STUDIES WITH BACTERIOPHAGES ACTIVE AGAINST MUCOID STRAINS OF BACTERIA[1]

MORRIS L. RAKIETEN, ARNOLD H. EGGERTH, AND TONY L. RAKIETEN
Department of Bacteriology, Long Island College of Medicine, Brooklyn, New York

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Bacteriophages acting on mucoid strains of *Klebsiella*, *Aerobacter*, and *Escherichia* are strikingly different from most of the other lytic agents in two important respects (1) the plaques which are produced have an unusual and distinct morphology; and (2) they are highly type-specific in their action, this type-specificity closely paralleling their serological behavior.

The plaques, which appear when appropriate dilutions of phage are layered over previously inoculated Savita agar plates of a mucoid strain, consist of a clear area (the true plaque) varying in size from pin point to four millimeters in diameter, which is surrounded by a translucent or "ground glass" band (hereafter designated as "the zone"). These zones vary in size from one to five millimeters upon first appearance, and with further incubation the width of the zone increases until it may cover the entire plate culture (see figures 1, 2 and 3). The organisms found in the zone are not dead; they have, however, been deprived of their capsules and are avirulent. Bacteriophage can invariably be isolated from all parts of the zone as well as from the plaque itself.

Of secondary importance, is the possibility that phages may be very useful in classifying members of the genus *Klebsiella* into their respective types—since, with the limited number of strains that we have employed, the phages acting on *Klebsiella pneumoniae* Type A are restricted in their action toward these strains, having no lytic effect on "S" cultures of Types B and C. Phages

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acting on Type B strains have no demonstrable action on Type A and C strains. Phages lysing the two Type C strains are also specific.

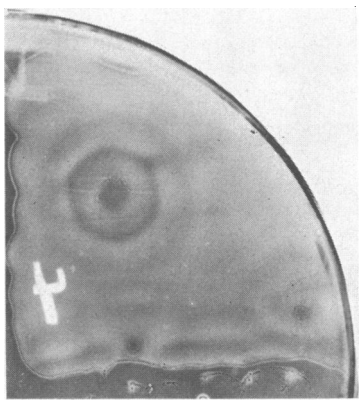

FIG. 1. PHAGE AMag ACTING ON KLEBSIELLA PNEUMONIAE TYPE A
Appearance of plaque with surrounding zone. Incubated at 35°C. for 18 hours.

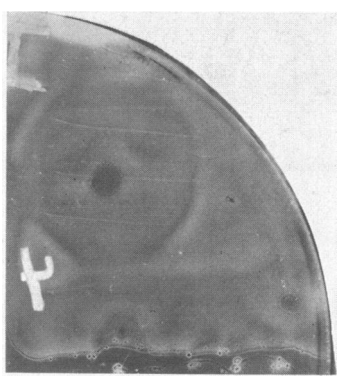

FIG. 2. APPEARANCE OF THE SAME PLAQUE AFTER INCUBATION FOR ANOTHER TWENTY FOUR HOURS AT 35°C.
Note increase in the size of the zone, but no increase in the size of the plaque.

Bacteriophages active against *Klebsiella* organisms were first reported by Caublot (1924), and by Hadley (1925). Both of these investigators mentioned that secondary cultures were devoid of capsule and lacked pathogenicity. Kimura (1925) produced mucoid variants of *Escherichia coli* which tended to be phage-

resistant, and he postulated that the presence of mucoid material may serve to prevent phage from attacking the bacterial cell. Others have, through the action of phage, produced mucoid variants, which themselves were lysogenic (Bordet and Ciuca, 1921; Gratia, 1921; d'Herelle and Beecroft, 1932). It has been assumed that the presence of capsule probably endows the organism with a protective coating that prevents bacteriophage from coming in contact with the cell surface. However, most strains of bacteria that produce capsule do so in the "S" state of their development,

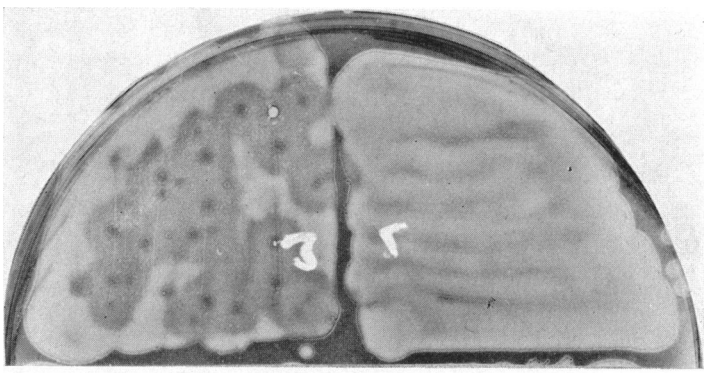

FIG. 3. ACTION OF A BACTERIOPHAGE ACTING ON A KLEBSIELLA PNEUMONIAE TYPE B STRAIN

All the plaques surrounded by zones, which have begun to coalesce. Incubation at 35°C. for 18 hours.

and it is precisely in this phase that organisms are usually most susceptible to bacteriophagy.

All of the phages used in this study were isolated from sewage. Briefly, the method consists in adding to 900 ml. of sewage water—made slightly alkaline by the addition of NaOH—100 ml. of ten-times concentrated Savita broth, pH 7.5. This mixture was then divided into a series of Erlenmeyer flasks—approximately 200 ml. per flask—and to each portion was added 10 ml. of an 18-hour broth culture for which a phage was being sought. After incubation at 35°C. for 18 to 22 hours a generous amount was filtered through an L_5 Chamberland candle. The filtrate was then tested for lytic action against homologous and other cultures. This method was generally successful whereas when the

sewage was filtered directly or incubated after adding only broth without culture, any phage that was isolated was a weak one.

The medium employed throughout this study was Savita broth and Savita agar (1 per cent).

PHAGES ACTING ON AEROBACTER AEROGENES STRAINS

Seven strains of bacteria belonging to this group were employed. Six phages isolated for strain Soil # 3 and one phage isolated for strain AW1 were tested for activity against our *Aerobacter* cultures as well as organisms belonging to the genus *Klebsiella*. All seven strains of *Aerobacter* were Voges-Proskauer positive; failed to produce indole and when tested by an India ink method,

TABLE 1

Activity of phages against Aerobacter aerogenes strains

PHAGE	AER. S #3	AER. #11	AER. AW1	AER. AF11	AER. M. STATE	AER. BYRD	AER. RVS.
Eg S3/37	4+	0	0	3+	0	0	0
Eg S3 1/38	4+	2+	0	3+	0	1+	3+
Eg S3 5/19	4+	0	0	2+	4+	0	0
Eg S3 111	4+	0	0	1+	0	0	0
Eg S3 111M	4+	0	0	3+	3+	0	3+
Eg S3 11/21	4+	0	0	1+	2+	0	0
Eg AW1	0	0	4+	0	0	0	0

4+, complete lysis; 3+, presence of a few colonies; 2+, plaque with many colonies; 1+, plaque covered with colonies; 0, no observable action.

all seven strains showed capsules. Strain S # 3 injected intraperitoneally into white mice killed them regularly within a period of 10 to 18 hours. The lytic effect of the various phages on the *Aerobacter* strains, when tested by the "cross test" (Asheshov, 1933) is set forth in Table l.

All of the S3 phages also lysed a strain of *Klebiella rhinoscleromatis* (R-243) and a few strains of *Klebsiella pneumoniae* Type B. None of these phages attacked strains belonging to Types A and C. The results listed above are those obtained when the *primary* sewage filtrate is used. When one of these filtrates is passed serially against strain S # 3 it loses its activity for all other organisms except the homologous one. This is due, undoubtedly, to the

dropping out of phage types, a phenomenon which is commonly encountered in developing sewage filtrates against single strains of enteric organisms. In contrast to the polyvalency of the primary filtrates isolated for *Aerobacter* strain S #3 the phage isolated for *Aerobacter* strain AW1 lysed only this strain and a culture of *Klebsiella granulomatis*.

The sewage filtrates containing active lytic principle for strain S #3 when plated against this strain on Savita agar gave rise to several different kinds of plaques. They varied from pin-point in size with an accompanying zone 1 to 3 mm. in diameter, to plaques having a diameter of 3.5 mm. encircled by a zone often extending 5 mm. from the edge of the plaque. Plaques without zones were also observed. Thirteen pure-line phages, using the technique described in an earlier paper (Rakieten, *et al*, 1937) were isolated from five of the sewage filtrates acting on strain S #3. While they were originally classified as pure-line on the basis of plaque morphology, it was found on further study that these thirteen types fell into six groups based on activity toward secondary cultures. This latter evidence signifies that in sewage filtrates certain phage types keep recurring. This is particularly interesting since in some instances several months elapsed between our attempts to isolate phages from sewage against this culture.

It became evident early in this study that the zones surrounding the plaques grew larger and eventually coalesced, but that the plaques, regardless of the length of time that the plates were incubated, did not increase perceptibly after the first twenty-four-hour incubation period. The increase in the zone occurs as regularly at room temperature as at incubator temperatures, and steadily progresses day by day until the entire plate culture has been acted upon. This consists chiefly in the conversion of the mucoid growth into one that is translucent. From the plaque itself, as well as from the culture within the zone, phage could be isolated. It is important to keep in mind that the zone progressively spreads through a culture which is many days old; but if one places phage on a twenty-four-hour old plate culture, in order to see whether any action can be observed, no change in the culture takes place where the phage has been deposited. When

phage is plated on non-mucoid susceptible variants of *Aerobacter* strains, plaques develop, but these plaques are not surrounded by a zone.

PHAGES ACTING ON KLEBSIELLA PNEUMONIAE TYPE B

All of the strains in this group are pathogenic for mice; all are indole-negative and are agglutinated by Type B anti-serum. They possess capsules and grow luxuriantly on Savita agar and in Savita broth. These cultures with the exception of strain EGS (from Dr. Julianelle) were from the collection of Miss Osterman, Department of Bacteriology, Yale University. Strain EGS was

TABLE 2

Action of sewage filtrates isolated against K. pneumoniae Type B strain Egs, on cross test

Klebsiella pneumoniae

PHAGE	TYPE B									TYPE A				TYPE C F10	AERO-BCT.		KL. RHINOSCL.
	Egs	Caroli	13	7	2328	17	coll	114	15	So	F5	Hlp.	Mag.		S #3	M. state	
B	4+	4+	4+	4+	4+	4+	3+	4+	4+	0	0	0	0	0	2+	4+	2+
B 11/24	4+	4+	4+	3+	4+	4+	4+	4+	4+	0	0	0	0	0	0	4+	4+
B 12/20	4+	4+	4+	4+	4+	4+	4+	4+	2+	0	0	0	0	0	0	4+	4+
B (x)	4+	4+	4+	4+	4+	4+	4+	4+	4+	0	0	0	0	0	2+	4+	4+
B 10/21	4+	4+	4+	2+	4+	4+	3+	4+	4+	0	0	0	0	0	0	3+	4+

considered as the type strain in this group, and five phages have been isolated against this strain from sewage by the use of the same method employed for *Aerobacter* phages. The results listed in table 2 indicate the action of these phages on homologous type strains as well as on others.

An inspection of the results listed above makes it quite clear that five different sewage filtrates isolated against one strain of Type B (EGS) exhibit strong lytic action on eight other Type B strains. No action is noted against Type A and two Type C strains. Three strains of *Aerobacter* were lysed by these filtrates (the two listed above) and strain Byrd. *Klebsiella rhinoscleromatis* strain R-243 is also susceptible to these filtrates. The

polyvalency of these filtrates is not lost when they are serially passed against strain EGS, as contrasted with what takes place when sewage filtrates active against *Aerobacter* strains are serially passed against *Aerobacter* strain S #3.

All of these phages produce plaques varying from 1 to 4 mm. in diameter, surrounded by zones extending 2 to 5 mm. beyond the edge of the plaque, when they are plated on smooth, mucoid Type B cultures. The plaques themselves, on continued incubation, remain fixed in size, but the zones continue to spread throughout the entire plate culture, converting the mucoid growth to one that is vitreous in appearance. This vitreous growth carries phage, does not possess capsule, and no longer is pathogenic for white mice. If a plate contains a large number of plaques when observed after 18 hours incubation, the mucoid portion not touched as yet by phage may be converted into translucent growth within another 6 to 7 hours incubation, by the consolidation of the adjacent zones (see fig. 3).

The secondary cultures isolated from within the zone do not regain the ability to produce mucoid colonies upon repeated inoculation into broth. Their biochemical activities parallel those of the parent mucoid strains, but in some instances certain changes have been observed. The secondary culture produced through the action of phage B on strain EGS became Methyl-red negative, whereas the smooth culture is Methyl-red positive, and the secondary culture produced by the action of phage B11/24 on strain 15 is Voges-Proskauer negative, while the smooth strain is Voges-Proskauer positive. Randall (1939) has also reported that certain of his variants produced by growing *Klebsiella* strains in lithium-chloride peptone water had biochemical reactions different from the original strains.

Pure line bacteriophages producing plaques with accompanying zones were isolated from some of the original sewage filtrates, using culture Egs as host strain. These pure-line phages produced plaques which did not differ from those observed when the original filtrates were used, the zones having the same ability to diffuse throughout the culture, so long as it remained mucoid. When the cultures were held at room temperature after the

plaques had appeared, while the zones diffused a little more slowly than at incubator temperature they ultimately spread through the mucoid culture until only translucent material remained. If only a few plaques were present originally, this process at room temperature took as long as three weeks—long after a culture is considered to be susceptible. Again, as with *Aerobacter* phages acting on secondary, non-capsulated strains, phages acting on *Klebsiella* Type B secondary cultures produce plaques but the plaques do not have any accompanying zones.

PHAGES ACTING ON KLEBSIELLA PNEUMONIAE TYPE A STRAINS

Four strains of Type A were employed. Two of these, Sc., and F5 were from Dr. Julianelle's collection, while the other two, Hlp. and Mag. were isolated in this laboratory. All four strains agglutinated with Type A anti-serum; were pathogenic for white mice; and produced large capsules on Savita medium. None of these strains produced indole. Six phages active against these strains were isolated from sewage—two of these using culture Sc. as the host strain; three using culture F5 as host and one using culture Mag. as the strain added to the sewage broth mixture. The activity of these sewage filtrates against the various cultures is indicated in table 3.

TABLE 3

Action of sewage filtrates isolated against Klebsiella pneumoniae Type A strains

PHAGE	TYPE A				TYPE B			TYPE C F10	KL. RHNO-SCL.	KL. OZENA.	KL. GRNU-LOMTIS.
	Sc	F5	Hlp.	Mag.	Egs	2328	17				
A Sc/1.....	4+	4+	4+	1+	0	0	0	0	2+	0	0
Eg Sc.....	4+	4+	1+	1+	0	0	0	0	0	0	0
Eg AF5/1....	4+	4+	4+	4+	0	0	0	0	1+	0	0
Eg AF5/2....	4+	4+	0	2+	0	3+	3+	0	4+	2+	0
Eg AF5/3....	4+	4+	0	4+	0	0	2+	0	1+	3+	0
Eg AMag.....	4+	4+	0	4+	0	0	2+	0	4+	2+	0

Klebsiella pneumoniae

While all of the primary sewage filtrates lysed the Type A strains certain of them also acted positively on two Type B strains. In sharp contrast to all of our other sewage filtrates three of the six produced against Type A strains as host also had lytic

power against a strain of *Klebsiella ozenae*. All of these filtrates also lysed the single strain of *K. rhinoscleromatis* that we tested. On passing any of these filtrates against their homologous culture the ability to lyse strains other than Type A, and *K. rhinoscleromatis* was lost. In other words, the filtrate became type specific.

These filtrates when properly seeded on a plate previously layered with any of the smooth, mucoid Type A strains, after an incubation of 12 hours exhibited plaques having large zones (see figs. 1 and 2). The spread of these zones on increased incubation, either at room temperature or that of the incubator, duplicated in every detail the description of the spread of zones surrounding plaques of Type B phages.

Secondary cultures within the zones, or produced in broth at the expense of one of these phages, did not possess capsules, and were in most instances avirulent for white mice when injected intra-peritoneally. However, heart-blood cultures from mice that did die after being injected with secondary culture did not produce mucoid colonies on plates, and were not susceptible to the phage that was responsible for their growth. At intervals of 10 to 16 days, mice injected with secondary cultures produced from pathogenic Type A strains and which showed no ill effects, were sacrificed and their entire spleens removed aseptically. The spleen was placed in a tube of broth and incubated for at least five days before being discarded as negative. Growth when it was observed appeared by the third day, and this growth when studied was found to consist of the secondary culture to which the animal was originally subjected.

PHAGES ACTIVE AGAINST KLEBSIELLA PNEUMONIAE TYPE C

We have only two strains of *Klebsiella pneumoniae* Type C, and utilizing culture F10 (from Dr. Julianelle's collection) as host strain we have isolated three phages from sewage filtrates. The activity of these filtrates on the Type C strains and certain other cultures is summarized in table 4.

These filtrates lost their ability to lyse *Aerobacter aerogenes* strain #11 when they were passed serially three times against either of the two Type C cultures. However, they retain their

activity to lyse the strain of *K. rhinoscleromatis*. These phages also produce plaques with surrounding zones when plated against smooth, mucoid, homologous culture. The spread of these zones parallels in all respects that observed with the other phages previously described.

We have also isolated four mucoid strains of coliform bacilli from individuals with cystitis and colitis. All four strains produced indole and were Methyl-red positive, and one strain in addition was Voges-Proskauer positive. Phages isolated from sewage against these four strains were strain specific, lysing only the particular strain of *E. coli* that had been added to the sewage-water-broth mixture prior to incubation. These phages when

TABLE 4

Action of sewage filtrates isolated against Klebsiella pneumoniae Type C F10

Klebsiella pneumoniae

PHAGE	TYPE C		TYPE A SC	TYPE B EGS	AER. S #3	AER. #11	KL. RHINOSCL.
	F10	Thck.					
Eg C/1.....	4+	4+	0	0	0	3+	3+
Eg C/2.....	4+	4+	0	0	0	3+	3+
Eg C/3.....	4+	4+	0	0	1+	3+	3+

placed against their homologous cultures produced plaques with zones which on continued incubation diffuse through the mucoid growth in the same manner as we have noted previously for other phages acting on mucoid strains.

SUSCEPTIBILITY OF SECONDARY CULTURES

The phages that were utilized for the production of secondary cultures from smooth, mucoid susceptible strains were either type or strain specific. A phage was considered to be a type-specific one when it lysed only cultures belonging in that particular group, as for example a phage that lysed only *Klebsiella pneumoniae* Type A strains. As described in an earlier section, this high degree of specificity could be obtained when any of the sewage filtrates was passed serially against a susceptible member of that type. Certain of the phages on first isolation were strain specific,

particularly those acting on mucoid varieties of *E. coli*, the one active against *Aerobacter aerogenes* strain AW1, and the one lysing *Klebsiella granulomatis*. For the production of secondary cultures the following method was used: To each tube of Savita broth (10 ml.) was added one drop (0.05 ml.) of a fresh 18-hour broth culture of the smooth strain and five drops (0.25 ml.) of phage. The tubes were kept in the incubator until growth appeared. In general complete clearing in the tube containing phage and culture lasted approximately 48 hours and then growth appeared. With certain of our Type B phages and those active against *Aerobacter aerogenes* S #3 frequently two weeks elapsed before any secondary growth was noted. On plating these cultures non-mucoid, translucent colonies appeared. Capsules could not be demonstrated by an India ink method. When these

TABLE 5

Action of phages on smooth and secondary culture of Klebsiella pneumoniae Type B

	BP. B11/24	BP. AF5/1	BP. C/1	BP. ABR. 83
Secondary culture EGS.....	0	2+	2+	3+
Original culture EGS.....	4+	0	0	0

secondary cultures were tested for susceptibility to phages other than those that produced them we noted that, in contrast to the high degree of phage specificity exhibited by the smooth culture, most of the secondaries were susceptible to phages that had no effect on the smooth parent strain. An example of the marked difference in susceptibility between a secondary culture of Kl Type B and the original mucoid strain is set forth in table 5.

The secondary cultures of Type A were not found to be susceptible to phages to which smooth parent strains were resistant, but this class of secondary cultures was the exception, most of the other secondaries that we produced giving results similar to those above.

DISCUSSION

Bacterial strains which under ordinary conditions of growth produce mucoid colonies and which when viewed under the mi-

croscope are encapsulated, are as susceptible to bacteriophagy as organisms that are commonly regarded as non-mucoid, or non-capsulated. The phages that attack such strains are usually found in sources that are rich in lytic principle for all of the members of the enteric group of bacteria. When placed in proper dilution on plate cultures of smooth, mucoid varieties of bacteria belonging to the genera *Aerobacter*, *Klebsiella*, and *Escherichia*, these phages produce plaques varying in size, and surrounding the majority of these plaques are zones, which in most instances are considerably larger in size than the plaques.

A considerable number of investigators have studied phages from the standpoint of plaque morphology and have observed plaques which have accompanying zones. We have especially observed this when studying sewage filtrates for their ability to produce plaques on cultures of *E. coli*. Such zones, however, do not increase in size on further incubation over a period of several days. Furthermore, when plaques accompanied by zones were picked for further purification with the idea of eventually making a pure line phage, it was observed that with each passage and plating the zone diminished in size and that often by the third serial passage the plaques that appeared did not possess zones.

In 1929 Sertic described a bacteriophage that acting upon a strain of *E. coli* produced plaques with zones, and that with increased incubation time the zones spread throughout the culture. The material in the zone was found to contain a lytic principle, but this was not transmissable in series; and acted on dead as well as living culture. This material which Sertic designated as the *lysin* of the phage will pass through ultra-filters that will retain the phage. This very interesting report of Sertic has been corroborated by Asheshov (personal communication) and Lominski (1937, 1939). More recently Sertic (1935) has described other phages that produce plaques with surrounding zones, and from which a lysin may be demonstrated. Dr. Sertic has very kindly sent us his susceptible strain of *E. coli* (Fb) with a pure line phage (FCZ) and we have in the main been able to repeat his experiments. However, on numerous occasions when we studied other phages isolated by ourselves which produced plaques with

surrounding zones on strains of *E. coli*, on attempting to isolate a lysin from these zones, we failed. In those instances only phage, and not lysin was obtained.

The zones surrounding plaques produced by phages that we isolated for mucoid strains of bacteria will not disappear when these phages are passed serially. So long as the cultures remain in the "S" phase the phages always reproduce plaques with zones. The plaques have never been observed to increase in area after the first twenty-four hours, regardless of the length of incubation period. In distinct contrast to this, the zone surrounding the plaque regularly increases in size so long as there remains mucoid growth on the plate. The course of the zone is easily followed since there is a difference in the appearance of the culture within the zone. Here the culture is vitreous looking, whereas outside of the zone culture, that has as yet remained untouched, it is mucoid and opaque. So long as any mucoid growth remains on the plate the zone continues to spread until the culture has lost completely its mucinous character. It is important to keep in mind that a few weeks may elapse before the entire plate culture has been acted upon by the phage, the period of time depending on initial number of plaques and the incubation temperature. With a single plaque it may take as long as three weeks at incubator temperature; with many plaques the zones coalesce and the process of decapsulation may be only a matter of several hours after plaques have appeared.

Some of these phages produce plaques that are free from secondary colonies and from within the plaque phage may be re-isolated, passed in series against homologous culture and the final filtrate may again give rise to plaques when plated. From the zone, however, living culture can always be isolated. This culture is free from capsule, does not produce mucoid colonies, and always carries bacteriophage. That this is phage and not lysin is evidenced by showing that filtrates of these cultures produce plaques; that the filtrate can be passed in series against susceptible mucoid strains; and that the filtrate is inactive against the vitreous culture within the zone. In a later report experimental evidence in detail, dealing with our attempts to prove that the zone surround-

ing a plaque is something other than phage, will be given. When one attempts to demonstrate plaques by placing phage (from broth) on a twenty-four-hour old culture of a mucoid strain it generally results in failure. And yet in cultures that are weeks old, the phage manifests itself by the increase in the spread of the zone through the mucoid growth.

The phage obtained from the zone will lyse only smooth mucoid culture, but not the culture from the zone (which is its secondary). It is very remarkable, however, that the phage obtained from the plaque lyses (on cross tests) not only the smooth mucoid culture, but the secondary from the zone as well.

At first glance it would appear that we were therefore dealing with two different phages; only one being present in the zone, but both being present in the plaque. Yet every attempt to separate two phages from the plaque itself has failed. The usual replating procedures for separating pure line types failed; every plaque obtained showed both types of phage action. Attempts to isolate a component that lyses the secondary from a component that does not, by propagating the phage against secondary cultures, have also failed; after many such serial passages, the resulting phage produces plaques that have the same phage-composition as the original plaques (or, are identical in every way with the original plaques).

We are forced to conclude, therefore, that if we are actually dealing with two phages, then there is one in the plaque, which lyses both cultures; and that the one in the zone is continually derived from this parent phage by some process analogous to antigenic phase variation in certain bacteria.

Plaques are regularly produced by phage against secondary cultures of these mucoid strains, but no zones characterize these plaques.

The reason for the continued spread of the phage on mucoid cultures is not known. The change brought about by the action of the phage is not, as far as we can observe, lysis of the cell body, but only a decapsulation, a "melting away" of the mucinous substance; and what remains is living secondary culture. This may be because of the relatively low degree of susceptibility of plate cultures many days old, resulting only in an incomplete action on

the part of the phage. Or the possibility exists that even on a plate culture many days old some cell proliferation is going on and the phage multiplying against this susceptible portion of the culture gives the appearance of diffusion. However, one can demonstrate the high degree of diffusibility of these phages by seeding plates in such a manner with susceptible culture that between the

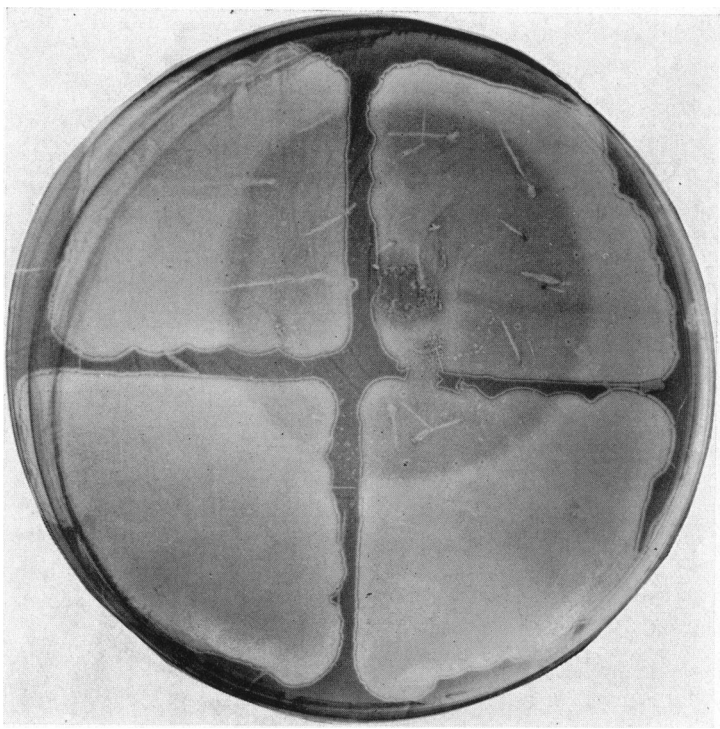

FIG. 4. BACTERIOPHAGE B ACTING ON TYPE BEgs.
Eight days incubation at 35°C. Note the spread of the phage (Zone) across bare uninoculated agar.

areas of culture considerable uninoculated agar separates them. Touching only one of the inoculated culture areas with phage, one may observe after several days of incubation the zone surrounding the original plaque has spread across the uninoculated bare agar and coming in contact with mucoid culture has decapsulated it and continues to do so as long as mucoid growth exists (fig. 4). If one cuts out of the plate a portion of the bare unino-

culated agar in the path of the diffusing phage, and places it in a tube of broth with susceptible culture bacteriophagy occurs. Finally the rate of spread on a plate after two weeks at room temperature is just about the same as that which one observes during the first three days. If the increase in the zone is due to the presence of some enzyme system in the phage, acting particularly on the capsular substance of the organism we have not been able to demonstrate it.

That the nature of the bacterial capsule does play some role in a culture's susceptibility to bacteriophage is evidenced by the type-specific action of phages attacking members of the *Klebsiella pneumoniae* groups. So long as these strains remain encapsulated they are susceptible to phages active against only that particular type. Without capsule they become susceptible to phages acting on other types. Type A strains may however, prove to be an exception to this statement, secondary cultures of Type A strains that we have worked with being susceptible only to phages that act on the smooth strains in this group. Providing one has a specific phage acting on a single type of *Klebsiella pneumoniae* this phage may be useful in classifying organisms belonging in this group.

CONCLUSIONS

The findings that have been described in this report may be summarized thus:

1. Bacteriophages acting on mucoid strains of bacteria are type-specific when acting on members of the *Klebsiella* group of organisms, strain specific for mucoid varieties of *Escherichia*, and may be strain or fairly group specific for organisms of the genus *Aerobacter*.

2. These phages have an unusually high degree of diffusibility, spreading through plate cultures long after the organisms have reached their most active period of proliferation.

3. As a result of this action, the mucoid culture loses its capsule, becomes susceptible to phages which may not act on the mucoid phase, and is no longer pathogenic for white mice.

4. The conversion of a smooth encapsulated pathogenic species

to what may be termed on "R" state may be brought about within a short period of time by the action of a bacteriophage.

5. Specific phages may be useful in classifying *Klebsiella* strains into their respective types.

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