal volume of a sterile solution of the specific inhibitor containing 0.2 mg. per cc. was now added to each of the ten viruses. The tubes, together with the proper controls, were incubated at 37°C. for 24 hours and then plated using Phase II *Sh. sonnei* as the host. One hundred micrograms of inhibitor per cc: appears to be the optimal minimum amount of inhibitor necessary to give complete inhibition of all susceptible particles.

If the viruses obtained from the ten plaques had regained the property of susceptibility, the inhibition experiments performed at this one concentration of inhibitor should suffice to determine whether the newly generated viruses were resistant or susceptible. Despite the fact that the ten newly formed clones of viral particles had originated from single particles which were resistant (assuming, of course, that the particles themselves are not mixed aggregates), it can be seen from Table VII that the progeny had regained susceptibility. In each instance the viruses derived from the ten plaques were as susceptible to the action of the inhibitor as was the parent stock from which the resistant virus had been obtained. These experiments reveal that the property of resistance is not transmissible under the environmental conditions employed, and therefore it must be considered an unstable characteristic.

A second attempt was made to produce a resistant stock of virus by infecting colon bacilli actively growing in broth containing 1 mg. per cc. of the specific inhibitor, with a suspension of the resistant viral particles. Under these condi-

tions it was thought that an environment unfavorable to the development of the susceptible particles but favorable to the development of resistant variants would be furnished.

3 cc. of nutrient broth containing 1 mg. of the Phase II antigen per cc. was inoculated with *E. coli* B. When growth had reached approximately 5×10^7 bacilli per cc. the tube was infected with a total of 3×10^5 particles of the resistant virus T_3R_2 obtained as described above. After approximately 2 hours of aeration the tube had cleared. The contents were filtered and the virus then placed at 37°C. and assayed at the end of 24, 48, and 72 hours. It was found that the titer diminished progressively from approximately 3×10^{10} particles to 5×10^8 particles in this time interval. A determination of the resistance pattern of the remaining virus showed that some 65 per cent of the viral particles were still susceptible to the action of the specific inhibitor. Repetition of the experiment, using the residual virus, yielded essentially the same results.

TABLE VII

Susceptibility of Virus Isolated from Ten Plaques Originating from Resistant Virus T_3R_2

Virus from plaque No.	Per cent virus inhibited by $10^{2}\gamma$ of Phase II antigen
1	97
2	94
3	97
4	95
5	97
6	98
7	90
8	96
9	93
10	93

The above experiment is further demonstration of the fact that infection of a bacterial population with the resistant variant of T_3 yields progeny which is predominantly susceptible. The rapid drop in titer of the virus after the final burst is due to gradual inactivation of newly formed susceptible particles by the inhibitor. The fact that the rate of inactivation is relatively slow makes it possible to observe this phenomenon. That the titer of the virus drops only approximately 2 log units and leaves a population containing some 65 per cent of susceptible particles, is to be explained on the basis that the concentration of inhibitor is simply not sufficient to bring about complete inactivation of all susceptible particles. Two successive passages of the virus in the presence of inhibitor were made. Each time the virus was permitted to stand first for 72 hours at 37°C. before infecting new host cells. Each time the virus was passed, the newly formed virus showed on standing the same relative drop in titer as did the virus of the first passage. Although the environment was deemed favorable for the development of resistant virus, still it was not possible to obtain by this means a stock containing a preponderance of the resistant variant.

A Variant of T_3 Specific for E. coli B.—If one assays a freshly prepared stock of T_3 on Phase II *Sh. sonnei* and at the same time on *E. coli* B, it will be noticed that a slightly higher count is obtained on plates infected with the latter microorganism. If enough plates are counted to be of significance, it is found that there are approximately 5 to 10 per cent more plaques on those plates for which the colon bacillus served as the host cell. If one now makes a differential count of the residual T_3 virus described above (*i.e.*, of the virus which is no longer inhibitable by the Phase II antigen) it is found that the ratio of the number of plaques formed on Phase II to those appearing on *E. coli* B has increased from 1:1.12 to 1:40 (see Table VI). The original T_3 stock, in addition to containing a variant resistant to inhibition by the Phase II antigen,

TABLE VIII

Assay of Virus Isolated from Six Plaques Originating from an E. coli B Specific Variant of T_3

Plaque No.	Average plaque count when plated on		
	E. coli B	Phase II	Ratio $\frac{\text{E. coli B}}{\text{Phase II}}$
I	101	57	1.79
II	116	78	1.49
III	101	59	1.71
IV	105	69	1.52
V	105	73	1.44
VI	114	74	1.54
T_3* (control)	210	188	1.12

* Original T_3 stock before treatment with specific inhibitor.

yet capable of infecting this cell, appears to contain still another variant. The latter is not inactivated by further addition of the Phase II antigen, and appears to be capable of infecting only *E. coli* B.

The so called resistant virus T_3R_2 was diluted so that on the average only one plaque was formed when 0.1 cc. of the dilution was plated with Phase II *Sh. sonnei*. When plated with *E. coli* B, this same dilution yielded an average of 41 plaques. Six of these plaques were now isolated and the virus recovered as described above. Each virus stock was assayed on *E. coli* B, then diluted to contain 1×10^8 particles per cc. 0.1 cc. of each dilution was now plated on Phase II *Sh. sonnei* and on *E. coli* B using four plates in each instance. After 12 hours' incubation the plaques were counted and the results tabulated (Table VIII). Since the resistant virus had been so diluted that the probability of selecting a plaque arising from a viral particle infective only for *E. coli* B, and not for Phase II *Sh. sonnei*, was 40:1, the chance was slight that one of the six plaques selected would be reactive with both organisms.

The results presented in Table VIII indicate quite clearly that transmission of the property of specificity for *E. coli* B, under the environmental conditions selected, cannot be achieved. The clone of viral particles in the six plaques

under investigation probably all arose from individual viral particles capable of infecting only *E. coli* B. Yet the new viral population in each instance consisted of particles capable of infecting both those cells and they occurred essentially in the same ratio as that in the virus stock from which the so called resistant virus had originally been prepared. In this respect the second variant shows unstable characteristics similar to those noted above.

DISCUSSION

When a susceptible bacteriophage is brought into contact with a sufficiently high concentration of a specific inhibitor, the two substances appear to interact and the virus is rendered incapable of infecting the host from which the inhibitor was derived. The nature of the chemical union between inhibitor and phage is not understood, nor is it yet known whether the virus itself is irreparably altered. Furthermore, it has not yet been ascertained whether infectious virus can be released from the inactive complex. Although the mechanism of its action is obscure, the fact remains that the somatic antigen of Phase II *Sh. sonnei* can specifically inactivate *in vitro* three of the T viruses, T_3, T_4, and T_7. It has been suggested therefore that this substance, distributed on the surface of the dysentery bacillus, serves as the receptor for the phages in question at the instant of inception of the infectious process. From immunological evidence presented in this communication it has been shown that an antigen similar to that of Phase II *Sh. sonnei* is present on the cell surface of *E. coli* B, and it is suggested that this antigen serves in a similar capacity when the colon bacillus undergoes infection with T_3, T_4, or T_7.

That the two microorganisms possess a similar surface antigen is substantiated by the fact that the colon bacillus agglutinates in an antiserum to the chemically purified dysentery antigen, and by the fact that the latter is precipitated by an antiserum to the colon bacillus. These antigens are not identical, however, for some, but not all, of the antibodies are removed from Phase II antigen immune serum by absorption with *E. coli* B. Since the two antigens show immunological crossing they must likewise be related chemically. It is reasonable to think, therefore, that these two substances can function similarly as receptors for the same viruses. The isolation of the new antigen from *E. coli* B has not been undertaken. It cannot be said therefore whether it is a single chemical entity, or a part of a more complex molecule which has affinity for others of the T series of viruses.

There are many examples among antigen-antibody reactions for which it has been shown that part but not all of the antibody protein combines with an heterologous antigen. One example was reported from this laboratory many years ago (12). It was shown that the native capsular polysaccharide of Type I pneumococcus possesses a labile acetyl group. When the latter was removed, the resulting deacetylated polysaccharide was still capable of reacting with Type I antibody. When sera were absorbed with the deacetylated polysaccha-

ride, a residue of antibody remained which failed to react with the chemically altered carbohydrate but was still reactive with the native polysaccharide. It is conceivable that the isolated antigen of Phase II *Sh. sonnei* has suffered alteration during its chemical purification. As finally isolated it may no longer represent an exact replica of the bacterial antigen as it occurs in its native state and hence cannot carry to completion the inactivation of all the T_3 viral particles in a given population.

Assuming this to be so, one might anticipate finding in a virus-inhibitor mixture only residual particles capable of infecting both host cells, Phase II *Sh. sonnei* and *E. coli* B. This is not the case, however, for it has been seen that the partially inhibited residual virus T_3R_1 is but slightly affected by fresh bacterial antigen. The decrease in titer which takes place is slight and equals the residual susceptible viral particles. The virus which remains, T_3R_2, is no longer affected by the specific inhibitor. Moreover, it contains a variant having a specificity different from that of the majority of the original viral particles.

Thus it has been shown that stocks of T_3, after treatment with the inhibitor, contain at least two variants, neither of which is inhibited by further treatment with the Phase II antigen. One of these combines with and infects both the Phase II dysentery bacillus and *E. coli* B; the other infects only the latter microorganism. This fact suggests the hypothesis that both microorganisms possess still other receptors through which these variants, and indeed other viruses, as well, can combine and bring about the infectious process. The nature of these receptors is not yet known.

Those who have studied the inhibition of phages by extracts of microorganisms (13) and by specific antisera (14) have come to the conclusion that viral populations are probably not homogeneous but may contain a small proportion of particles which are relatively resistant to these agents. Several investigators have demonstrated that the property of resistance cannot be transmitted to new generations of viral particles. In certain respects the action of the Phase II antigen of *Sh. sonnei* on the T_3 virus is comparable to that of a specific antiserum. When virus and inhibitor are mixed the majority of particles are rendered incapable of infecting the host cell but a small portion remains which is resistant to the inhibitor. In the case of the resistant virus T_3R_1 and the residual virus T_3R_2, which exhibits specificity only for *E. coli* B, it cannot be said whether these variants arise as a result of the action of the specific inhibitor on normal virus particles, or whether they are naturally occurring phenotypic variants. One fact seems to be clear, however, and that is neither of these properties appears to be transmissible to new generations of virus particles.

During the course of this investigation the author has discussed it often with Dr. Frank L. Horsfall, Jr., and with Dr. Ruth Sager. He wishes to express his appreciation for their interest and for their many stimulating suggestions.

SUMMARY

By serological means it has been shown that *E. coli* B contains an antigen closely related to the protein-lipocarbohydrate complex of Phase II *Sh. sonnei*. Lysis of *E. coli* B by three of the T viruses, T_3, T_4, and T_7, can be inhibited by the Phase II dysentery antigen. It has been suggested that the receptor of *E. coli* B with which these viruses combine is this newly described antigenic component. Two variants of the virus T_3 have also been described, in stocks which have been treated with the Phase II antigen. One of these variants infects both Phase II *Sh. sonnei* and *E. coli* B, and the other infects only the latter microorganism; neither of the two variants is inhibited by concentrations of the Phase II antigen of 1 mg. per cc. The distinctive properties of the variants are not hereditary.

APPENDIX

A Bacteriophage Plaque and Colony Counter

(IN COLLABORATION WITH MR. JOSEPH BLUM)

The counting of plaques can become a chore of no mean dimension in bacteriophage research. In this laboratory we have occasion to count as many as a 100 plates daily

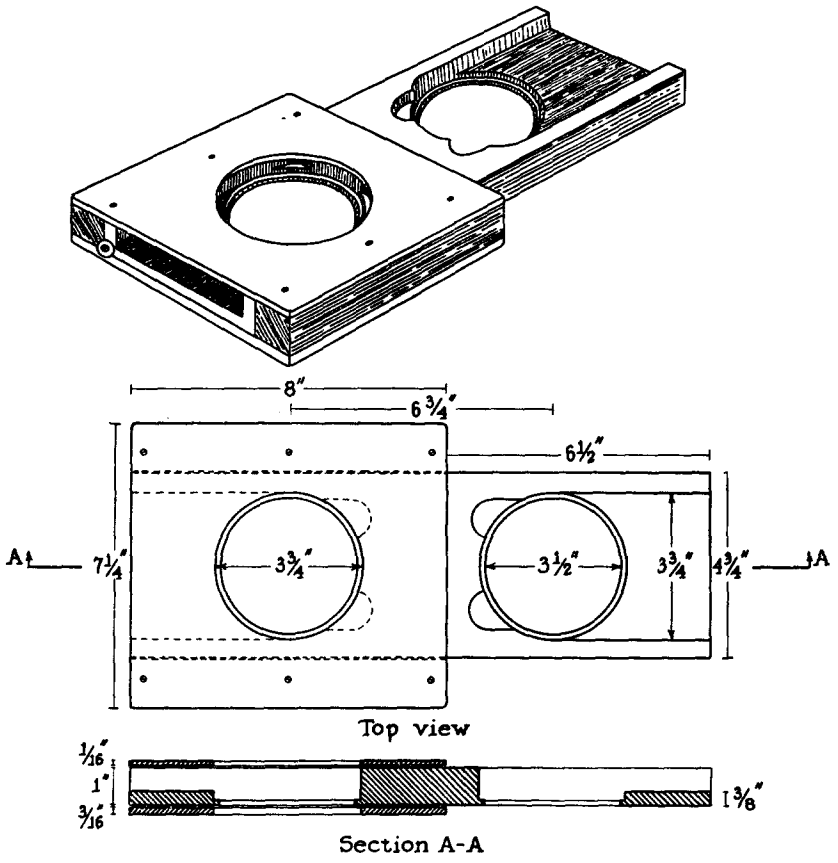

FIG. 3. Petri dish adapter.

and mechanical assistance in the execution of this task is essential. The conventional Quebec counter is helpful, and the addition of an electrically operated counting device described by Varney (15) is a decided improvement. Unfortunately this equipment is no longer available commercially nor to our knowledge is any other mechanical device for counting bacterial colonies. A simple apparatus has therefore been devised which has been of great assistance in counting accurately and rapidly bacteriophage plaques.

A 4 × 5 inch DeJur professional model photographic enlarger, having the light and

condenser housing separated from the bellows, was fitted with a sliding bakelite adapter built to carry two Petri dishes (Fig. 3). A 150 watt bulb served as the light source and an F 4.5 anastigmatic lens was fitted to the lens board. An electrical impulse reset counter manufactured by the Production Instrument Company, Chicago, model EC-94, was mounted adjacent to the enlarger post at an angle of 45°. An electrical contact switch in the form of an aluminum pencil holder in which a soft lead

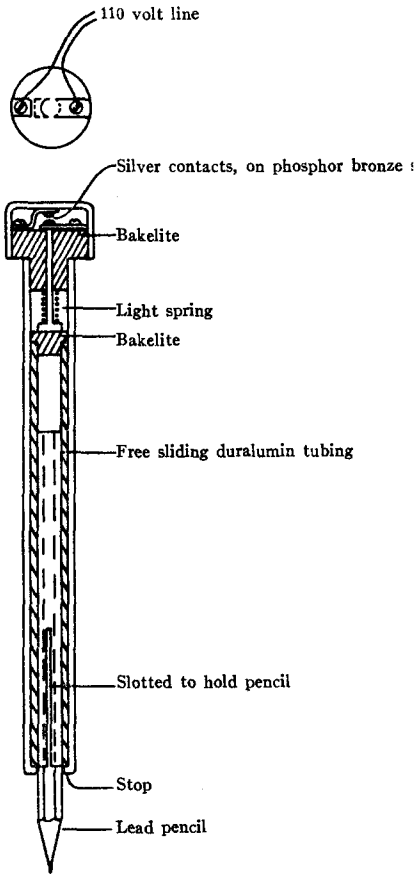

FIG. 4. Electric switch pencil holder.

pencil was inserted was wired in series with the counter and was operated from the 110 volt A.C. circuit (Fig. 4). When the tip of the pencil is touched to the paper, contact is made within the switch and the impulse is registered in the counter. The Petri dish without its lid, is inserted face downward in the adapter. The image of the dish, magnified two and one-half fold, is projected on a sheet of paper placed on the enlarger easel board. The images of the plaques can be counted either by touching individually, or by streaking five at once. A plate with 100 plaques can be counted in less than a minute.

If the Petri dish holder is constructed to hold the dish without its lid and with the agar half facing the lens, an image is had which extends to the very edge of the dish. Thus it is possible to see all the plaques on the agar surface except those which, because of the pouring technique, have crept to the perpendicular edge. If the pouring is carefully done, there will be few plaques in this position, and any error introduced by plaques which are not visible is small. This can of course be corrected by counting the latter in the conventional manner. This equipment can also be used to count bacterial colonies, to examine the morphology of phage plaques and bacterial colonies as well. Permanent negative records can be made by projecting the image on sensitized paper and developing the latter.

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