THE SPECIFICITY OF BACTERIOPHAGES FOR DIFFERENT STRAINS OF ESCH. COLI AND ESCH. COMMUNIOR

By

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John O. Boyd, Jr.

INTRODUCTION

Although bacteriophage was discovered some twenty-odd years ago, little is yet known of its real nature. It has been investigated extensively, but is still best known by its action on micro-organisms susceptible to it.

When a small amount of bacteriophage is added to a young culture of susceptible organisms it brings about their dissolution, or lysis within a few hours. The time required for this phenomenon is variable, but the importance of the result is readily recognized and was immediately of great interest to medical men. At first it appeared that in this substance had been found a "cure-all" for all bacterial diseases, and it has been from the therapeutic as well as the physico-chemical standpoints that it has been investigated.

The subsequent work with this material did not bear out the glowing reports of D'Herelle. The results from its therapeutic use were so inconsistent that it was not long before its use had been generally discarded. Many explanations were offered for the variable clinical results. Although D'Herelle (8) states that body fluids do not inactivate phage, Colvin (5 &6) reports to the contrary. The idea of greater specificity of the phage to the susceptible organism has also been advanced, but very little work has been done to demonstrate the degree of specificity that does exist.

In this work, the author has assumed that a specificity comparable to serological specificities does exist and has endeavored to show this with different strains and species of the genus Escherichia. The proof of a species or strain specificity in this genus would warrant the hypothesis that such specificity does also exist in other pathogenic genera and thus a better explanation for the therapeutic inconsistencies resulting from the use of commercially prepared phages might be advanced.

HISTORY

It was in 1915 that bacteriophage was first recognised. Twort isolated a filterable substance from the exudate of a boil which when added to a young culture of the staphylococci isolated from this boil produced the dissolution of the young culture. Two years later, D'Herelle (8) isolated from the feces of a patient recovering from dysentery a filterable substance which when added to a young culture of Shiga dysenteriae produced the clearing of this culture. These workers were working independently and although Twort first described the phenomenon, D'Herelle deserves equal credit for his work. It is for this reason that it is frequently referred to as the Twort-D'Herelle phenomenon, but D'Herelle gave it the name of bacteriophage.

D'Herelle first made this substance the subject of an extensive study. He found that bacteriophage could be isolated from sewage which would be active for many different organisms, and his publications attracted wide attention among bacteriologists and medical men.

Much of the work with bacteriophage has been concerned with its therapeutic use in disease. Being a physician, it is natural that this aspect of the phenomenon appealed to D'Herelle, and he isolated many different races of phage active against a number of different pathogens.

His results were most remarkable, and he attributed to phage a role of utmost importance in the recovery of patients from bacillary infections, particularly those of the gastro-intestinal tract. In cases of typhoid fever and bacillary dysentery he noted that recovery was first evidenced by the appearance in the feces of a particularly active phage against the pathogenic organism concerned in the disease. He found that doses of phage by mouth to patients suffering from typhoid or dysentery produced rapid recovery.

The above results of D'Herelle naturally attracted much attention, and phage came into prominence in the treatment of many diseases. However, the reports in the various medical journals differed widely, some being very favorable while in other cases the results were entirely negative. Therefore, it is understandable that in recent years the use of phage therapeutically has become almost unheard of.

Nevertheless, the advantages of phage, if the phenomenon could be constantly produced, has continued to hold the interest of many, and numerous men have continued to work with this substance in the effort to discover more about it, and if possible the reasons for the inconsistent results obtained in its therapeutic use. Among the most plausible explanations is the possibility of its inactivation in the body. D'Herelle (8) says that body fluids (blood serum, pleural exudate, etc.) do not

have a harmful effect on bacteriophage, but that bile is unquestionably inhibitory. Colvin (5 & 6) reports definitely to the contrary. He has found that serum tends to inactivate phage, and moreover that strains grown in serum become phage resistant (the existance of phage-resistant strains has long been recognized). (4, 15 & 19). Although this sheds much light on the inconsistency of the results obtained, it does not account for those many favorable results which have been reported, and it is in this connection that the question of specificity of the phage used for the pathogen concerned has arisen.

The specificity of antibody for antigen is a phenomenon that is recognized and accepted by bacteriologists and clinicians. Evidence for such a definite specificity in the case of bacteriophages is not as strong, but specificity, nevertheless, may have an important bearing in the question of its therapeutic use. Dutton (10) in speaking of streptococcal infections treated with stock streptococcal phages says that the inconsistent results obtained may be explained by the fact that the phage used was active against streptococci normally occurring in the throat, and that the pathogen concerned was resistant to the phage used. More recently, Craigie and Brandon (7) have isolated a typhoid bacteriophage which they have found to be specific for only those organisms containing the Vi antigen. They have used this phage

in the isolation of pure V cultures of this organism, and have found that it is specific for only this V form.

Still other workers have been interested in the specificity of bacteriophage. Burnet (3) has worked out a classification of coli-dysentery phages on the basis of the resistant forms produced by their action as well as any serological differences in the phages themselves. Physiologically he has recognized four definite groups of coli-dysentery phages, and serologically, he has found that they fall into twelve groups. The above work of Burnet seems to establish beyond a doubt a definite specificity, but in most cases not to the degree found in antigen antibody reactions generally.

From the time of D'Herelle's original paper, until the present day, there has been much dispute among the investigators of bacteriophage as to its mode of action, and its nature. There have been a number of workers who have accepted and attempted to substantiate D'Herelle's conception of bacteriophage as a virus. Another school is of the opinion that bacteriophage is not a virus, but rather an enzyme which is capable of causing the dissolution of susceptible bacteria and in this process greatly multiply itself. Several extensive reviews of the literature (2, 4, 13) have been made by competent workers with this substance and the study of these leads one more toward the concep-

tion of phage as an enzyme than as a virus. Only this year, Northrop (14, 15) has isolated a protein much more active and more highly concentrated than anything previously described, which he considers to be the pure bacteriophage.

PURPOSE OF INVESTIGATION

Powers (16) has approached the study of specificity among the coli-form organisms more closely than any other worker, except Burnet (3). In his work he attempted to show by the action of different phages on related coliform organisms, any specificity demonstrable by means of a plate technique. He has found that as a whole there is some specificity between Escherichia and Aerobacter, and that by phage tests "intermediates" are more closely related to the genus Escherichia than to Aerobacter. Of the forty-three phages studied by him, most of them showed different specificities and, therefore, he concludes that there are many different races of phage, some of whih are monovalent (active only to coli-aerogenes strains) while others are polyvalent (active against a wider range of organisms).

Evans (11) has utilized different races of bacteriophage in the classification of hemolytic streptococci and has found that the results obtained by the differentiation of these organisms on the basis of their susceptibility to phages are closely comparable to those obtained by serologic classification. By this means, a different group may be established leading to the assumption of new and heretofore unidentified species. Craigie (7) (mentioned above) has also used a race of phage in the identi-

fication of a specific strain of Eberthella typhosus.

It is the purpose of this investigation to determine whether any specificity exists in the several different phages which have been isolated from sewage effluent, as well as in one which was obtained from Dr. Emory Ellis, on various species and strains of the genus Escherichia which have been obtained from different sources. The demonstration of such specificity would lead to:

1. The use of phage in the identification and differentiation of closely related species or even strains.

2. An explanation for the inconsistencies reported by clinicians in the therapeutic use of bacteriophage.

EXPERIMENTAL

Physiological:- Specimens of fresh feces were obtained from three different humans, a dog, and a chicken, as well as a saline solution of menstrual fluid. These specimens were emulsified in broth and then plated on eosin-methylene-blue agar. After incubation for twenty-four hours, three typical Esch. coli colonies were picked from each plate, and transferred to agar slants. Thus, it was assumed that eighteen different strains of Esch. coli were obtained, and they were designated A through R as shown in Table 1.

After several rapid transfers to bring the cultures to a state of maximum activity, they were subjected to the physiological tests shown in Table 1. This was done in order to differentiate them in so far as was possible, physiologically, as well as to definitely establish all strains as members of the genus Escherichia. In all cases not requiring special media, a beef extract peptone broth base, pH 6.6 - 6.9, was used with one per cent of the carbohydrate added.

Some of the tests shown in Table 1 were made in order to rule out the possibility of strains intermediate between the genera Escherichia and Aerobacter. A study of this table shows that all strains were indole positive, Voges-Proskauer negative, citrate negative, and with only three

TABLE 1 PHYSIOLOGICAL REACTIONS OF VARIOUS SPECIES AND STRAINS OF ESCHERICHIA ISOLATED FROM DIFFERENT SOURCES

	Chick			Dog			Menstrual Fluid			Human 1				Human 2			Human 3	
Cultures / Sugars	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Methyl-red	+	+	+	−	+	+	+	+	+	+	+	+	±	+	±	+	+	+
Sucrose	−	−	−	⊕	⊕	⊕	−	−	−	+ / ⊕	+ / ⊕	+ / ⊕	−	−	−	⊕	−	−
Salicin	− / ⊕	⊕	+ / ⊕	+	− / ⊕	− / ±	−	−	−	⊕	+ / ⊕	−	⊕	⊕	− / ⊕	− / ⊕	⊕	⊕
Rhamnose	+	+	+	⊕	⊕	⊕	⊕	⊕	+ / ⊕	+	+	+	⊕	⊕	⊕	+	⊕	⊕
Raffinose	−	−	−	⊕	⊕	⊕	−	−	−	⊕	⊕	⊕	−	−	−	⊕	−	−
Dextrin	−	−	−	− / +	−	−	− / +	+ / −	+	−	−	− / +	− / +	− / +	+ / ⊕	− / ⊕	−	−
Dulcitol	⊕	⊕	⊕	⊕ / −	⊕ / −	⊕ / −	− / ⊕	−	−	⊕	⊕	⊕ / −	⊕	⊕	⊕	−	⊕	⊕
Starch	−	−	−	− / +	−	−	−	− / +	− / +	− / +	− / +	− / +	− / +	−	−	− / +	− / +	− / +

Legend:

+ - acid
⊕ - gas
− - no change or alkaline

(a) Upper reading at end of 24 hours
(b) Lower reading at end of 72 hours
(c) Single reading when result was same at 72 hours as at 24 hours

1. All cultures negative on:
   a. Citrate
   b. Voges-Proskauer
   c. Gelatin
   d. Insulin

2. All cultures on:
   a. dextrose
   b. lactose
   c. levulose
   d. xylose
   e. arabinose
   f. sorbitol
   g. mannose
   h. glycerine
   i. maltose
   j. mannitol
   k. galactose

All cultures:
   a. Litmus milk acid
   b. Indole positive
   c. Nitrates reduced

exceptions Methyl-red positive. According to Standard Methods of Water Analysis (17), these tests show that all strains belong to the genus Escherichia and that there were no "intermediates" among them.

Table 1 also shows the physiological reactions run in the attempt to separate cultures into species and, if possible, into strains. In addition to the tests shown on this table, it was found that every culture produced acid on litmus milk, and acid and gas on the following: dextrose, lactose, levulose, xylose, arabinose, sorbitol, mannose, glycerine, maltose, mannitol, and galactose. It was also found that none of them liquified gelatine, and that all reduced nitrates in twenty-four hours.

From the information in Table 1, each culture was assigned a species name with the aid of Bergey's Manual. These are given in Table 2, together with the individual strain variation from the type species, as well as differences in tests other than those listed in Bergey's Mannual.

The cultures A, B and C appear to be identical, and vary only slightly from the identical cultures Q and R. M, N, and O differ between themselves, so that there appears to be a total of five different strains of Esch. coli isolated from two sources.

D, E, F, J, and K were found to be of the species communior. Of

TABLE 2 SPECIES OF ESCHERICHIA, ISOLATED FROM DIFFERENT SOURCES, CLASSIFIED FROM THEIR PHYSIOLOGICAL REACTIONS ACCORDING TO BERGEY

SPECIES	SOURCE	CULTURE	STRAIN VARIATION	
			From Bergey	Other Tests
coli	Chick	A	Dextrin, Raffinose — —	Rhamnose +
		B	Dextrin, Raffinose — —	Rhamnose +
		C	Dextrin, Raffinose — —	Rhamnose +
	Human	M	Methyl-red ± Dextrin, no gas Raffinose —	Starch + Rhamnose ⊕
		N	Dextrin, no gas Raffinose —	Rhamnose ⊕
		O	Methyl-red + Raffinose —	Rhamnose ⊕
		Q	Dextrin — Raffinose —	Rhamnose ⊕ ; Starch +
		R	Dextrin — Raffinose —	Rhamnose ⊕ ; Starch +
communior	Dog	D	Methyl-red -; Salicin-nogas; dextrin-nogas	Rhamnose ⊕ ; Starch +
		E	Dextrin —	Rhamnose ⊕
		F	Salicin-nogas Dextrin —	Rhamnose ⊕
	Human	J	Dextrin —	Rhamnose + ; Starch +
		K	Dextrin —	Rhamnose + ; Starch +
gruenthali	Menstrual Fluid	H	Typical	Starch + Rhamnose ⊕
		I	Typical	Starch + Rhamnose ⊕
formica	Menstrual Fluid	G	Typical	Rhamnose ⊕
anindolica	Human	L	Indole —	Starch + Rhamnose +
pseudo-coloides	Human	P	Typical	Starch + Rhamnose +

these, J and K are the only identical ones, so that there are four strains of this species out of the five isolated from the two sources.

According to the physiological reactions, G appears to be Esch. formica, H and I Esch. gruenthali, L Esch. anindolica and P Esch. pseudocolloides.

Phage propagation:- The next step in this investigation was the isolation of phages for the organisms obtained. This was done according to Hyde (12) and Powers (16) with minor modifications. Sewage effulent was obtained from the Imhoff tank of the Blacksburg sewage disposal plant. To a tube containing 10 c.c. of nutrient broth, inoculated with the organism for which the phage was desired, was added 2 c.c. of effluent. This enrichment tube was incubated at 37° C. for 18 to 24 hours. The contents of this tube were then filtered through a Seitz E - K filter. Two c.c. of this filtrate were then added to a tube containing 10 c.c. of Difco nutrient broth (pH 6.7) to which had been added .1 of a c.c. of 18 hour broth culture of the strain with which the enrichment tube had been inoculated. In most cases, lysis occurred within five hours. This tube was filtered as soon as lysis was complete and 2 c.c. of the filtrate added as before to .1 of a c.c. of 18 hour broth culture. It was found that 1 c.c. of

this filtrate produced lysis within three hours and a half, and this filtrate was powerful enough so that two drops were sufficient to produce lysis in the same time. The phages obtained were reproduced in this manner, .1 c.c. of the preceding filtrate to .1 of a c.c. of an 18 hour broth culture, for at least eight times. The last filtrate was the one in each case which was used in the test for [redacted] and for propagating the phage to be used in the serological work.

For isolating the phages, it was decided to work with strains A, D, G, J, M, and P, as each of these was from a different source and among them were both Esch. coli and Esch. communior.

It is commonly supposed that any true strain specificity would be demonstrated only after many passages of phage on homologous culture. Therefore, it was hoped that the phages first obtained would not be specific and that from them could be propagated phages for all 18 of the cultures. However, this did not prove to be true, and with only the few exceptions shown in Table 3, the phageswere specific as soon as they had been propagated sufficiently to be really active. (3rd passage).

Although at some time or another, lysates were obtained for each of the above mentioned strains, they were not strong enough to be maintained, and were lost in the subsequent effort to build up their

titres. In addition to the strains mentioned in the preceding paragraph, E and F were used and these were the first strains for which a strong phage was obtained. At a later date, a phage for M was obtained through the procedure outlined above.

In addition to phages propagated on E, F, and M, a phage was used in this work which was obtained from Dr. Emory L. Ellis of the California Institute of Technology, at Pasadena. Along with this phage, Dr. Ellis sent the strain of Bach. coli upon which it had been propagated together with directions for its propagation. This was carried out as directed, except for the use of the Seitz filter; he recommended filtration through a sintered glass filter.

Cross lysis tests:- It was decided that these four phages would be used in this work without further effort to obtain those for the other strains. The next step was to determine the titre of these four phages. This was done according to the technique for titre determination as given in Zinsser and Bayne-Jones (19), except that nutrient broth was used as the medium instead of peptone water. The titres of these phages were as follows: E had a titre of 1×10^7; F a titre of 1×10^8; M a titre of 1×10^7; and Ellis a titre of 1×10^6.

These phages were then tested qualitatively (at a dilution of

1-100) on all of the other cultures of Escherichia isolated. The results are shown in Table 3. Even in high concentrations, each of the four phages acted on only two to four of the nineteen cultures. The phage for culture H appeared to be specific for four cultures in the coli species. Since Q and R were shown to be identical physiologically (Table 2), this phage can be said to be specific for three strains of Esch. coli.

The two communior phages were specific for two of the five cultures of Esch. communior (E and F). Cultures E and F were shown (Table 2) to be nearly identical physiologically so that there is a possibility that the phages were also identical. They were peculiar in that they also lysed one culture of Esch. coli (Ellis). The phage for this same culture of Esch. coli also lysed one of the Esch. communior strains (E). Thus it would seem that in certain cases in high concentration species is not even a barrier to lytic action. However, it must be noted that the degree of clearing is not nearly so great and that the titre to which it is evidenced is much lower than with the homologous strain (Table 4). In spite of this discrepancy a high degree of specificity seems to be demonstrated.

In order to further differentiate specificity, titres were determined between the phages and those cultures shown to be sensitive in Table 3.

T A BLE 5 SPEC IFICITY OF PHAGES FOR VARIOUS S PECIES AND S TRAINS OF ESCHERICHIA
(P HAGES USED IN 1-100 DILUTION)

CULTURES / PHAGES	ESCH. COLI.									ESCH. COMMUNIOR.					ESCH FORMKA	ESCH GRUENTHAL		ESCH. ANINDO-LICA	ESCH PREU COLOI
	A	B	C	M	N	O	Q	R	Ellis	D	E	F	J	K	G	H	I	L	P
E	—	—	—	—	—	—	—	—	++	—	++	+	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	++	—	++	+	—	—	—	—	—	—	—
M	—	—	—	++	++	—	++	++	—	—	—	—	—	—	—	—	—	—	—
ELLIS	—	—	—	—	—	—	—	—	++	—	++	—	—	—	—	—	—	—	—

Legend:
++ equals total clearing
\+ equals definite clearing
All controls were negative (no clearing)

This was done by the same method employed in determining the original titre of the phages themselves. The results of these tests together with the original titres are shown in Table 4.

The study of this table shows very little more than has already been evidenced by the table of the qualitative differentiation. It does show that the titre of phage E on culture E is greater than on culture F and likewise phage F titre is greater on culture F than on culture E. This might indicate a slight strain variation between cultures E and F in spite of physiological similarity.

The cross lytic action of strains of Esch. coli and Esch. communior is shown to be only slight compared with that within the species. That there could be any action at all, however, is an interesting observation.

Serological:- For this work, only those strains of Esch. coli and Esch. communior were chosen that had been classified according to their physiological reactions. Nine cultures of these two species were obtained in this way - A, D, E, F, J, K, N, 4, and Ellis; B and C were not used, because they were physiologically identical with A and A was taken as being representative of this group (strain); J was taken as representative of the strain as embodied in J and K as they are identical physiologically; and 4 was taken as the

TABLE 4 TITRES TO WHICH PHAGES LYSED OTHER CULTURES FOUND TO BE PHAGE SENSITIVE

PHAGES \ CULTURES	ESCH. COMMUNIOR		ESCH. COLI.				
	E	F	Ellis	M	N	Q	R
E	7 (11)	6 (9)	(6)				
F	6 (11)	8 (10)	(4)				
M				7 (11)	9 (11)	8 (11)	8 (11)
Ellis	(4)		6 (8)				

Legend:

4 - dilution of 1 x 10^4
6 - dilution of 1 x 10^5
7 - dilution of 1 x 10^7
8 - dilution of 1 x 10^8
9 - dilution of 1 x 10^9
10 - dilution of 1 x 10^{10}
11 - dilution of 1 x 10^{11}

Complete lysis up to and through dilution shown by uncircled numbers. Incomplete lysis up to and through dilution shown by encircled numbers. All controls were negative. (no clearing).

representative strain as represented by the two cultures ~ and R which are identical. O was not used because it was not lysed by any of the phages.

The nine aforementioned cultures were transplanted to Kolle flasks and incubated at 37° for 24 hours. The cultures were then harvested in 100 c.c. of sterile saline, transferred to sterile flasks and placed in a water bath at 60° C. for one hour. The suspensions were standardized against a set of MacFarland bacterial standards and it was found that they had a turbidity of 1,500,000 organisms per c.c. Vaccines thus prepared were used throughout in the agglutinin production in rabbits.

The rabbits were healthy, at least four months old, and weighed more than three pounds at the beginning of the experiment. The injections were made daily for a period of seven days, the initial dose being .1 c.c. in each case and this was increased by .1 c.c. on each succeeding day during the period of injections. A week after the seventh injection they were bled, but the titres of the agglutinins formed were not sufficiently high and further injections were then made. In these injections, .7 c.c. was used, the first being given eleven days after the preceding injection, and the second two days later. A week after this last injection, the animals were bled, the

titres found to be satisfactory, and the animals then bled to death.

These injections were made into the marginal vein of the rabbit's ear. When the final blood was taken after the desired titres had been reached, the rabbits were bled out by exposing one of the carotid arteries and collecting the blood directly in large sterile tubes. The blood obtained was centrifuged and 5 c.c. of the serum was transferred to a flask where it was diluted with 95 c.c. of a .5 percent carbolic saline solution. This serum of 1:20 dilution was then used in the cross agglutination tests.

At the same time that the vaccines were being injected for agglutinin formation, phages were injected into different rabbits in the attempt to stimulate the formation of precipitins. The titres of the phages used in the injections are shown in Table 5. In this case, the injections were made in the same sequence as the above, but the dose initiated at .2 c.c. and increased daily by .2 c.c. so that on the fifth day the dose had reached 1 c.c. and this dosage was maintained in subsequent injections. It was felt that the stimulation of precipitins would be more difficult than the stimulation of agglutinins, and, therefore, 13 injections were made. However, two weeks after the last injection when the rabbits were bled, no precipitation was obtained in a dilution of one to forty, so that precipitin reactions of the phages were

TABLE 5 PHAGE TITRE DETERMINATIONS FOR SEROLOGICAL INJECTIONS

DILUTIONS / PHAGES	1×10^{2}	7×10^{3}	1×10^{4}	1×10^{5}	1×10^{6}	1×10^{7}	1×10^{8}	1×10^{9}	CONTROL
E	+ +	+ +	+ +	+ +	+	+	+	+	—
F	+ +	+ +	+ +	+ +	+	+	+	+	—
M	+ +	+ +	+ +	+	+	+	+	+	—
ELLIS	+ +	+ +	+ +	+ +	—	—	—	—	—

Legend:

++ = total clearing
+ = partial clearing
— = no clearing

In these preparations the media was .3% peptone and .5% NaCl.

not obtained.

Cross agglutinations were set up using each of the anti-sera obtained against each of the nine cultures used. Seven dilutions of the sera were made so that the final dilution after the addition of the antigen ran from one to eighty to one to 5,120. These tubes were placed in a water bath at 55° C. for two hours and the results taken at the end of this time; on the following morning, any further evidence of agglutination was noted and recorded, but in no instance was the degree of agglutination markedly changed in any of the tubes by standing overnight.

Results of agglutination tests:- The results of these tests are shown in Table 6. An analysis of this table shows that A is very different serologically from the other four strains of Esch. coli shown in this table (M, N, Q, and Ellis). This would be expected from the physiological variations shown in Table 2. The least difference is that between A and Q (vary only in the fermentation of starch and production of gas on rhamnose) and yet they have no apparent antigenetic relationship. On the other hand, even though M, N, and Q showed more physiological differences they all seem to be antigenetically alike and similar to Ellis. It would seem from this data that physiological variation is not necessarily correlated with antigenic variation,

TABLE 6 CROSS AGGLUTINATION REACTIONS OF SPECIES AND STRAINS OF ESCHERICHIA

Species / anti-sera / antigen		*Esch. communior*				*Esch. coli*		
		D	E	F	J	M	N	Ellis
coli	A	0	0	+1	0	0	0	0
	M	+1	0	+5	+4	7	6 ⑦	5 ⑥
	N	0	0	2 ④	2 ③	7	7	6 ⑦
	Q	0	0	4 ⑥	+5	7	7	6 ⑦
	Ellis	0	0	0	0	4 ⑤	4 ⑤	7
communior	D	7	0	+6	0	0	0	0
	E	+7	5 ⑥	7	+5	3 ④	+5	+4
	F	+7	5 ⑥	7	+3	3 ④	+4	+4
	J	+1	0	+6	7	0	0	0

Dilutions:
1 = 1:80
2 = 1:160
3 = 1:320
4 = 1:640
5 = 1:1280
6 = 1:2560
7 = 1:5120
Controls Neg.

Legends:
(a) Uncircled numbers indicate complete agglutination up to and through tube indicated.
(b) Encircled numbers show partial agglutination through tube indicated.
(c) before number shows partial agglutination from first tube through the one shown.
(d) Suspension density between MacFarland's #2 and #3.

and vice versa.

In the group of four strains of Esch. communior, E and F are alike by serological test and practically so physiologically. D and J showed some antigenic similarity to E and F which is correlated with slight fermentation differences. There appears to be some relationship between Esch. coli (Ellis) and cultures of Esch. communior (E and F) as already shown in Table 4. This is borne out by the serological data shown in Table 6. However, the close antigenic similarity of this (Ellis) culture to other coli cultures (M, N, and Q) established it as Esch. coli beyond reasonable doubt. It in no way explains the action of this coli phage on a strain of communior but it does make it seem more reasonable.

Division of all the organisms worked with into two species on the basis of physiological tests becomes a bit confusing in the agglutination reactions. Some strains within either one of the species coli or communior showed considerable antigenic similarity for strains in the other species even though the two species differ physiologically. Still other strains showed the expected lack of antigenic similarity between species.

The action of two communior phages (E and F) on a strain of coli (Ellis) and also the action of a coli phage (Ellis) on a strain of communior (E) has been mentioned in the preceding paragraph. The

antigenic similarity of these various cultures was also pointed out. The data in Table 6, however, also shows antigenic similarity between strains of different species, which strains were not effected by the same phages. It would, therefore, appear that phage specificity is not necessarily correlated with antigenic relationship.

The organisms in this investigation have been studied from the standpoint of fermentation and agglutination reactions as well as phage lysis; and it has been found that phage specificity is not correlated with physiological reactions, and not necessarily with antigenic relationship.

SUMMARY

In this work, a selected number of strains of the genus Esch. coli and Esch. communior were obtained from different sources, and four phages were obtained which were active against one or more of them; three of these phages were isolated from sewage effluent while the fourth was obtained from another laboratory. Cross lysis tests were performed in order to determine whether the isolated phages were specific for one or more of the strains used. From the results it would seem that such specificity does exist, for the phages prepared for two strains of Esch. communior (E and F) were almost equally effective in lysing either of these cultures; but, with the exception of some lytic action on one strain of Esch. coli, had no effect on the other seventeen cultures of the six species in the genus Escherichia which had been used in the investigation. Three other strains of Esch. communior (D, J, and K) appeared to be somewhat different both physiologically and serologically, so that the specificity exhibited by the phages for the first two strains of Esch. communior (E and F) appeared to be correlated with their fermentation and agglutination reactions. This is not strictly true, however, because there was antigenic similarity between certain of the coli cultures (M, N, and Q) and these two communior strains.

The phage developed for one of the Esch. coli cultures (M) was lytically active against four of the nine strains of this species with little difference between them. These strains were antigenically alike but differed materially in fermentation reactions.

One strain of Esch. coli (Ellis) mentioned above cross lysed to a slight degree in high concentration with Esch. communior and not with other strains of Esch. coli in spite of antigenic similarity to the latter.

The specificity that has been demonstrated in this work is much more marked than has ever before been shown, although Burnet (3) states that occasionally, one does run into phages which show a strain specificity. It must be borne in mind, therefore, that in dealing with such a small number specific phages may have been isolated by chance. It does seem that this would be highly improbable if they are so rare as Burnet would lead one to believe, but nevertheless the possibility remains.

CONCLUSIONS

1. Lysogenic tests with phages, prepared from strains of the species coli and communior isolated from various sources, exhibited marked specificity for only two to three cultures out of a total of nineteen.

2. There appeared to be no direct relationship between phage specificity and physiological variation in strains.

3. Serological relationship and identity was not necessarily correlated.

4. Some evidence was obtained that organisms of different species may be lysed by the same phage (although not to the same extent) and that the phage is specific for only those cultures.

5. Such specificity has been shown as to cast serious doubt on the beneficial use of phage therapy unless it is first shown that the specific pathogen is susceptible to the phage administered.

The results of this investigation are sufficient to indicate a high degree of phage specificity, but further work would be necessary to establish that this specificity is independent of physiological, antigenic, or species relationship.

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