BACTERIOPHAGY IN URINARY INFECTION[1]

PART II. BACTERIOPHAGY IN THE BLADDER

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Bacteriophage therapy of many infections, occasioned by various organisms and occurring in different tissues, has been attempted with a fair degree of success. Numerous publications bearing upon the treatment of dysentery, typhoid and paratyphoid fevers, urinary infections and plague, and of localized infections caused by the staphylococcus, streptococcus and other organisms have appeared in the literature within the past three years. Nearly all of these communications testify to the success of this method of treatment, but for the most part the work has been empirical. Reasoning that a substance capable of bringing about the complete dissolution of a given bacterium when inoculated into a broth culture or suspension of the organism in a test tube should bring about the cure of an infection because of a similar occurrence in the body of an infected individual, the chief concern of a majority of these investigators has been to bring the bacteriophage into contact with the bacterium. However striking the results obtained by such a method may be, until the mechanism of the reaction is better understood, and until methods based upon demonstrated facts have been devised, it cannot come into general acceptance as a scientific procedure.

The problems confronting the investigator bent upon determining the behavior of the bacteriophage in the body depend to a considerable extent upon the location and nature of the infection. It is perfectly obvious that each infection and each tissue infected

[1] This paper is taken from a dissertation presented for the degree of Doctor of Philosophy in the Department of Pathology and Bacteriology, Yale University.

presents a different problem with respect to the action of the bacteriophage. Hence, it is not logical to infer that because bacterial dissolution occurs in the test tube in the presence of bacteriophage, it will likewise take place in a given tissue. That bacteriophage is extremely susceptible to changes in the environment has been shown by numerous investigators.[2] Consequently, within the tissues or in contact with various body fluids, it must be modified to an extent impossible to predict. Not only may the bacteriophage be modified by various physical and chemical conditions, but such physiological factors as the leucocytes as shown by d'Herelle and Eliava (d'Herelle, 1921), may become stimulated in the presence of the principle. Because of these facts it is evident that to administer bacteriophage as a therapeutic agent in infectious diseases without a knowledge of the limitations imposed by the environment is to eliminate the possibility of determing its rôle as a factor in the result.

So long as investigators interested in the therapeutic application of the bacteriophage confine their efforts to a study of the material they are using and its effects upon the bacterium *in vitro* and neglect the modifications which bacteriophagy may undergo *in vivo*, there will be little progress in the use of bacteriophage as a therapeutic agent. The realization of this fact was responsible for the work set forth in the following pages. *Bacillus coli* infections of the urinary tract offer unusual opportunities for the study of bacteriophagy within the body. The location and character of the lesions, the variations in the bacterium, and the environmental conditions provided by the secretion of urine and of mucus provide almost limitless material for investigation. Consequently, infections of this character were selected for study.

Having found in a previous investigation[3] that normal urine; that is, urine not known to contain bacteria, was free of bac-

[2] Gratia (1921), Eliava and Pozerski (1921), Scheiddiger (1923), Davison (1922.) and Reichert (1924). Hydrogen ion concentration.

Da Costa Cruz (1923a and b). Electrolytes.

Doerr (1922), Doerr and Berger (1922), Nakumura (1923a and b), Hauduroy (1923). Viscosity of medium.

Hauduroy (1925). Bile.

[3] Bacteriophagy in urinary infection. Part I (in press).

teriophage, while this principle occurred in 25 per cent of urines derived from infected bladders or kidneys, and that colon bacilli susceptible to lysis occurred in a like percentage of cases, although not necessarily in the same cases, the question arose as to the source of the bacteriophage and the reason for the existence of the different types of *Bacillus coli*. Other questions developed in the course of the investigation such as; the effect upon the bacteria of bacteriophage introduced into the bladder; the reason for the absence of bacteriophage in normal urines; the explanation of the infectiousness of the colon bacillus; and the effects of the presence of urine and of mucus upon the bacterium and upon the bacteriophage. The attempts to answer these questions through experiments and the results obtained are described in the following pages.

EXPERIMENTAL WORK

As to the source of the bacteriophage found in many infected urines it is evident that it is in some manner connected with the presence of bacteria since it is never present in normal urine. In addition to the eleven human urines reported in a previous paper, examinations of the urines of about twenty-five normal rabbits, five guinea-pigs, and three dogs have given the same result. Since the presence of bacteria was always associated with the presence of bacteriophage, attempts were made to infect rabbits by introducing colon bacilli into the bladder. The outcome of these efforts is shown in table 1.

Although not providing conclusive evidence, the above results tend to indicate the so-called lysogenic strains of *Bacillus coli* as responsible for urinary infection. By lysogenic strains is meant, strains capable of carrying the bacteriophage, that principle existing in symbiosis with the bacterium and subject to all of the laws which govern such a condition. Infection with such strains results in the appearance of bacteriophage in the infected region. Such a result has been demonstrated repeatedly by other investigators and needs no further consideration here. One must, however, eliminate the other possibilities in order to show that the lysogenic bacterium is alone responsible for the presence

of bacteriophage in the urine. This has been done to a considerable extent in the present investigation.

To indict the *lysogenic* strains of *Bacillus coli* as responsible for the urinary infections caused by this bacterium, is a matter of some

TABLE 1

RABBIT	SUSPENSION INTRODUCED INTO BLADDER*	DATE	RESULT
I	10 cc. Coli S†	5/10/24	Urine sterile 8/11/24; no bacteriophage
II	5 cc. Coli S	5/ 9/24	Urine contained Coli S 7/12/24; no bacteriophage
III	5 cc. Coli S	7/ 9/24	Urine contained Coli S 7/21/24; no bacteriophage. Urine sterile 8/11/24; no bacteriophage
IV	5 cc. Coli S	7/12/24	Urine contained Coli S 7/21/24; no bacteriophage Urine sterile 8/11/24; no bacteriophage
V	2.5 cc. Coli S (intravenously)	7/12/24	Urine contained Coli S 7/18/24; no bacteriophage. Died 7/23/24, Abscesses in kidneys and bladder. No bacteriophage
VI	1 cc. Coli S	7/ 6/24	Urine contained Coli S 7/7/24 and 8/6/24; no bacteriophage
VII	10 cc. Coli R‡	5/10/24	Urine sterile 8/11/24
VIII	10 cc. Coli R	10/21/24	Urine contained Coli R 10/29/24; no bacteriophage. Urine sterile 11/4/24; no bacteriophage
IX, X, XI, XII	5 cc. Coli R	8/19/24	Urine sterile 9/9/24; no bacteriophage
XIII	20 cc. Coli S	10/10/25	Urine sterile 10/12/25; no bacteriophage
XIV	20 cc. Coli R	10/12/25	Urine sterile 10/15/25; no bacteriophage

* A suspension prepared by washing an eighteen-hour agar slant growth with 5 cc. of broth. Introduced into bladder by catheter.

† Colon bacillus capable of being dissolved by bacteriophage, race 3620 in six hours.

‡ Colon bacillus not visibly affected by bacteriophage.

significance. The longer the duration of symbiosis, the greater becomes the resistance of the bacterium to dissolution by any bacteriophage (d'Herelle, 1926), consequently, it is not to be

TABLE 2

RABBIT	STATE OF BLADDER*	PROCEDURE	RESULTS
I	Normal	1 cc. bacteriophage intravesicularly	Disappeared twenty-four hours later
II	Normal	5 cc. bacteriophage intravesicularly	Disappeared twenty-four hours later
III	Normal	10 cc. bacteriophage intravesicularly	Disappeared twenty-four hours later
IV	Normal	2 cc. bacteriophage intravenously	Appeared in urine in twelve minutes; disappeared in one and a half hours
V	Normal	2 cc. bacteriophage intravenously	Same as above
VI	Normal	10 cc. bacteriophage + 20 cc. Coli S intravenously	Bacteriophage and bacteria present after twenty-four hours; disappeared after forty-eight hours
VII	Normal	10 cc. bacteriophage + 25 cc. Coli S intravenously	Same as above
VIII	Normal	10 cc. bacteriophage + 20 cc. Coli R	Bacteriophage disappeared after twenty-four hours; bacteria disappeared on third day†
IX	Contained Coli R. Present for for 6 days	10 cc. bacteriophage intravesicularly	Bacteriophage disappeared after twenty-four hours; bacteria disappeared after five days†
X	Had contained Coli S for 2 months	10 cc. bacteriophage intravesicularly	Bacteria and bacteriophage absent 3 days later

* Urines were alkaline pH 7.6–7.8.

† The disappearance of the bacteria occurs within this time in the absence of bacteriophage.

expected that bacteriophage can be used successfully in cases of long standing. This statement assumes, what has yet to be demonstrated, that dissolution of the bacterium is alone responsible for any beneficial results from the therapeutic use of bacteriophage. We will return to this question later.

It is interesting to note the difficulty of producing *Bacillus coli* infections in rabbits. In practically every instance the bacteria could not be demonstrated in the urine after forty-eight hours. Helmholz and Beeler (1918) were able to produce pyelitis in about 50 per cent of rabbits by introducing colon bacilli into the bladder. These investigators were working with strains isolated from rabbits having a naturally acquired infection, however. It may be that such strains have a greater virulence for rabbits than do strains isolated from urines from human pyelitis cases.

The work so far described is concerned primarily with the influence of bacteria upon the appearance of bacteriophage in the urine. There is, however, interest in another phase of the same problem. Disregarding the bacteriophage already present, what is the fate of bacteriophage artificially introduced into the bladder? As a basis for the consideration of this problem, table 2 is presented.

That bacteriophage is rapidly eliminated from the bladder is shown by the above table. The presence of susceptible organisms slightly increases the persistence of the principle, but in any event its residence in the bladder is brief. Apparently it made no difference whether the colon bacilli had been in the bladder for some time previous to the bacteriophage instillation or whether bacteria and bacteriophage were introduced simultaneously. Obviously, this result fortifies the hypothesis advanced above that the presence of bacteriophage is due to an infection with the "lysogenic type" of colon bacilli, for we know that in infection of man, bacteriophage can be demonstrated repeatedly in certain cases.

The results reported in table 2 have been confirmed in a number of instances in treatment of pyelitis and cystitis in man. No protocols are given here since the cases have already been de-

scribed,[4] but it was always true in the cases studied that bacteriophage introduced into the human bladder was eliminated within twenty-four to forty-eight hours. This fact has its application to therapy, for in those cases where disappearance of the organisms did not occur within forty-eight hours after intravesicular instillations of bacteriophage it indicates that their subsequent disappearance could not be attributed to *dissolution* of the bacteria. If the principle is at all concerned with the ultimate reduction of the infection it must have operated in some manner other than dissolution.

The observations so far reported have been concerned with the presence or absence of bacteriophage in the urine. It has been suggested, however, that given a susceptible colon bacillus and an active bacteriophage in the urinary tract, the phenomenon of lysis might be considerably modified as compared to the manifestations of bacteriophagy *in vitro*. Indeed, the only direct evidence that bacteriophagy occurs at all in the bladder is furnished by Marcuse (1924), who infected guinea pigs by introducing colon bacilli into the bladder and observed the disappearance of the organisms after bacteriophage was instilled. The colon bacilli disappeared in the treated animals but remained in the controls. The disappearance of colon bacilli following introduction of bacteriophage has been reported by many investigators notably Hauduroy (1924), Philibert (1923–24), and d'Herelle (1926), treating human cases, and, in the present investigation, when rabbits were treated. In such observations, however, the only control was the previous condition in the bladder. The work of Marcuse is fairly conclusive so far as the disappearance of the organisms is concerned, but it does not exclude the possible intervention of other factors activated by the bacteriophage. Such a problem is exceedingly difficult but is of extreme importance. Should it prove that recovery from infection subsequent to bacteriophage introduction was dependent upon some factor other than dissolution, such as the intervention of leukocytes, urine, mucus, or some other substance the methods, as well as the concepts, of therapy would be materially modified.

[4] Bacteriophagy in urinary infection. Part I.

The work to be reported in the following pages represents an attempt to supply the answer to these questions. That it affords no basis for definite conclusions is admitted, yet it does indicate the possibilities and establishes certain points.

It is first necessary to determine the possibility of bacterial dissolution within the bladder cavity, and to observe whether the rate of lysis is influenced by the site of the reaction, whether the dissolution, if it occurs, is accompanied by any temperature changes in the animal, and what if any differences exist in the appearance of the colon bacilli during dissolution *in vivo* and *in vitro* as determined by examination of smears.

Since it had been found that it was difficult if not impossible to infect rabbits by introducing *Bacilli coli* into the bladder, the experiments were accomplished by instillation of 20 to 25 cc. of a suspension of the bacilli by catheter. Two to 10 cc. of bacteriophage were instilled immediately after the bacilli. The catheter was retained, closed by means of a pinch cock, and opened at intervals to remove specimens. A control animal received the same treatment except that no bacteriophage was introduced. The progress and degree of lysis was determined by comparison of the turbidity of the samples removed simultaneously from each rabbit as well as by examination of smears in which counts of the number of bacilli per field provided an index of the degree of dissolution. The study of these smears likewise provided other data which will be considered later. The results of these experiments appear in the protocols in table 3.

It is noticeable in all of these experiments that there occurred a decrease in the number of bacilli per field in smears of specimens taken from the bladder. This decrease followed an initial increase which reached its maximum between the second and the third hours. This fact admits of two explanations. Either the decrease was apparent only, being due to the dilution caused by the continued secretion of urine, or it was real, due to the operation of some agent other than the bacteriophage, operative in the control as well as in the test animals. The evidence afforded by the experiment in which a test tube control was used seemed to indicate that dissolution was more rapid in the rabbit, for the

TABLE 3

TIME	APPEARANCE OF URINE†		BACTERIAL COUNT PER FIELD		TEMPERATURE‡ °F.	
	I	II	I	II	I	II
	Experiment I. *B. coli* susceptible					
p.m.					°F.	°F.
3.00*	Clear	Clear	0	0	99.8	99.6
3.50	Cloudy	Cloudy	4	4	98.4	98.6
4.15	Cloudy	Cloudy	4	4	98.2	98.2
4.45	Cloudy	Cloudy	27	15	98.4	98.6
5.00	Cloudy	Cloudy	25	20	97.2	98.8
5.20	Turbid	Turbid	40	30	97.2	98.4
6.00	Turbid	Clearing	50	30	97.2	98.4
6.20	Turbid	Clear	40	0	97.2	97.2
	Experiment II. *B. coli* susceptible					
p.m.						
12.00*	Clear	Clear	0	0	99.0	97.8
12.45	Turbid	Turbid	22	20	98.4	97.5
1.15	Turbid	Turbid	20	15	98.8	96.4
2.15	Turbid	Turbid	30	25	98.7	96.3
2.45	Turbid	Turbid	15	10	98.6	96.3
3.15	Turbid	Clear	6	3	98.8	95.2
3.45	Turbid	Clear	5	0	99.0	96.2
4.15	Turbid	Clear	2	0	99.4	96.7
	Experiment III. *B. coli* resistant					
p.m.						
3.45*	Clear	Clear	0	0	99.1	100.5
4.45	Turbid	Turbid	10	10	99.3	98.8
5.45	Turbid	Turbid	30	12	99.6	99.8
6.30	Turbid	Turbid	30	20	99.7	99.3
7.30	Turbid	Turbid	30	30	99.7	99.4
	Experiment IV. *B. coli* resistant					
p.m.						
12.00*	Clear	Clear	0	0	95.9	97.3
1.00	Turbid	Turbid	4	8	93.6	96.0
2.00	Turbid	Turbid	6	20	93.6	95.0
3.00	Turbid	Turbid	18	50	92.0	95.4
4.00	Turbid	Turbid	30	50	92.3	97.1
5.00	Turbid	Turbid	10	25	92.0	97.7
6.00	Turbid	Turbid	10	30	92.8	98.8
7.00	Turbid	Turbid	12	20	92.5	99.2
8.00	Turbid	Turbid	2	20		
a.m.						
10.00	Clear	Clear	0	0		

TABLE 3—*Continued*

TIME	APPEARANCE OF URINE†		BACTERIAL COUNT PER FIELD		TEMPERATURE‡ °F.	
	I	II	I	II	I	II
Experiment V.§ *B. coli* susceptible						
a.m.					°F.	°F.
10.55*	Clear	Clear	0	0	99.9	98.6
11.30	Turbid	Turbid	10	15	98.5	98.6
12.00	Turbid	Turbid	15	30	97.8	98.6
p.m.						
1.00	Turbid	Turbid	60	60	98.3	98.6
1.45	Turbid	Turbid	15	70	98.3	98.6
2.15	Clearing	Turbid	3	50	98.8	98.6
2.45	Clear	Turbid	0	40	99.0	98.6
a.m.						
8.00		Clear		2		98.6

* Animals catheterized.

† In experiment I, I is control animal, II is test animal. In experiment V, II is control tube.

‡ Temperatures were taken every 15 minutes but showed no great variations.

§ Instead of a control animal in this experiment a control tube was used and bacteriophage added. The amounts of culture and bacteriophage being the same as in the rabbit.

striking difference in the time required for complete clearing of the medium—less than four hours in the bladder and nearly twenty-four hours in the test tube—could hardly logically be attributed to dilution. As further indication of the operation of other factors in the bladder, the time required for the elimination of all bacteria, whether susceptible or resistant and whether bacteriophage was present or absent, was so brief that dilution alone could scarcely account for the occurrence.

When the factor of dilution, and in fact the presence of urine in the bladder was removed by ligation of both ureters of rabbits, lysis proceeded at the same rate in the bladder and in the test tube. In the control animal no diminution in the number of organisms occurred during two and one-fourth hours. Since, however, this period was less than the time usually required for reduction in the number of organisms, the experiment is far from conclusive. Owing to the extreme urgency exhibited by rabbits

so shortly after the operation necessary for placing the ligatures, and to the consequent impossibility of introducing sufficient fluid to continue the experiment over a longer period, other experiments were devised which will be described later.

The above experiments do, however, show that bacteriophage can effect dissolution of bacteria in the bladder and confirm to this extent the results of Marcuse. They further show that no significant temperature reaction accompanies dissolution. The examination of the smears beside yielding the information as to the progress of dissolution afforded some other interesting data which will be considered later.

To return to the question of the effects of factors other than the bacteriophage upon colon bacilli in the bladder and to the effects of factors present upon the bacteriophage and upon bacteriophagy, it has been seen that ligation of the ureters did not provide a satisfactory experiment. Removal of the bladder would accomplish the elimination of the urine as a factor, but would also eliminate any substances supplied by the blood.

Any substances affecting either the bacteria or the bacteriophage within the bladder must have their origin in the bladder tissue itself, in the blood supplying the bladder, in the urine, in the mucus present in the urine, or in the products of bacterial metabolism. As a source of such substances, the blood can be eliminated, for not only is blood excluded fron direct contact with the bacteria or bacteriophage, but such elements as can leave the blood vessels, red blood cells and leukocytes, never appeared in the specimens examined in preceding experiments. Thus by removal of the bladder, the effect of bladder tissue itself—either alive or dead—and the effects of bacterial metabolism can be studied. The urine and the mucus can be investigated independently.

In table 4 are given the results obtained by introducing bacteriophage and colon bacilli into bladders removed from rabbits and suspended in Ringer-Locke solution, oxygenated, and kept at 37°C.

Apparently living bladder tissue has little or no effect upon bacteriophagy, lysis occurring in the same interval in the control

tube as in the bladder. On the other hand, dead tissue not only increases the rate of the reaction, but when added to a broth culture is able to bring about a degree of dissolution, although

TABLE 4

BLADDER	BLADDER CONTAINED				AMOUNT OF LYSIS AFTER HOUR:				
	Broth	Urine*	B. coli S	B/P 3620	First	Second	Third	Fourth	Twenty-fourth
	cc.	cc.	cc.	cc.					
I	25	0	2	1	0	0	+++	++++	++++
II	0	25	2	1	0	0	0	0	++++
III	25	0	5	1	0	++	−	−	++++
IV	0	25	5	1	0	0	−	−	++
V	25	0	5	1	0	++	++	++++	++++
TEST TUBE + BLADDER TISSUE									
I	25	0	2	1	0	0	+++	++++	++++
II	25	0	5	1	0	++++	−	−	++++
III	25	0	5	0	0	0	−	−	+
IV	25	0	5	1	0	+	++	++++	++++
V	25	0	5	0	0	0	+	+	++++
VI		25	5	1	0	0	0	0	++
TUBE	CONTENTS OF TUBE				AMOUNT OF LYSIS AFTER HOUR:				
	Broth	Urine	B. coli S	B/P 3620	First	Second	Third	Fourth	Twenty-fourth
	cc.	cc.	cc.	cc.					
I	25	0	2	1	0	0	+++	++++	++++
II	0	25	2	1	0	0	0	0	++++
III	25	0	2	0	0	0	0	0	0
IV	0	25	2	0	0	0	0	0	0
V	25	0	5	1	0	+	−	−	++++
VI	0	25	5	1	0	0	−	−	+

* Rabbit urine used.

no bacteriophage has been added. The presence of urine actually retards bacteriophagy to a marked degree.

Each of the latter two observations merits further investigation, the effect of urine in particular, since, although dead bladder tissue cannot play much of a rôle in the bacteriophage therapy of

cystitis, urine is especially significant because it is the medium in which bacteriophagy, if it takes place, must occur. The question of the effect of urine is still under investigation.

Demonstrations of bacteriolytic substances in tissues have been frequent. McKinley (1923) found a principle in tissues, both normal and infected, which he proved to be bacteriophage. Fleming and Allison (1922) describe a bacteriolytic substance in tissues and in secretions such as tears. They claim that this principle is not a bacteriophage and call it "lysozyme." Petrovanu (1924) isolated a substance from dried and ground up intestines capable of dissolving certain cultures of Cholera vibrios. In the present investigation, the substance found to cause dissolution in one of the tubes (V, indicated in table 4) was capable of producing plaques, and was still active in a filtrate after five contacts with *Bacillus coli* and intervening filtrations, a fact which is much in favor of its being bacteriophage. Further, four bladders from guinea pigs gave a similar principle which was apparently not present in equal amounts and strength in the different bladders, for in all but one instance it was necessary to make several contacts of filtrates and colon bacilli before disclosing the principle. To prove conclusively that this principle is given off only by dead tissue is a difficult problem, for few means are available for keeping a tissue surviving while submitting it to treatment necessary to extract the bacteriolytic agent. Yet so long as the bladder tissue survived in the Ringer-Locke's solution used in these experiments, bacteriophage was not to be found. Thus, although the evidence is slight, there is some indication that living or surviving tissue retains bacteriophage which may be released upon the death of the tissue. Several other hypotheses are possible, but the application of the observations to the problem at hand is not hypothetical. It is quite evident that the living bladder tissue itself contributes little to the phenomenon of bacteriophagy *in vivo* at least to the extent of accelerating the process.

The work thus far described, however, leaves the question still unanswered as to what, if anything, causes more rapid lysis in the bladder than in the test tube. There remains only the mucus

to be considered. The secretion of this substance is particularly abundant in rabbits.

In the examination of the smears prepared from specimens removed from the bladder in the living animal, a peculiar phenomenon was observed. The bacilli were at first clumped, giving the appearance of typhoid bacilli in a microscopic Widal test.

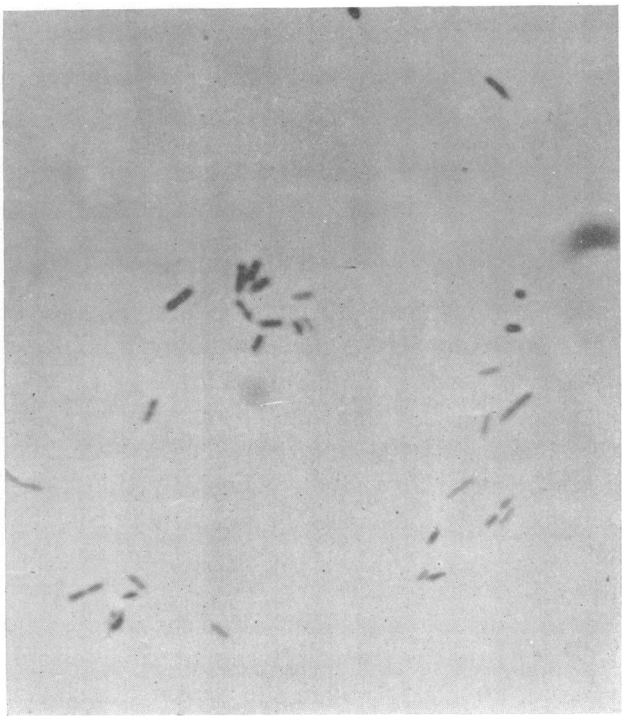

FIG. 1. × 1200

These clumped bacilli then became swollen and stained irregularly. The swelling was so great that the bacilli became from four to six times their normal dimensions. The two microphotographs presented here illustrate the change (figs. 1 and 2).

The first is of a smear prepared from a culture containing the same number of bacilli per cubic centimeter as were in the specimen from which the second smear was prepared. The colon

bacilli shown in figure 1, however, were developing in a test tube, those shown in figure 2 were growing in the bladder of a rabbit. Bacteriophage was present in both cultures. Other smears showed the same phenomenon, however, in the absence of bacteriophage. Since making these observations, many instances of a similar occurrence in man have been observed. In many

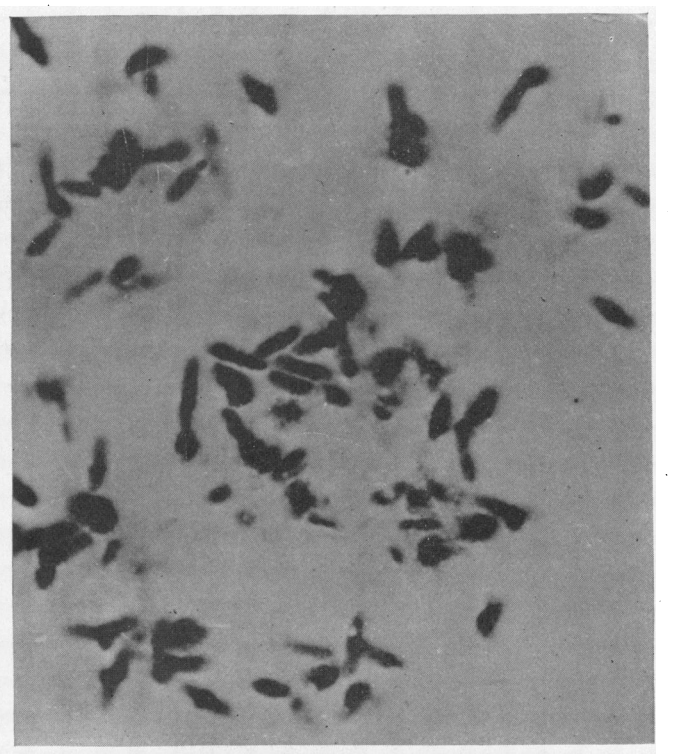

FIG. 2. × 1200

urines from cases of pyelitis and cystitis, the bacilli are clumped, lose motility, and tend to stain irregularly and to evidence more or less pleomorphism.

It has already been remarked that rabbit urine is particularly apt to contain large quantities of mucus, and it was in those urines in which mucus was most abundant that swelling and clumping were most marked. Some mucus obtained in a speci-

men of rabbit urine was washed several times with saline, and added to about 5 cc. of broth in two tubes. One was kept as a control of the sterility of the mucus, the other was inoculated with *Bacillus coli*. Both tubes were incubated at 37°C., the control remaining sterile. The colon bacilli from the other tube reproduced the phenomenon previously observed. Clumping and swelling became marked. Although there was no appreciable increase in the number of colon bacilli during several days of incubation, the organisms did not disappear but remained viable. Neither the inoculated tube nor the control contained demonstrable bacteriophage. It would appear from this observation that the mucus present in the bladder can exercise considerable influence upon bacteria invading the organ. Although it is not bactericidal or bacteriolytic, it has an inhibitory action which may be entirely mechanical. Through the agglutination which it causes it may aid in the normal process of removal of bacteria from the bladder. Certainly this clumping can account for a considerable proportion of the apparent decrease in organisms observed in smears. The question as to the effect of this mucus upon bacteriophage and upon bacteriophagy is at present under investigation.

CONCLUSIONS

1. Bacteriophage is not found in rabbits' urine when bacteria of any type except the lysogenic strains are put into the bladder and maintained there for varying periods of time.

2. The introduction of colon bacilli into the body by the enteral or intravenous route fails to cause bacteriophage to appear in the bladder.

3. Damage to the bladder wall by means of hydrochloric acid does not result in the appearance of bacteriophage in the urine.

4. When introduced into the bladder, bacteriophage is eliminated within twenty-four to forty-eight hours.

As a result of these findings it is suggested that infection with lysogenic strains of *Bacillus coli* is alone responsible for the existence of bacteriophage in the urine.

5. Lysis of colon bacilli through the action of the bacteriophage can take place in the bladder.

6. Urine exercises an inhibitive action upon bacteriophagy.

7. Mucus, although apparently not affecting bacteriophagy, acts upon the colon bacilli in such a manner as to promote their removal from the bladder.

8. Surviving bladder tissue has no effect upon bacteriophagy.

9. Dead bladder tissue releases a principle resembling bacteriophage.

While it is impossible, on the basis of these experiments to state through what agency and to what extent modifications occur, it is obvious that bacteriophagy is not the same in the bladder as it is in the test tube.

REFERENCES

DA COSTA CRUZ, J. 1923a A influencia dos electrolytos sobre a lyse pelo bacteriophago. Brazil-med., Rio de Jan., **37**, 341.

DA COSTA CRUZ, J. 1923b Sur la nature du bactériophage. Influence des électrolytes. Compt. rend. Soc. de biol., **89**, 759.

DAVISON, W. C. 1922 Observations on the properties of Bacteriolysants (d'Herelle's phenomenon, bacteriophage, bacteriolytic agent, etc.). Jour. Bacteriol., **7**, 475, 491.

DOERR, R. 1922 Die Bakteriophagen (Phänomen von Twort und d'Herelle). Klin. Wchnschr., **1**, 1489, 1537.

DOERR, R., AND BERGER, W. 1922 Studien zum Bakteriophagenproblem. III. Mitt. Die antagonistische Wirkung von Galtine und Agar auf den Ablauf der Bakteriophagenreaktion. Zeitschr. f. Hyg. u. Infektionskrankh., **97**, 422.

ELIAVA, G., AND POZERSKI, E. 1921 De l'action destructive des sels de quinine sur le bacteriophage de d'Herelle. Compt. rend. Soc. de biol., **85**, 139.

FLEMING, A., AND ALLISON, V. D. 1922 Observations on a Bacteriolytic substance ("Lysozyme") found in secretions and tissues. Brit. Jour. Exper. Path., **3**, 252.

GRATIA, A. 1921 Influence de la reaction du milieu sur l'autolyse microbieene transmissible. Compt. rend. Soc. de biol., **84**, 275.

HAUDUROY, P. 1923 Le rôle du bacteriophage dans la fièvre typhoïde; sa présence dans les selles. Compt. rend. Soc. de biol., **89**, 791.

HAUDUROY, P. 1924 Les applications du bactériophage de d'Herelle en therapeutique et en hygiene. Ann. de méd., Par., **16**, 340.

HAUDUROY, P. 1925 Action de la bile sur le bactériophage et importance de cette action. Compt. rend. Soc. de biol., **92**, 1442.

HELMHOLZ, H., AND BEELER, C. 1918 Experimental pyelitis in rabbits. Jour. Urol., **2**, 395.

D'HERELLE, F. 1921 Le Bactériophage, son rôle dans l'immunité. Maisson & Cie., Paris, 1921. English translation, The Williams & Wilkins Co., Baltimore.

D'HERELLE, F. 1926 The Bacteriophage and its Behavior. English translation, The Williams & Wilkins Co., Baltimore.

MCKINLEY, E. B. 1923 Further notes on d'Herelle's phenomenon. Jour. Lab. and Clin. Med., **9**, 185.

MARCUSE, K. 1924 Grundlagen und Aufgaben der Lysintherapie (d'Herelle's bakteriophagen). Deutsche. med. Wchnschr., **50**, 334.

NAKAMURA, O. 1923a Die Hemmung der Bacteriophagenwirkung durch Gelatine. Arch. f. Hyg., **92**, 61.

NAKAMURA, O. 1923b Der Verhalten von Bakteriophagen in Gelatine. Wein. klin. Wchnschr., **36**, 86.

PETROVANU, G. 1924 Recherches sur la présence du principe lytique vis-à-vis du Vibrion cholerique dans la paroide l'intestin grêle. Compt. rend. Soc. de Biol., **91**, 754.

PHILIBERT, A. 1923–24 Le principe bactériophage (bactériophage de d'Herelle), immunité; vaccination. Médecine, Par., **5**, 191.

REICHERT, F. 1924 Untersuchungen über das d'Herellesche Phänomen. Centralbl. f. Bakt., I. Orig., **91**, 235.

SCHEIDEGGER, E. 1923 Studien zum Bakteriophagenproblem. IV. Mitt. Der Einflus der Wasserstoffionenkonzentration auf das lytische Agens und den Bakteriolyse. Zeitschr. f. Hyg. u. Infektionskrankh., **99**, 403.