BACTERIOPHAGY IN URINARY INFECTION[1]

PART I. THE INCIDENCE OF BACTERIOPHAGE AND OF BACILLUS COLI SUSCEPTIBLE TO DISSOLUTION BY THE BACTERIOPHAGE IN URINES. PRESENTATION OF CASES OF RENAL INFECTION IN WHICH BACTERIOPHAGE WAS USED THERAPEUTICALLY

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INTRODUCTION

The extensive and rapidly increasing literature on the subject of bacteriophagy contains but few instances of a complete and thorough investigation of the rôle played by the bacteriophage in a given disease. No investigator, with the exception of the discoverer of the phenomenon of bacteriophagy, d'Herelle, has attempted to ascertain the exact conditions obtaining in a localized infection with respect to the bacteriophage or to the type of bacterium encountered, although in his studies of typhoid fever, barbone and fowl typhoid he has indicated in a very positive manner the value, as measured by results, of an extensive inquiry into the conditions prevailing in a particular infection. That no one has elected to follow d'Herelle's lead is perhaps not surprising in view of the enormous number of attractive problems resulting from the discovery of the phenomenon of bacteriophagy. Therapeutic work with the bacteriophage undertaken without the knowledge gained by studies such as have been made on typhoid fever, barbone and fowl typhoid must, however, be at best empirical. Realizing the importance of a knowledge of the conditions prevailing in a given disease as a basis for therapeutic experiments with bacteriophage, an inves-

[1] This paper forms part of a thesis presented for the Degree of Doctor of Philosophy in Yale University.

tigation of infection of the urinary tract has been undertaken; the results of which will be presented in a series of papers.

The present contribution is concerned with a statement and consideration of the results obtained from a systematic search for races of bacteriophage in urines of infected and non-infected individuals, and from a study of the state of the colon bacilli present with respect to their degree of susceptibility to dissolution by the bacteriophage. Subsequent papers dealing with the action of the bacteriophage in the body, and the effects of tissues and excretions, especially the urine, upon the bacteriophage, will be presented. The results provide some information as to the occurrence, permanence, source and fate of the bacteriophage in the urine, and the character and behavior of the *Bacillus coli* concerned in urinary infections. Such a study should contribute materially to a better conception of the rationale of bacteriophage therapy in pyelitis, cystitis, and associated infections.

PROCEDURE

In order to detect the presence of bacteriophage in a given medium, it is necessary that there should be provided a bacterium capable of undergoing dissolution as a result of the activity of the bacteriophage. Lysis, as it has been termed, whether evidenced by inhibition of bacterial growth, clearing of a turbid culture, or the formation of plaques on solid media, is the only criterion at present available for the detection of bacteriophage. When, after the contact of material in which bacteriophage is sought with a bacterial strain, lysis is found to occur, and when this lysis is reproducible in series, the conclusion that bacteriophage is present is justified. On the other hand, the failure to obtain such evidence of its activity is never proof that the principle is absent. It can only be said, that bacteriophage active for the bacterial strain provided and of sufficient virulence to display its activity, is not present. In searching for bacteriophage in a medium in which its presence is suspected, however, the greater the number of bacterial strains provided, and the more numerous the contacts, after successive filtrations,

the more likely becomes the conclusion, when no activity is apparent that no bacteriophage is present. At best, however, negative results can ill support interpretation, although positive findings provide a safe basis on which to build.

In searching for races of bacteriophage in urines, a technique was devised which provided, insofar as was possible in a routine study, the conditions most favorable for the detection of bacteriophage. Urines from patients with infections of the urinary tract were incubated for 18 hours at 37°C. after the addition of alkaline extract broth[2] the proportion of broth to urine being about 1:1. When, after eighteen hours of incubation, growth was apparent, and when smears proved that the organism was a Gram-negative bacillus, subcultures were made by streaking Endo plates, and the original culture subjected to filtration. The filtrate was the medium in which bacteriophage was sought.

To tubes of alkaline extract broth containing about 10 cc. of medium, these filtrates were added in amounts of 1, 0.5, and 0.1 cc. To the fourth tube no filtrate was added. Each of the four tubes was then inoculated with a drop of a suspension of *Bacillus coli*[3] which has been found to be susceptible to dissolution by a number of filtrates containing bacteriophage. This strain of *Bacillus coli* was, then, the indicator. As has been explained, bacteriophage might be present without having an activity for this particular colon bacillus, for d'Herelle (1924) has shown that *Bacillus coli* is an heterogeneous species insofar as susceptibility to the dissolving action of bacteriophage is concerned. Consequently, it is quite apparent that one strain of *Bacillus coli* is not a sufficient indicator. In order to increase the chance of obtaining a positive result, a second series of tubes was prepared, identical with the first except for the bacterium used. In this instance the strain of *Bacillus coli* isolated from the

[2] Alkaline extract broth was used in all experiments dealing with the bacteriophage. It was made by adjusting the reaction of plain extract broth to a pH of 7.8, which concentration of hydrogen ions has been shown to be favorable to the manifestation of bacteriophage activity.

[3] Throughout these experiments, the suspensions of *Bacillus coli* used to inoculate the tubes was obtained by washing the growth from the surface of an eighteen-hour slant with 10 cc. of alkaline extract broth.

patient's urine, if such a strain was available, was utilized. A third series was arranged in which the bacterium used as indicator was *Bacillus dysenteriae* Flexner which experience had shown to have a wide range of susceptibilities including susceptibility to most races of colon-bacteriophage. Immediately after the inoculation, a loopful of the material from each tube in each series was streaked on agar slants, and both the slants and the broth tubes were incubated at 37°C. for eighteen hours. Readings were made at various intervals from three to eighteen hours.

As has been stated the action of bacteriophage on a bacterium may be indicated by inhibition of growth, dissolution of a growth sufficient to cause turbidity in broth, or the formation of plaques. In the event of the dissolution or inhibition, the result is noted by comparison with the control tube in which a normal culture of the bacterium has been inoculated. The degree of turbidity in the broth may vary from a state scarcely to be differentiated from the control to one of complete limpidity. In making readings then, it is sometimes convenient to note the degree of activity of the bacteriophage. Although the analyses to be made in the present instance depend upon the presence or absence of the bacteriophage and not upon the degree of virulence manifested, the readings are presented as they were made. A completely limpid tube indicated the presence of bacteriophage of activity noted as $++++$, while slight turbidity, but markedly less than the control was regarded as evidence of $+++$ activity. The agar slants corresponding to the tubes in which such readings were obtained remained sterile. Bacteriophage of low virulence was recorded as of $++$ activity when there was only a very slight difference between the tube containing the filtrate and the control, and as of $+$ when the appearance of plaques on the agar slants was the only evidence of the presence of the lytic substance.

There is no necessity for including in the table of results of the above investigation a statement of the further steps which must be undertaken to verify the existence of a true bacteriophage. It is well known that the phenomena of bacterial dis-

solution and of plaque formation have been observed as resulting from substances undoubtedly not bacteriophage. As examples Flemming's lysozyme, Twort's bacteriolysin, and the pyocyaneus "bacteriophage" of Hadley might be cited. Serial activity, when demonstrated together with plaque formation and bacterial dissolution in broth are, however, the criteria which serve to identify bacteriophage. Serial activity was demonstrated with a considerable proportion of the active filtrates in this series, the number of passages varying from 2 to 10.

It is further to be noted that repeated contacts of a filtrate in which one is seeking evidence of bacteriophagic activity, with a susceptible bacterium, each contact secured by a separation of bacterium and fluid through filtration and the subsequent use of the subculture of the bacterium which has not been in previous contact with the bacteriophage, may result in enhancing the virulence of any bacteriophage present to such an extent that its activity becomes apparent. If after several contacts there is no evidence of bacteriophage action, it is safe to conclude that the filtrate does not contain the agent in a form in which its activity will ever become apparent. Obviously in routine procedures it is extremely tedius to employ such a method. Nevertheless, when the conclusions are based upon the absence of bacteriophage, no other method is permissible. On the other hand, when the presence of bacteriophage is the basic condition, failure to demonstrate it after one contact does not lead to serious errors in the results. In the present instance, when absence of bacteriophage was the important consideration, successive contacts were employed, but in the opposite condition a single contact only was made.

As a corollary to the search for bacteriophage in urines, a study of the *Bacillus coli* encountered was made to determine whether the bacterium was susceptible to the action of bacteriophage or whether it was resistant. To this end a procedure similar to that already described was followed. The conditions were reversed however. Two filtrates were placed in contact with the organism isolated from the urine. The first filtrate was known to contain a highly virulent bacteriophage for several

strains of *Bacilli coli*. The second was the filtrate of the urine from which the colon bacillus was isolated. In obtaining the bacterium from the urine, single colonies were taken from the Endo plates which had been streaked when the urine was first cultured, and alkaline agar slants inoculated. The procedure and methods of reading were identical with those described for the detection of bacteriophage.

Table 1 presents the results of these studies together with some related data, the significance of which will appear later. Although 102 tests are shown in this table, more than 200 urines were studied. The tests not recorded were made on specimens of urine obtained by catheterization of the ureters at the same time that bladder specimens were obtained. In every case the results of the tests of kidney urines were identical with those of the specimens secured from the bladder. Such tests were not without significance, however, for they indicated that the conditions in the kidney pelves were identical with those found to exist in the bladders insofar as the activity of the bacteriophage and the type of the bacterium were concerned.

The diagnoses in every case were made by the attending clinician and were obtained from the case histories. When several tests are described for the same patient these tests were made on specimens obtained at different times and the results are arranged in chronological order.

It is evident from the preceding table that we have six possible conditions with relation to the incidence of bacteriophage and the type of *Bacillus coli* in the urine. One can consider the presence of bacteriophage when susceptible colon bacilli, resistant colon bacilli and no bacteria, respectively, are found, and, on the other hand, the incidence of the two types of *Bacillus coli* in the absence of bacteriophage. The sixth possible condition is the absence of both bacteria and bacteriophage which is the condition in normal urines. It is likewise obvious that two means of interpretation of the results are available since two different units may be considered. Urines, regardless of the individuals from whom they were collected, may be studied, or, one may consider only the individuals, without reference to

TABLE 1

CASE DESIGNATION	SEX		URINE LYTIC FOR			TYPE OF B. COLI PRESENT
			S	F	H*	
			−	−	−	R
			−	−	−	R
BK	F.	Pyelitis-cystitis, acute and chronic	+++	0	++++	++++
			++	−	−	R
			−	+++	−	R
			−	−	−	R
LS	F.	Pyelitis-cystitis, acute—abortion (therapeutic)	++++	++++	++++	++++
			−	−	++++	++++
LB	F.	Pyelitis-cystitis, acute and chronic (pregnant)	++++	0	++++	++++
			−	−	−	R
			++	−	−	R
OS	F.	Pyelitis, chronic; cystitis, acute	−	−	−	R
			−	−	++	++
			−	−	−	++++
			−	−	−	R
			−	0	−	R
BD	F.	Pyelitis, chronic (pyelonephritis chronic†)	−	0	−	R
			++	0	++	++
			−	0	−	0
			−	−	++	++
LS	F.	Cystitis, chronic	+++	0	++	++++
			−	−	−	R
			−	−	++++	++++
CMu	F.	Cystitis, acute	−	−	+	++++
			++++	−	−	++++
			0	0	0	++++
AF	F.	Ureteral stricture; pyelitis, acute	++++	−	−	+++
BMcG	F.	Pyelonephritis, acute	++++	0	++++	++++
TS	F.	Not made	++++	0	++	++++
CV	F.	Not made	+	−	−	+++
EH	M.	No history	++	0	++	++++
			−	−	−	0
CMi	F.	Pyelitis, chronic; cystitis, acute	−	−	−	++++
			−	−	−	R
DS	F.	Pyelonephritis, chronic; cystitis, acute (pregnant)	−	−	−	++
FW	F.	Pyelitis, acute; cystitis, chronic	−	−	−	++++
MWa	F.	Pyelitis; cystitis, acute	−	−	−	++++

* S, susceptible *Bacillus coli;* F, susceptible *Bacillus dysenteriae* Flexner; H, *Bacillus coli* isolated from urine tested; R, resistant *Bacillus coli;* 0, no organism present.

† Started as mixed infection.

TABLE 1—*Continued*

CASE DESIGNATION	SEX		URINE LYTIC FOR			TYPE OF B. COLI PRESENT
			S	F	H*	
MWh	F.	Pyelitis; cystitis, acute	−	0	−	+++
BD	F.	Pyelitis, chronic	0	0	0	++
RA	M.	Pyelitis, acute; cystitis	0	0	0	++
EN	F.	Cystitis ?; urethral stricture	−	−	−	R
		acute exacerbation	++++	++	−	R
GS	F.	Pyelitis; cystitis, chronic	−	−	−	R
			++	0	−	R
			−	0	−	R
			−	−	−	R
EF	M.	Pyelonephritis, acute (died)	−	−	−	R
			++++	0	−	R
ES	F.	Cystitis, chronic	++	−	−	R
HS	F.	Pyelitis; cystitis, chronic	−	−	−	R
			−	−	−	R
CR	F.	Pyelitis; cystitis, chronic	−	−	−	R
			−	−	−	R
HP	F.	Pyelitis; cystitis, chronic	−	−	−	R
LM	F.	Pyelitis; cystitis, chronic	−	−	−	R
AN	F.	Pyelitis; cystitis, chronic	−	−	−	R
			−	+	−	+
G	F.	Pyelitis; cystitis, chronic	−	−	−	R
			−	−	−	R
MP	F.	Pyelitis; cystitis, chronic	−	−	−	R
RN	M.	Pyelitis; cystitis, chronic	−	−	−	R
RH	M.	Pyelitis; cystitis, chronic	−	−	−	R
			−	−	−	R
			−	−	0	R
			−	−	−	R
			−	−	−	R
			−	−	−	R
			−	−	−	R
			−	−	−	R
AB	F.	Pyelitis; cystitis, chronic	−	−	−	R
WD	M.	Pyelitis; cystitis, chronic	−	−	−	R
WMQ	M.	Pyelitis; cystitis, chronic	−	−	−	R
			−	−	−	R
			−	−	−	R
JA	M.	Cystitis, chronic; prostate, hypertrophy	++	−	−	R
			−	−	−	R
JF	M.	Cystitis, chronic; prostate, hypertrophy	−	−	−	R

TABLE 1—*Concluded*

CASE DESIGNATION	SEX		URINE LYTIC FOR			TYPE OF B. COLI PRESENT
			S	F	H*	
ED	M.	Cystitis, chronic; prostatitis	–	–	–	R
HM	M.	Cystitis; pyelitis; prostatitis, chronic	–	–	–	R
FY	M.	Cystitis, chronic; prostatitis, hypertrophy	–	–	–	R
MC	M.	Cystitis, chronic; prostatitis, hypertrophy	–	–	–	R
VS	F.	Pyelonephritis, acute	–	–	–	R
			–	–	0	R
			–	–	–	R
			–	–	–	R
			–	–	–	R
			–	–	–	R
			–	–	–	R
			–	–	–	R
LH	F.	Pyelonephritis, chronic (died)	–	–	–	R
			–	–	–	R
AB	M.	Ureteral calculus; pyelitis, chronic	–	–	–	R
ES	F.	Carcinomatosis; cystitis, chronic	–	–	–	R
			–	–	–	R
DJ	F.	Pyelonephritis, acute and chronic	–	–	–	R
IR	F.	Pyelitis; cystitis, acute (Strep.)	–	–	–	++†
JS	M.	No diagnosis	++++	0	++	0
MC	F.		–	–	–	R
LBt	F.		–	–	0	R
RH	M.		–	–	–	R
GG	M.		–	–	–	R
ML	F.		–	–	–	R
WM	F.	No renal infection	–	–	–	R
BH	F.		–	–	–	R
LM	M.	Normal urine	–			
ML	M.		–			
P	F.		–			
W	F.		–			
HA	M.		–			
MC	M.		–			
D	M.		–			
AN	M.		–			
ER	M.		–			

‡ Coli contamination.

the number of specimens from a single patient. In the latter case, since positive findings may occur at times and negative results at others, it is necessary to adopt an arbitrary standard in order to classify the cases. This has been accomplished by including in the positive category all individuals whose urine contained the factors sought in any specimen of urine. Such variations as occur in the different specimens from the same patient will, however, receive consideration.

It is of some interest, however, to study the findings in urines without reference to the patients from whom they were obtained. Excluding the normal urines; that is the urines from individuals not known to have infection of the urinary tract 109 specimens are available for analysis. On the basis of the six conditions mentioned above, these urines may be distributed as follows:

	NUMBER	PER CENT
Bacteriophage and susceptible *Bacillus coli*	17	15.6
Resistant *Bacillus coli*	8	7.3
No *Bacillus coli*	1	0.9
Total	26	23.8
No bacteriophage and susceptible *Bacillus coli*	7	6.4
Resistant *Bacillus coli*	67	61.5
No *Bacillus coli* (normal urines)	9	8.3
Total	83	76.2
Susceptible *Bacillus coli*		24–25
Resistant *Bacillus coli*		75–75

Because of several facts among which are the meagerness of the material, and the variation in the number of specimens from different individuals, it is not safe to draw too many inferences from these data. It is, however, quite obvious that susceptible colon bacilli occur in infected urines, that the bacteriophage is likewise found in a fair proportion of the specimens, that bacteriophage is not found in uninfected urines, and that its pres-

ence may be coincident with either resistant[4] or susceptible bacteria. The single case in which bacteriophage was found in a urine in which no organism was demonstrated should not be regarded as an exception to the statement that bacteriophage is not found in uninfected urines, for this individual was undoubtedly infected although the organism had not been isolated.

It is the individual, i.e., the patient, however, in whom one is interested rather than in the urine, consequently an analysis on the basis of persons is far more significant than that based on urines. Adopting the suggestion previously made that the occurrence of bacteriophage or susceptible bacteria at any time in the urine from a single individual would place that person in the positive group with respect to the presence of these factors, the following analysis is presented:

	NUMBER OF INDIVIDUALS	PER CENT
Bacteriophage and susceptible *Bacillus coli*	13	25.0
Resistant *Bacillus coli*	5	9.6
No *Bacillus coli*	1*	1.9
Total	19	36.5
No bacteriophage and susceptible *Bacillus coli*	8	15.4
Resistant *Bacillus coli*	25	48.1
No *Bacillus coli*	0	0.0
Total	33	63.5
Susceptible *Bacillus coli*	21	41.0
Resistant *Bacillus coli*	30	59.0

* The case mentioned above which was certainly an infected individual.

In this study, the normal non-infected group of individuals is not included, since it was disposed of in the preceding table.

As in the preceding instance, the number of individual factors

[4] This statement appears to be contradicted later. Let the word resistant here be understood as meaning not completely susceptible to dissolution. Also let susceptible be understood to mean not resistant. It will be seen that the above statement is not true for strains of *Bacillus coli* that are completely resistant or completely susceptible.

in the group is too small to permit of making strong assertions, yet it is evident that a susceptible bacterium occurs, at some time or at frequent intervals during an infection, in a fairly large percentage of the cases considered. The same statement applies to the bacteriophage. Neither bacteriophage nor susceptible bacteria are found consistently in all of the specimens from a single individual however. The possible significance of such a result will be discussed later.

Not only did the specimens studied come from individuals; they came from males as well as females, and the cases represented both acute and chronic conditions of infection. Analyses of the results obtained in males as contrasted to those found in females as well as those found in chronic cases as distinguished from acute cases present some facts worthy of consideration. These analyses are contained in the following tables:

	MALES		FEMALES	
	Number	Per cent	Number	Per cent
Bacteriophage and susceptible *Bacillus coli*	1	5.9	11	30.5
Resistant *Bacillus coli*	3	17.6	3	8.5
No *Bacillus coli*	1	5.9	0	0.0
Total	5	29.4	14	39.0
No bacteriophage and susceptible *Bacillus coli*	1	5.9	8	22.2
Resistant *Bacillus coli*	11	64.7	14	38.8
No *Bacillus coli*	0	0.0	0	0.0
Total	12	70.6	22	61.0
Susceptible *Bacillus coli*	2	12.5	19	52.8
Resistant *Bacillus coli*	14	87.5	17	47.2
Total	16			

	ACUTE		CHRONIC	
	Number	Per cent	Number	Per cent
Bacteriophage and susceptible *Bacillus coli*	7	38.8	2	7.1
Resistant *Bacillus coli*	2	11.8	3	10.7
No *Bacillus coli*	0	0.0	0	0.0
Total	9	50.0	5	17.8
No bacteriophage and susceptible *Bacillus coli*	8	44.4	3	10.7
Resistant *Bacillus coli*	1	5.6	20	71.5
No *Bacillus coli*	0	0.0	0	0.0
Total	9	50.0	23	82.2
Susceptible *Bacillus coli*	15	83.3	5	17.9
Resistant *Bacillus coli*	3	16.7	23	82.1
Total	18	100.0	28	100.0

It is obvious that the incidence of bacteriophage and of susceptible colon bacilli is greater in females than in males, but it is also apparent that in acute conditions of infection the same factors are present to a much greater extent than in chronic states. The male patients who provided the material for this study were almost without exception subject to a chronic infection. Such a condition is generally true. Male patients suffering with urinary infection have at the time they undergo treatment, chronic infections. They are less often seen by the clinician when their infections are in the acute stages than are the females. On this account it can not be argued from the results presented here that males are less subject to infection with susceptible bacteria than are females nor that they less frequently harbor a race of bacteriophage in the infected urine.

DISCUSSION

In the introductory paragraph it was stated that the investigation might contribute to a better understanding of the source occurrence, permanence and fate of the bacteriophage in the

urine and also that information of value in the bacteriophage therapy of urinary infections might be provided. These questions will now be considered from the standpoint of the data reported.

With regard to the occurrence of bacteriophage in the urine, but little is to be found in the literature. We are not concerned here with the appearance of the principle when artificially introduced into the body as after injection, but with its natural occurrence. Almost the only reference to this question is made by d'Herelle (1922) who found it in the urine. He does not state, however, whether this urine contained bacteria but claims that the principle was able to pass through the intestinal mucosa into the blood and thence to the urine through the kidneys. Such a sequence of events may occur, but only when there is an infection in the urinary tract; in spite of the comparatively small number of normal urines studied in this series, it seems safe to state that the normal, uninfected urine never contains the principle naturally. When injected into the circulation bacteriophage may be detected in the normal urine but except under such artificial conditions it is not likely to be found. Nevertheless, when an infection is present in the urinary tract the bacteriophage is detectable in many urines and further, it is found in nearly all specimens when the infection is acute. What then can be the source of this bacteriophage?

Before attempting the answer to the above question, it is necessary to consider the occurrence of the two types of *Bacillus coli* found. It is not conceivable that a susceptible bacterium could maintain its existence in a fluid medium in the presence of the bacteriophage. It would be dissolved. On the other hand it is well known that a type of bacterium exists which acts as a carrier of the bacteriophage. Such an organism has neither complete susceptibility nor complete resistance. It will be seen, however, that in a considerable percentage of the urines studied bacteriophage and organisms apparently capable of being dissolved were present together. These bacteria, then, being of the carrier or lysogenic type were responsible for the presence of the bacteriophage in the urine. They introduced

and maintained the principle in the bladder and kidneys. In a subsequent study dealing with animal experiments and the action of bacteriophage in the body it will be shown that bacteriophage does not remain in the bladder except when a carrier or lysogenic type of bacterium is present.

With regard to the permanence of the principle in the urine, an examination of the first table shows that it seldom endures throughout the course of the infection. The bacteriophage appears and disappears. The disappearance is quite explainable; the lysogenic state being instable, is capable of change to a state of absolute susceptibility or resistance. When either of these conditions is reached, the bacteriophage disappears. Its reappearance can only be accounted for by reinfection with a lysogenic bacterium.

The fate of the principle depends upon the outcome of the struggle between the bacteriophage and the bacterium. If the bacterium is able to oppose an increasing resistance to the dissolving action of the principle, the time arrives when it becomes impossible to demonstrate bacteriophagy, either because the bacteriophage is no longer present, having been eliminated mechanically since there was nothing permitting its reproduction, or, as is more likely, because it has lost its virulence through association with a resistant bacterium. On the other hand, if the bacterium becomes less resistant, or if the bacteriophage gains in virulence, the bacterium is dissolved, and the bacteriophage, no longer having anything to retain it, is mechanically removed.

The results of the studies of the acute cases lend some support to these suppositions, since here we find bacteriophage and moderately susceptible bacteria. The condition cannot remain static. The struggle between the bacteriophage and the bacterium goes on, and, according to whether the bacterium or the bacteriophage gains the ascendency, the case becomes chronic or recovers. Might not this fact account for a number of spontaneous cures of pyelitis and cystitis?

Beyond a doubt there are other factors which play a rôle and influence the course of events, for we are not dealing solely with

the relationship between the bacteriophage and the bacterium. The presence of tissue, bladder and kidney, and the presence of urine contributes something to the reaction—the possible modifying factors which will be considered later.

CASES TREATED

From time to time during the prosecution of this investigation opportunities for the treatment of cases of pyelitis and cystitis presented themselves. Inasmuch as the work already completed had furnished ample justification for undertaking therapeutic experiments, and since the reports of cases to be found in the literature provided not only further justification but descriptions of methods, the opportunities were accepted. It will be seen from a consideration of the cases presented that various means of administration of the bacteriophage were adopted. The procedures were suggested by the published records of treatments undertaken by Bazy (1925), Zdansky (1925), Courcoux (1922), and Alphonsi (1924). These cases are not presented with any claim as to the efficacy of the principle administered, nevertheless, they add to the number of instances of this nature in which bacteriophage therapy has been employed, and, regardless of the interpretation which one may choose to make of the results, it is obvious that improvement in the condition of the patients followed the administration of bacteriophage. Such a statement is not intended, however, to imply cause and effect.

Case 1. L. B., female, aged thirty-three years. This patient was referred from the Dispensary with a diagnosis of pregnancy complicated by pyelitis. After a cystoscopic examination on October 4, 1923, the following notes were made: Patient seven and one-half months pregnant complaining of frequency of urination; loss of weight; localized dull pain in right abdomen; burning and smarting associated with urination. The examination itself revealed the following conditions: Generalized cystitis; pus and bacilli in the right kidney pelvis; left kidney, normal. A kidney lavage of 2 per cent boric acid was given and the patient was asked to return for examination later. On October 9, the condition was unchanged and the treatment was repeated. Sub-

sequent treatments with little or no improvement were continued until December 7 when delivery occurred.

Eleven days after delivery, examination showed that the condition of the bladder had changed but little. The left kidney had, however, become infected by this time. Cultures from the right and left kidneys and bladder were positive for *Bacillus coli*. The organism isolated proved to be susceptible to dissolution by a stock filtrate containing bacteriophage. On December 18, 5 cc. of bacteriophage were introduced into *each* kidney pelvis. The patient was discharged the next day and cautioned to report any return of symptoms. On January 17, she returned to the Dispensary and reported complete freedom from urinary troubles.

During the past two years she has been seen at intervals of five or six weeks because of conditions not associated with the genito-urinary tract. During this period she has had no return of her urinary troubles.

Case 2. M. W., female child, aged eight months. This child was admitted on October 26, 1924, to the Pediatric service. Her previous history was negative. Four days prior to admission, the mother noticed the first signs of sickness. These early symptoms, which consisted of vomiting and restlessness, persisted and were present on admission, when further observation indicated a dry and protruding tongue and dry lips. The urine was found to contain many pus cells and bacteria which proved to be *Bacillus coli*. A diagnosis of pyelitis was made as a result of these findings.

The treatment consisted of administration of large quantities of fluid, mostly by subcutaneous inoculation and nasal drips. Sodium bicarbonate, saline and glucose were given. These measures apparently producing little effect, a blood transfusion was given which was followed by a drop in temperature to 37.5 degrees, lasting for 24 hours. During this time, however, the patient's condition was not good. She continued to be apathetic and restless.

On October 30, the patient having grown progressively worse, and a blood culture having shown the presence of *Bacillus coli*, bacteriophage treatment was instituted. The filtrate used had not been shown to be active for the *Bacillus coli* causing the infection, nevertheless, 10 cc. of bacteriophage were introduced into the bladder by catheter at 5:30 p.m. An immediate drop in the temperature was observed and the patient improved clinically so that by the next morning a very noticeable change for the better was observed.

In the twenty-four hours succeeding the first treatment a race of bacteriophage had been found which was capable of producing lysis of the colon bacillus in question. Consequently the treatment was repeated on October 31, following which defervescence occurred. The patient continued to improve and was discharged a week later as cured. The temperature record is presented here.

The laboratory study of this case was not as complete as could be desired, yet studies of the urine after the treatment revealed a remarkable increase in the number of pus cells and a clumping of the bacteria with loss of motility. The pus persisted for several days and gradually

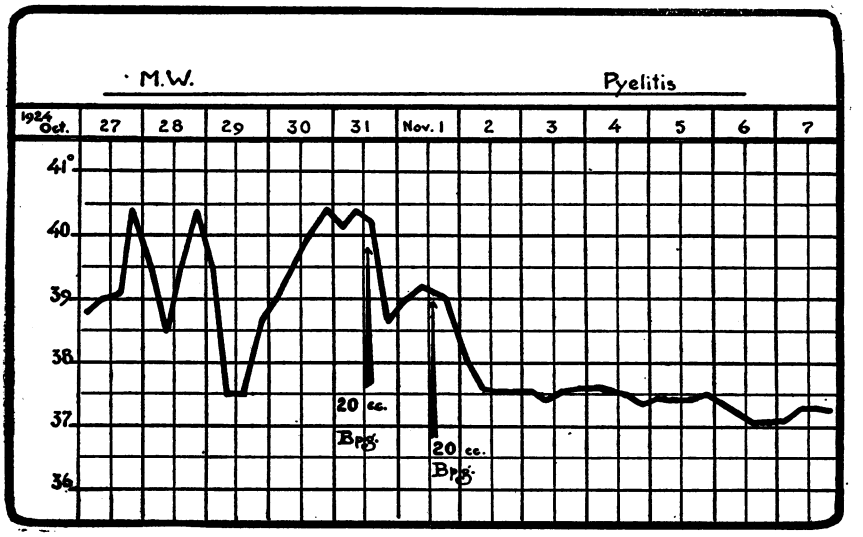

FIG. 1

disappeared. The bacteria likewise could be seen in smears for several days, remaining clumped and non-motile. They gradually decreased in numbers, although at the time the patient was discharged the urine still contained bacteria. The blood leukocyte count increased from 22,000, which it was upon admission, to 40,000 shortly after the administration of the bacteriophage. It had not returned to normal upon the discharge of the patient.

Two weeks after leaving the hospital the child was brought in for examination. At that time it was apparently well. The urine was free of bacteria. About nine months after the patient's discharge, the mother returned for the purpose of reporting that the child had been in excellent health since leaving the hospital.

Case 3. E. R., female, aged thirty. This patient was admitted to the hospital on April 9, 1925, with a diagnosis of pyelitis complicating pregnancy. She was in the seventh month of her pregnancy and reported an attack of severe pain in the right lower quadrant. This pain was first noticed five days prior to admission and was persistent, being associated with chills and fever. An examination of the urine showed pus cells and many bacteria which were motile and not clumped.

A colon bacillus susceptible to lysis with a stock filtrate containing bacteriophage was cultured from the urine. On april 12, three days after admission, the patient was given a subcutaneous inoculation of

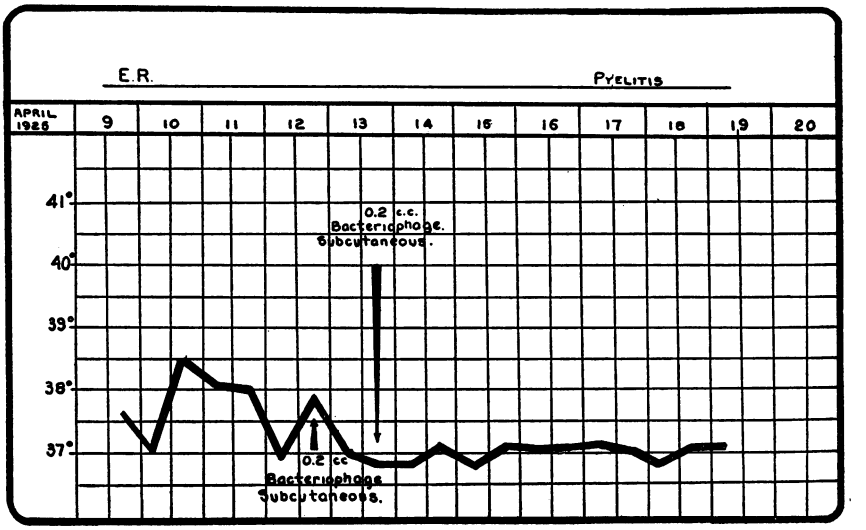

FIG. 2

2 cc. of this bacteriophage. A slight rise in temperature followed, but within twelve hours the temperature had fallen, and it continued to drop, reaching 37°C. twenty-four hours after the first inoculation. At this time a second inoculation of bacteriophage (2 cc.) was given which was in turn followed by a rise in temperature amounting to one degree. The temperature quickly dropped to normal, however, and from this time until the discharge of the patient, six days later, it remained so. The pain which had been persistent, relieved only by posture, disappeared shortly after the treatment was begun and did not reappear. The patient was discharged a week after the first treatment. She was delivered on July 1, at which time she had no urinary symptoms and

the urine was free of bacteria nor had she had urinary symptoms in the interval.

The laboratory findings in this case were as follows: There was no change in the leukocytic count following inoculation of bacteriophage. The urine, however, contained large numbers of pus cells which increased after administration of the bacteriophage and then gradually decreased. The bacteria presented the same phenomenon observed in the preceding case. Clumping with loss of motility was marked. At the time of discharge the urine still showed an occasional bacillus, but in July when the patient came in for delivery there were no bacteria in the urine. The temperature record is presented as it shows a sharp drop following inoculation of the bacteriophage.

Case 4. A. R., female, aged fourteen months. In this case the urinary infection followed a protracted, intermittent diarrhea and, at the time of admission, the child was in a decidedly malnourished condition having a thrush infection and a bilateral otitis media in addition to the pyelitis. There was also a question of respiratory infection. On account of the many complications, the temperature record in this case is of little value in indicating the effects of bacteriophage therapy for the urinary infection. The urine contained many pus cells and large numbers of motile Gram-negative bacilli which on culture proved to be *Bacillus coli*. When subjected to *in vitro* tests the bacillus was dissolved by the race of bacteriophage used in the treatment of the previous cases. A specimen of urine obtained by catheterization at the time of the second treatment showed many clumped, non-motile bacilli and many pus cells. Forty-eight hours after the second treatment a catheterized specimen of urine contained an occasional bacillus, non-motile, and very few pus cells. Two days later, the urine was sterile. No bacilli were seen in smears and no growth occurred in cultures of the urine. No pus cells were present. The patient remained in the hospital for several weeks during which time no bacteria were found in repeated specimens of urine, and at the time of discharge the urine was sterile. No further discussion of these cases will be presented since it is the opinion of the writer that interpretation of the results of treatments of so few cases is unwarranted.

SUMMARY

Routine studies of urines from patients having urinary infections revealed the fact that bacteriophage was present in about 25 per cent of the urines while *Bacillus coli* susceptible to the

action of bacteriophage was present in the same proportion of the specimens. The urines in which the susceptible colon bacilli were found were not necessarily the same as those in which bacteriophage was demonstrated. Normal urines, that is, urines not known to contain bacteria, were found to be free of bacteriophage.

When individual cases rather than urines were considered it was found that over 36 per cent of the cases studied had bacteriophage in one or more of the specimens of urine examined, while over 40 per cent (not necessarily including the above 36 per cent), were infected with a colon bacillus capable of being dissolved by a race of bacteriophage.

Almost without exception, the chronic cases provided urines in which only resistant bacteria were found, while the acute were seldom due to this type of colon bacillus. Bacteriophage too was found exclusively in the urines from individuals having acute infections.

The incidence of bacteriophage and susceptible colon bacilli in males and females was affected by the above condition. In practically every instance the males were suffering with chronic infections. Consequently the males, except in one case, were never a source of bacteriophage or susceptible bacteria.

Four patients subjected to treatment with the bacteriophage showed definite improvement after the treatment.

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