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INACTIVATION OF ANTISTREPTOCOCCUS BACTERIOPHAGE BY ANIMAL FLUIDS

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INTRODUCTION

If bacteriophage could destroy bacteria *in vivo* as it does *in vitro*, it would offer a remarkable cure for infections with those bacteria for which an active phage has been found. With this intriguing theoretic possibility of the value of phage in the treatment of disease, many trials have been made of its therapeutic properties in a great variety of human diseases. While the nature of the trials has been usually that of an experiment without controls, the recovery of the patient has been often ascribed to the therapeutic virtues of the phage. In some of the trials there appears to be good evidence that beneficial results followed the use of phage. An example is MacNeal and Frisbee's recent report of seven recoveries out of 15 cases of staphylococcus bacteremia treated with phage, whereas such infections are known to result generally in a higher average percentage of mortality. On the whole, however, the results of the use of phage as a therapeutic agent have been disappointing. Certainly they have been less successful than test tube experiments seemed to promise. A review of the literature led Larkum in his recent summary of the information on the use of phage in clinical medicine to state that if its effectiveness is dependent on lysis *in vivo*, its application is limited to enteric infections, with some slight hope for kidney and bladder conditions. To that disillusioned view it must be added that even in enteric infections the results with phage have been worthless in the opinion of some investigators who have tried it.

There are two kinds of experiments which should enlighten the use of bacteriophage in human disease: First, its efficacy can be determined in the treatment of experimentally infected animals, with adequate controls; and second, the effect on bacteriophage of the various fluids and cells with which it comes into contact when introduced into the body can be determined *in vitro*.

REVIEW OF THE LITERATURE

With only a few dissenting reports, there is agreement in respect to the experiments in which phage has been used in the treatment of experimentally infected animals. In reviewing the results ob-

tained, Bronfenbrenner (1928) states that it has almost universally failed to influence the course of the disease. The work done subsequently to his review agrees generally with that statement.

The more recently reported results may be summarized briefly. Flu could not save guinea pigs inoculated with minimal lethal doses of plague bacilli by treatment with phage simultaneously, nor by administering the phage from one to three days after the inoculation. White rats also failed to show protection when injected with phage immediately after injection with the plague bacillus.

After a search for a phage highly active against the agent of a current epidemic of plague, Naidu and Avari found a strain which caused lysis in broth cultures in less than two hours. Yet it was of no benefit when injected into rabbits experimentally infected with the sensitive plague organism. On the contrary, the phage lowered the percentage of recoveries when injected together with an antiserum which possessed the property of reducing the mortality among experimentally infected rabbits. Thirty-three human patients were treated with this highly active strain of phage and every one died.

Eliava found that phage specific for the Shiga bacillus, staphylococcus, or colon bacillus had no therapeutic effect in animals; and Cowles and Hale reported that phage injected into the body appeared to have a detrimental, rather than a beneficial effect in experimental anthrax in white mice.

On the contrary, Walker reported a marked reduction in the mortality of mice when bacteriophage was injected into the peritoneal cavity simultaneously with the colon bacillus. Later he carried out experiments with phage in the treatment of staphylococcus and streptococcus infections. The cocci were injected intracutaneously and the specific phage injected previously or simultaneously with the culture had no effect on the lesion. Likewise, intravenous injection of phage subsequent to intracutaneous injection of staphylococci had no observable effect on the lesions. It was only when the phage was mixed with the staphylococci previous to injection that a beneficial effect was observed.

In a recent publication the conclusions of MacNeal, Frisbee, and Slavkin in regard to the beneficial effects of bacteriophage in experimental infections disagree with those of the majority of investigators. They studied the multiplication of staphylococci in experimentally infected rabbits treated with bacteriophage and in control animals. They reported that one immediate effect of intravenous injection of phage in bacteremia is to favor more rapid phagocytosis of the bacteria which are circulating in the blood. They also reported that the phage tends to restrain the further growth of the bacteria which have lodged in the internal organs, and that it favors the more rapid and efficient intracellular digestion of the phagocyted bacteria. These

investigators, however, apparently did not carry out experiments to compare the mortality in phage-treated and in control animals, all of their animals having been killed to follow the progress of the disappearance of the cocci. In the same number of the same journal containing the report of MacNeal, Frisbee, and Slavkin, a diametrically opposite conclusion is reached by Krueger, Lich, and Schulze, who also studied the multiplication of staphylococci in experimentally infected rabbits. Their work is referred to again further on.

In agreement with the majority of investigators who tested the efficacy of bacteriophage in controlled animal experiments are the results of Dresel and Lewis, who studied the behavior of bacteriophage in tissue cultures with mouse typhoid bacilli. Cultures with phage were compared with cultures without phage in susceptible mouse tissue and in resistant chicken tissue. In neither case could any difference in behavior be observed in the multiplication of the bacilli in cultures with or without phage.

A more detailed review of the limited experimentation with antistreptococcus bacteriophage is in order. It was used therapeutically in human cases of streptococcus infection reported by McKinley, by Dutton, and by Raiga. It appears that both McKinley and Raiga treated a single case. Dutton treated five cases, but apparently he used a very weak phage. Hence the statement can be made that the treatment of human cases of streptococcus infection with specific bacteriophage has never received an adequate trial. The negative results obtained in animal experiments offer no hope that such treatment would be beneficial.

Clark and Clark infected rabbits with a streptococcus, isolated from spontaneous rabbit infection, which was lysed by their strain of phage in high titer. Treatment with phage was given either before or after the bacterial inoculations. It was given *per os*, or by intraperitoneal or intravenous injections. In no case did the treated animals do better than the untreated controls. On the contrary, in every instance in which a large amount of the phage was used, the treated animals died before the controls.

Colvin also studied the effect of bacteriophage on streptococcus infection in the natural host of the particular streptococcus strain used, namely, in lymphadenitis in guinea pigs. Treatment with a phage highly virulent for this streptococcus revealed no therapeutic value. On the contrary, as in the Clarks' experiments, there was evidence that it was not entirely harmless. The writer's experiments reported in detail further on are in complete agreement with those of the Clarks and those of Colvin in showing that the therapeutic use of antistreptococcus phage did not ameliorate experimental streptococcus infections, and some of the data indicate that the disease was more severe in animals injected with the phage.

From the foregoing review of the failures to cure experimental infectious diseases, it is evident that there is something in the body which inhibits bacteriophagy. Opinions are contradictory, however, as to what the inhibiting agent may be. Several writers (Bruynoghe and Maisin; Applebaum and MacNeal; Colvin) have noted the inhibitory action of pus, and some (Bruynoghe and Maisin; Eliava) believe that the leucocytes are responsible for the inactivation. On the contrary, Bier and Cunha assert that leucocytes do not inhibit the action of phage. There are some writers who believe that the leucocytes are responsible for the disappearance of bacteriophage from the blood. Burnet subscribes to that opinion in his recent review. Some writers (Gratia and Jaumain; Gratia and Mutsaars; Cowles and Hale; Applebaum and MacNeal; Colvin; Riding) have reported that blood serum is inhibitory. Others (Bruynoghe and Maisin; Eliava) have reported that it is not inhibitory. Krueger, Lich, and Schulze recently reported that phage injected into rabbits simultaneously with virulent staphylococci failed to prevent the multiplication of the staphylococci. The curves showing the bacterial content of the blood in the treated animals and in the controls exhibited no significant differences. These investigators believe that the inhibition of the phage is due to its adsorption by red blood cells, thus agreeing with Arloing, Langeron, and Sempé, whereas other investigators (Eliava; Applebaum and MacNeal) believe that red blood cells are not inhibitory.

Until recently scant attention has been paid to the possible inhibitory action of fluids other than blood or pus with which bacteriophage becomes diluted when used as a therapeutic agent. Calalb reported, however, that bile inhibits the action of bacteriophages active against typhoid and colon bacilli and staphylococci. (This work has been referred to in the literature as Hauduroy's.) In a recent paper, which was published after the completion of the experiments here reported, Colvin states that serum and pus inhibit the action of bacteriophage, and to a less degree ascitic fluid, cerebrospinal fluid, and urine are also inhibitory.

Colvin emphasizes, as did Gratia and Jaumain, that different bacterial species vary greatly in their response to the inhibitory action of body fluids on phage. He found that different races of phage also differ in their response to inhibitory agents, and that even different samples of a given fluid may differ in inhibitory activity.

In test tube experiments there are three variables concerned in the inactivation of bacteriophage—the strain of bacterium, the race of phage, and the body fluid. In animal experiments even greater variability is conceivable. The results of the experiments here reported which are limited to a single race of bacteriophage and to two strains of streptococci, can not be assumed to apply equally to all other races

of phage and all other bacteria. They do, however, offer an explanation why bacteriophage has been so disappointing as a therapeutic agent.

EXPERIMENTAL

The experiments recorded in this paper are concerned with the therapeutic action of antistreptococcus bacteriophage in experimentally infected animals, and its lytic action in the presence of animal fluids and cells *in vitro*.

For the test tube experiments the strain of streptococcus used was that known as Birkhaug E_1, originally from a case of erysipelas. For the animal inoculation experiments a highly virulent strain of streptococcus was used. It was received from the late Dr. F. B. Jennings, of Johns Hopkins Medical School, as one of his virulent cultures derived from a nonvirulent culture by selection of dissociating colonies. The Jennings strain was usually lethal to white mice in 1×10^{-8} c c of 24-hour broth culture, which contained only a few units of streptococci, the unit being a single coccus, a pair, or a chain from which a colony would develop on blood agar.

The bacteriophage used was the strain originally obtained from sludge by Clark and Clark. It was received through the courtesy of Dr. Gregory Shwartzman, of Mount Sinai Hospital, New York City. For general purposes the phage was propagated on the erysipelas streptococcus. The filtrates usually contained approximately 10^{10} particles of phage active for the homologous strain. When tested on the Jennings strain the titer was lower, lysing the culture in dilutions of 1 to 10^6 c c but not in higher dilutions. For some of the experiments in mice, and all the experiments in rabbits, a substrain of the phage was used which was produced by growth for 20 culture generations on the Jennings streptococcus. This treatment raised its titer for the Jennings streptococcus to 10^9.

EXPERIMENTS IN VIVO

Several sets of mouse inoculations with streptococcus and bacteriophage gave consistent results, with no evidence that the phage ameliorated the disease. In all the experiments the dose was 0.5 c c of undiluted phage and 0.5 c c of streptococcus culture, the dilution varying in the several experiments. In the preliminary experiment the inoculating dose was diluted to 1×10^{-5} c c. For 3 mice the phage and streptococci were incubated for 1 hour before inoculation; for 3 mice the phage and streptococci were injected simultaneously; and for 3 mice the phage was injected 1 hour after the streptococci. All nine mice died on the second or third day of the experiment, and streptococci were cultured from the heart blood of all.

TABLE 1.—*Protocol of an experiment showing that bacteriophage does not protect mice inoculated with approximately five minimal lethal doses of sensitive streptococci*

Inoculum	Mouse 1 of group	Mouse 2 of group	Mouse 3 of group
Controls: Culture + broth	Died, 52 hours	Died, 52 hours	Died, 72 hours.
Group I: Culture + phage, not incubated	Died, 46 hours	Died, 46 hours	Died, 52 hours.
Group II: Culture + phage incubated together 1 hour.	Died, 38 hours	Died, 65 hours	Died, 110 hours.
Group III: Culture + phage incubated together 2 hours.	Died, 46 hours	Died, 52 hours	Died, 62 hours.

Hemolytic streptococci were recovered from the heart blood of all the mice.

Another experiment was carried out using broth culture diluted to 1 part in 10^7. (See Table 1 for the protocol.) The inoculum of 0.5 c c therefore contained approximately five minimal lethal doses. Three mice of 1 group were injected with culture and phage simultaneously; 3 mice of another group were injected with culture and phage which had been incubated together for 1 hour; 3 mice of another group were injected with culture and phage which had been incubated together for 2 hours; and a control group of 3 mice received culture without phage. Plantings on blood agar plates showed that the inoculum of the 3 control mice and of the group receiving the culture and phage without incubation contained approximately 5 units of streptococci; plantings made just before inoculation of the mixtures of culture and phage showed that lysis of the streptococci had not occurred during the 1 or 2 hours of incubation, nor had multiplication of streptococci occurred. As in the first experiment the mice all died, and hemolytic streptococci were cultivated from the heart blood of all.

TABLE 2.—*Protocol of an experiment showing that bacteriophage does not protect mice inoculated with approximately one unit of sensitive streptococci*

Inoculum	Mouse 1 of group	Mouse 2 of group	Mouse 3 of group
Controls: Culture + broth	Survived	Survived	Survived.
Group I: Culture + phage, not incubated	Died, 52 hours [1]	Died, 62 hours [1]	Died, 89 hours.[2]
Group II: Culture + phage incubated together 1½ hours.	Died, 47 hours [1]	Died, 52 hours [1]	Died, 64 hours.[1]
Group III: Culture + phage incubated together 3 hours.	Died, 46 hours [1]	Died, 62 hours [1]	Died, 100 hours. [1]

[1] Pure culture of streptococcus from the heart blood.
[2] No growth in culture planted with heart blood.

The third experiment was similar to the second, except that the streptococcus culture was diluted still higher, to 1 part in 10^8. As it happened, the dilution was slightly too high, and none of the three control mice died. Yet every one of the nine mice receiving bacteriophage died, and streptococci were cultured from the heart blood of all except one. (See Table 2.) In this experiment the streptococcus culture was diluted so high that the inoculum would contain only a

very few units. By chance it might occasionally contain no streptococci. It is possible that the three control mice may not have received any streptococci. The protocol suggests, however, that in this experiment as in those of the Clarks and of Colvin, the phage was harmful, stimulating a sublethal dose of streptococci to produce a fatal infection.

Two further experiments were carried out to demonstrate the possible stimulation of a sublethal dose of the streptococcus by bacteriophage. In each experiment there were three control mice which received streptococci alone, and three mice which received streptococci and phage simultaneously. For one experiment the broth culture was diluted to 1 part in 10^8. One of the 3 controls and 1 of the 3 mice which received phage survived. In the other experiment the broth culture was diluted to 1 part in 5×10^7. One of the 3 control mice survived, but all 3 mice receiving phage died. Summarizing the 3 experiments in which the inoculum contained only a few minimal lethal doses, with the possibility that it might not contain a single unit of streptococci, 5 out of 9 control mice survived, whereas only 1 out of 15 mice receiving phage survived. These limited figures can not be taken as proof that the phage was harmful, but they demonstrate emphatically that it was of no benefit under the conditions of these experiments.

The possibility of a therapeutic property in the antistreptococcus phage was tested in rabbits also. The infecting dose of streptococcus in these experiments was so adjusted that the majority, but not all, of the control rabbits not receiving phage would die. Thus the experiment would not only demonstrate the possible beneficial effect of the phage, but also it would demonstrate any possible harmful effect. Two similar experiments were carried out, in each of which there were nine rabbits. Three had been injected intravenously with 2 c c of phage 3 days previously, 3 were injected with 2 c c of phage simultaneously with the infecting dose of streptococcus, and 3 without phage treatment served as controls. The infecting dose was 1 c c of the 1 in 10^3 dilution of a 24-hour broth culture injected intravenously. (In the course of another experiment on another phase of the bacteriophage problem not reported in this paper, it had been determined that the mentioned dose would kill the majority, but not all, of the rabbits.) One of the control rabbits died with an intercurrent infection. It is eliminated in the consideration of the results.

The protocols of the two experiments are combined in Table 3. In so far as can be judged from these limited data, the mortality rate was not appreciably influenced by the presence of phage in the body. The slight differences, which happen to favor the phage-treated rabbits, are within the limits of error.

TABLE 3.—*Combined protocols of two experiments showing that bacteriophage does not influence the death rate of rabbits inoculated with sensitive streptococci*

Treatment	Date of inoculation	Rabbit 1 of group	Rabbit 2 of group	Rabbit 3 of group	Total number of rabbits	Died	Survived
	1933						
None (controls)	Jan. 7	Dead, ninth day.[1]	Survived	Dead, sixth day.[1]			
	Jan. 19	Dead, twelfth day.[1]	Dead, eleventh day.[2]	Survived	[3] 5	3	2
Group I: 2 cc phage 3 days previously.	Jan. 7	Dead, fourth day.[1]	Dead, fifth day.[1]	Dead, sixth day.[1]			
	Jan. 19	Survived	Survived	Survived	6	3	3
Group II: 2 cc phage simultaneously.	Jan. 7	Dead, third day.[1]	do	do			
	Jan. 19	Survived	Dead, seventh day.[1]	Dead, sixth day.[1]	6	3	3

[1] Pure culture of streptococcus from the heart blood.
[2] An extraneous organism was cultured from the heart blood.
[3] One control rabbit died of intercurrent infection.

Although the mortality rate shows no evidence that the phage was harmful, there was evidence that the disease was unusually violent in those rabbits treated with phage which succumbed to the infection. Whereas the death of the control rabbits which received no phage occurred on the sixth to twelfth days, those treated with phage died earlier, from the third to seventh days. The control rabbits which died on the sixth and ninth days showed gross lesions only in the lungs. In the control rabbit which died on the twelfth day, in addition to the lung lesions, pus was found in the pleural and peritoneal cavities. In some of the phage-treated rabbits there were other lesions indicating a more violent disease. The one which died on the third day had been bleeding from the nose, and the liver was of an abnormally bright red color; in two rabbits (dying on the fifth and sixth days) there were necrotic areas on the liver; and in two rabbits (dying on the fifth and sixth days) a ruptured stomach or intestine was found.

The evidence here given that bacteriophage treatment may be harmful to experimentally infected mice and rabbits is in agreement with the results of other workers who reported a harmful effect of phage. (See the review of the literature.) In speculating why the disease should be more severe in the presence of phage, the observed stimulation of bacterial growth *in vitro* in the presence of phage under certain conditions comes to mind. Hetler and Bronfenbrenner found that 4 per cent agar or 50 per cent gelatin in culture medium prevents lysis by bacteriophage, and that on these media the bacteria grow more vigorously in the presence of phage. I have observed that strains of streptococci that are only slightly sensitive to bacteriophage sometimes grow more vigorously in broth cultures in the presence of phage.

The following experiment was carried out to determine the fate of the phage after introduction into the blood stream. A rabbit weighing approximately 2 kg was injected intravenously with 2 c c of a sample of phage with a titer of 10^9. Samples of blood taken from the ear vein after 2 hours, and after 1, 2, and 3 days, were tested for the presence of phage. The most successful method found for its demonstration was to allow 1 drop of blood to flow from the ear vein into a tube of broth. A larger quantity of blood in the broth inhibited the action of the phage. (The inhibitory action of body fluids is considered further on.) The broth containing the blood was inoculated with the streptococcus and incubated. On the following day, if there was any question whether partial lysis had occurred, the culture was filtered and the filtrate was tested for phage. Thus it was demonstrated in the blood samples taken at 2 hours, 1 and 2 days, but it had disappeared from the blood when the sample was taken 3 days after its injection.

The presence of phage in the rabbit's body three days after injection was of particular interest in connection with the experiment recorded in Table 3. Hence the rabbit was killed after the 3-day sample of blood had been taken, and the liver and spleen were examined for phage. To demonstrate the phage, a portion of the organ was crushed to a pulp in a mortar, then approximately 2 parts of broth were added and, after mixing, a drop of the emulsion was added to a tube of broth. The subsequent procedure was the same as for the demonstration of phage in the blood. A larger quantity of emulsion added to the broth gave negative results, due to its inhibitory action on the phage. By the described method, phage could be demonstrated in the spleen, but not in the liver. These results are in agreement with those of Appelmans, who found that phage injected into the blood stream of rabbits was retained in the spleen after it had disappeared from the other organs and from the blood.

EXPERIMENTS IN VITRO

The results of the foregoing experiments are in agreement with the results of other investigators in showing that phage is rendered ineffective when introduced into the animal body. An attempt was therefore made to determine what constituents of the blood cause the inactivation and what other body fluids have the same effect.

In these experiments the test for phage was always made in broth culture, because the plate method for the demonstration of antistreptococcus phage has been found to be less delicate. The diluent of the test substances was broth with a hydrogen ion concentration of pH 7.6. Incubation was at 37° C. The phage was always diluted to contain approximately 10^4 particles per c c of test substance. (Phage containing 10^{10} particles per c c was diluted to 1 part in 10^4, and 2

drops of this dilution were added to 10 c c of test substance.) The substance under consideration was tested undiluted and diluted to varying degrees with broth. For every test made, a control tube without phage demonstrated any possible inhibition of the growth of the streptococcus by the test substance itself. Control tubes of inoculated broth without and with phage demonstrated the multiplication of the streptococci in the one and the lysis by phage in an inert medium in the other. (A clear medium in a tube inoculated with streptococci and bacteriophage signifies more than inhibition of growth. It signifies lysis, for multiplication of the streptococci, producing turbidity, precedes lysis.) Streptococcus inoculations were always with one drop of 2-hour culture which had been planted heavily enough to develop faint turbidity during the short incubation. The cultures in the test substance with and without phage were incubated 24 hours, then were agitated to make uniform suspensions, and a loopful of each was spread on blood agar to demonstrate the relative number of streptococci in the corresponding tubes with and without phage. The blood agar plates were marked to divide them into halves; one-half was streaked with a loopful of the culture containing phage and the other half was streaked with the corresponding culture without phage. After the plates had been incubated 24 hours readings were made.

TABLE 4.—*Sample protocol of an experiment to show the inhibitory action of various substances on bacteriophage*

Test substance	Bacteriophage	Dilution of test substance			
		Undiluted	1:2	1:5	1:10
Broth	None	Myriads of colonies.			
	Present	Many colonies			
Pus	None	Alike. Myriads of colonies.	Myriads of colonies.	Myriads of colonies.	Myriads of colonies.
	Present		Colonies in ragged patches.	28 colonies	2 colonies.
Washed cells from pus.	None	Myriads of colonies.	Myriads of colonies.		
	Present	1 colony	About 80 colonies		
Ascitic fluid	None	Alike. Myriads of colonies.	Alike. Myriads of colonies.	Myriads of colonies.	Myriads of colonies.
	Present			About 160 colonies.	About 160 colonies.
Urine	None	Myriads of colonies.	Myriads of colonies.	Myriads of colonies.	
	Present	Colonies in ragged patches.	Sterile	13 colonies	

A sample protocol is given in Table 4. In recording these data each hemolyzed disk on the blood agar plates was counted as a colony, although on the plates streaked with culture containing active phage the streptococcal growth which produced the hemolysis might have disappeared by the time the plates were examined.

In the experiments recorded in Table 4 the broth control culture in which lysis had occurred was clear, yet the blood agar streaked

with it developed many isolated colonies—many more than developed as a rule from broth cultures in which lysis had taken place. Nevertheless, the contrast between the isolated colonies on the one half of the plate and the myriads of colonies on the other half gave a striking demonstration of the results of bacteriophagy. Other plate cultures recorded in Table 4 which developed colonies varying in number from 0 to 160 gave typical pictures of the results of lysis in inert media.

Partial inhibition of bacteriophagy by the test substance was sometimes demonstrated in the blood agar cultures by a reduced number of colonies evenly distributed; at other times by a patched appearance of the plates, with irregular areas of the plate hemolyzed, interspersed with irregular nonhemolyzed areas where no growth had taken place. In the experiment recorded in Table 4, partial inhibition occurred in the culture with phage added to pus diluted 1 to 2, and in the culture with phage added to undiluted urine.

When the blood agar streaked with culture to which phage had been added developed as many colonies as the control half of the plate streaked with culture without phage, the result was considered as complete inhibition.

According to these interpretations, Table 4 shows that in undiluted pus the action of phage was completely inhibited; in the 1 to 2 dilution there was partial inhibition; and in higher dilutions the pus exerted no inhibitory influence. The washed pus cells did not inhibit bacteriophagy. It was completely inhibited by ascitic fluid undiluted or in the 1 to 2 dilution, but not in higher dilutions. Undiluted urine partially inhibited it, but there was no action in dilutions of 1 to 2 and higher.

Repeated tests were carried out with various substances, using the technique and interpretations described above. For every substance investigated, the tests were carried out on at least two different occasions; and if the results were then uncertain, there was further repetition. The results of the tests are summarized in Table 5.

The blood and pus and their respective constituents were from rabbits, the pus being produced by injection of aleuronat into the pleural cavity; the ascitic fluid, urine, and saliva were of human origin; and the bile and milk were of bovine origin. The gastric juice was obtained from a dog under anæsthesia, through a canula inserted into the duodenum just beneath the pylorus.[1] A small amount of water was injected into the stomach to stimulate the flow of juice, which was thus diluted to approximately 1 part in 2.

The blood and pus cells were washed several times in saline solution. For the whole cells the final suspension was in broth. The suspensions were agitated at the beginning of the experiments, but

[1] The writer is indebted to Dr. S. M. Rosenthal, of the National Institute of Health, for obtaining the gastric juice.

not during the incubation period. For the hemolyzed blood cells, distilled water was added to the washed cells. For the autolyzed pus cells the washed cells were suspended in saline solution and incubated for several hours until autolysis occurred. The final suspensions of whole cells, and also of cells to be lysed, were made up to a density approximately the same as that in the blood or pus from which they were derived.

The samples of urine, saliva, and gastric juice were sterilized by passing through a Berkefeld N filter, the saliva having been first mixed with an equal quantity of broth and centrifugated to remove cellular material. The bile was sterilized by heat.

TABLE 5.—*Summary of the influence of various body fluids and cells on bacteriophage*

Test substance	Dilution			
	Undiluted	1:2	1:5	1:10
Blood	Complete inhibition	do	Partial inhibition	No definite inhibition.
Blood serum	do	do	do	Do.
Washed blood cells.	Different tests gave none, or partial inhibition.	do	do	
Hemolyzed blood cells.	Complete or partial inhibition.	Partial inhibition	No inhibition	
Pus	Complete inhibition.	do	do	
Pus fluid	do	do	do	
Washed pus cells.	No inhibition	do	do	
Autolyzed pus cells.	(Test substance inhibits growth of streptococci.)	(Test substance inhibits growth of streptococci.)	(Test substance partially inhibits growth of streptococci.)	Different tests gave complete, or partial, or no inhibition.
Ascitic fluid	Complete inhibition.	Complete inhibition.	Partial inhibition	No inhibition.
Bile	(Test substance inhibits growth of streptococci.)	Complete or partial inhibition.	do	(See discussion in text.)
Gastric juice	do	do	do	No inhibition.
Urine	[1]	No inhibition	No inhibition	
Saliva	do	Complete inhibition.	Partial inhibition	

[1] Undiluted urine sometimes inhibits the growth of the streptococci. If it does permit growth, there is partial inhibition of the bacteriophage.

According to Table 5 all the undiluted fluids tested, with the exception of urine, completely inhibited the action of bacteriophage. The gastric juice which had been diluted with an approximately equal part of water did not permit growth of the streptococcus; the 1 to 5 dilution partially inhibited the action of the phage. The diminished action of diluted blood, pus, serum, and ascitic fluid is of no practical interest; for when phage comes in contact with these fluids in the body, they are always in full strength. On the other hand, the diminished activity of diluted saliva, gastric juice, and bile permit a hope to be entertained that phage might be effective in the treatment of infections of the alimentary tract; and the partial inhibition of bacteriophagy by undiluted urine suggests that bladder instillations of phage might not be without effect.

There is no assurance, however, that phage could act under the conditions within the mouth or intestine; for in addition to the defi-

nite inactivation by undiluted or slightly diluted body fluids, unknown factors cause an irregular and uncertain behavior of phage even in test-tube experiments. This irregular behavior is termed the "zone phenomenon." It may be observed frequently when falling dilutions of phage are inoculated with a constant dose of the streptococcus, as in the titration of a sample of phage for potency. In the series of tubes containing phage, sometimes a single tube remains turbid. The turbid tube may be anywhere in the series. Sometimes the zone includes several tubes, which may be in the lowest dilutions or anywhere else in the series. Certain batches of phage exhibit the zone phenomenon with considerable regularity; other batches, prepared in the same manner, may be titrated repeatedly without showing it.

Since bile is always more or less diluted in the intestines, it was of interest to determine its action in a series of falling dilutions, with a constant amount of phage. The tests were carried out in the same manner as those already described, with the series of tubes containing bile in dilutions of 1 in 2, 1 in 5, 1 in 10, 1 in 20, 1 in 50, 1 in 100, etc. The series began with the 1 in 2 dilution because the undiluted bile itself inhibited the multiplication of the streptococcus. In the 1 in 2 dilution the phage was completely or partially inactivated; in the 1 in 5 dilution there was usually a partial inhibition; in the 1 in 10 dilution there was usually no evident inhibition. In several experiments the series was carried to high dilutions. There always appeared one zone, or sometimes two zones, of one or two turbid tubes in which the phage failed to act in the high dilutions of bile. These zones occurred anywhere in the series and on several occasions included the tube containing the 1 in 5,000 dilution.

TABLE 6.—*The zone phenomenon as exhibited in titrations to show the action of bile on bacteriophage*

Dilution of bile	Date tested	
	June 4, 1932	June 12, 1932
1 to 2	Complete inhibition	Almost complete inhibition.
1 to 5	Partial inhibition	Do.
1 to 10	No inhibition	Do.
1 to 20	Slight (?) inhibition	Slight (?) inhibition.
1 to 50	Partial inhibition	Almost complete inhibition.
1 to 100	do	Partial inhibition.
1 to 200	No inhibition	No inhibition.
1 to 500	do	Do.
1 to 1,000	Partial inhibition	Do.
1 to 2,000	Complete inhibition	Do.
1 to 5,000		Partial inhibition.
1 to 10,000		No inhibition.

To illustrate the zone phenomenon the results of two series of tests with the same sample of bile are given in Table 6. Since the zone phenomenon frequently occurs in a series of tubes of broth with falling dilutions of phage, that which occurred so commonly in the series

with constant quantities of phage and falling dilutions of bile can not be ascribed to a specific action of the bile on the phage, although it occurred more frequently, and involved more tubes of the series when bile was present than in titrations in broth. The important point in considering the activity of phage in the intestines is that, in addition to the definite inhibition in low dilutions of bile, the phage is very unstable and is easily and frequently inactivated by slight changes in conditions, for unexplainable reasons.

It appears that Calalb must have encountered the zone phenomenon without recognizing it as such; for in reporting that bile inactivates phage, he states that high dilutions were as effective as low dilutions.

The data given in Table 5 show that it is the fluid constituents of blood and pus which contain the most active inhibitory agent, the washed cells being almost or quite inert under the conditions of these experiments. In this respect the data presented here are at variance with the opinion commonly held, and supported by the reports of some investigators, that leucocytes are the active inhibitory constituent of blood. (See the review of the literature.) On the other hand, the lysed blood cells inhibited bacteriophagy completely or partially. Incidentally the complete inhibition of growth of the streptococcus by the autolyzed pus cells is of interest. There was partial inhibition of the growth of the streptococcus even in the 1 in 5 dilution of an autolyzed suspension of cells which before dilution was of approximately the same density as that of pus. It was therefore impossible to determine the effect of the autolyzed pus cells on bacteriophage in dilutions lower than 1 in 10. In this dilution the results were irregular, showing complete, or partial, or no inhibition.

SUMMARY

Antistreptococcus bacteriophage injected into mice inoculated with a minimal lethal dose of sensitive culture did not palliate the infection when the two doses were given simultaneously, nor when the phage and streptococci were incubated together previous to inoculation. There was some evidence that phage may activate a sublethal dose of the streptococcus in mice.

Bacteriophage injected intravenously into rabbits simultaneously with a dose of streptococcus which would kill the majority, but not all of the control rabbits, failed to influence appreciably the mortality rate. There was some evidence, however, that the course of the disease was unusually violent in the phage-treated rabbits which succumbed to the infection. The same results were obtained when bacteriophage was injected into rabbits three days before the infecting dose of streptococcus.

Bacteriophage injected intravenously into a rabbit could be demonstrated in the blood until the second day, but it had disappeared

from the blood on the third day. It could then be demonstrated in the spleen, but not in the liver.

In test-tube experiments bacteriophagy is completely inhibited by blood, pus, ascitic fluid, bile, and saliva. It is partially inhibited by urine.

The fluid portion of blood and of pus contains the active inhibitory constituent, the washed whole cells being almost or quite inert under the conditions of these experiments.

In addition to the definite inactivation of phage by undiluted or slightly diluted body fluids, there is an irregular inhibition in higher dilutions, caused by unknown factors.

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COURT DECISION RELATING TO PUBLIC HEALTH

State water commission act held unconstitutional.—(West Virginia Supreme Court of Appeals; Danielley et al. *v.* City of Princeton, 167 S.E. 620; decided Jan. 24, 1933.) Under the law relating to the State water commission, the city of Princeton was directed by the said commission to cease depositing sewage in a certain creek or to install either of certain systems which would reduce or eliminate the sewage pollution found to exist. The law provided that the circuit court should review any order of the commission, that such court could hear and consider any pertinent evidence offered, etc., and that it should determine all questions arising on the law and evidence and render such judgment or make such order upon the whole matter as law and equity required. On certification to the supreme court of appeals, that court held the statute to be unconstitutional because it committed executive powers to the judiciary. In the course of its opinion the appellate court, in part, said:

A hearing before the commission involves the determination (1) of whether the act complained of is a statutory pollution, and, if so, (2) of the proper sewage treatment or system of filtration to reduce the pollution. The first determination is quasi-judicial; the second is executive or administrative. An order of the commission properly determining these questions is an order on the whole matter. Upon appeal from the commission, the circuit court, in order to pass upon the whole matter, would have to review the identical questions primarily determined by the commission. A review of the system (for the regulation of the pollution) adopted by the commission and the approval of that or some other system by the court would require the court itself to exercise discretion; i.e., executive power. Whether the proceeding before the court be regarded as certiorari or appeal, the court cannot substitute its discretion for that of the commission lawfully exercised. [Cases cited.] The legislative, executive, and judicial powers under the Constitution (art. 5) are each, in its own sphere of duty, independent of and exclusive of the other; so that, whenever a subject is committed to the discretion of the legislative or executive department, the lawful exercise of that discretion cannot be controlled by the judiciary. * * *

DEATHS DURING WEEK ENDED APRIL 1, 1933

[From the Weekly Health Index issued by the Bureau of the Census, Department of Commerce]

	Week ended Apr. 1, 1933	Corresponding week, 1932
Data from 85 large cities of the United States:		
Total deaths	8,099	9,459
Deaths per 1,000 population, annual basis	11.3	13.5
Deaths under 1 year of age	595	685
Deaths under 1 year of age per 1,000 estimated live births [1]	52	58
Deaths per 1,000 population, annual basis, first 13 weeks of year	12.3	12.7
Data from industrial insurance companies:		
Policies in force	68,635,399	73,717,468
Number of death claims	14,432	18,540
Death claims per 1,000 policies in force, annual rate	11.0	13.1
Death claims per 1,000 policies, first 13 weeks of year, annual rate	11.2	10.4

[1] 1933, 81 cities; 1932, 80 cities

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Reports for Weeks Ended April 8, 1933, and April 9, 1932

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended April 8, 1933, and April 9, 1932

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932
New England States:								
Maine	----	1	[1] 96	7	4	246	0	0
New Hampshire	----	----	1	----	5	13	0	0
Vermont	2	3	----	----	17	73	0	0
Massachusetts	7	30	1	12	472	661	2	5
Rhode Island	4	5	----	----	----	133	0	1
Connecticut	4	6	19	19	275	112	0	2
Middle Atlantic States:								
New York	94	111	[2] 23	[2] 60	3,977	2,484	6	7
New Jersey	17	29	30	67	2,036	573	2	0
Pennsylvania	90	90	----	----	1,747	1,947	7	5
East North Central States:								
Ohio	29	35	16	71	865	820	1	1
Indiana	16	36	30	138	119	83	3	9
Illinois	22	104	43	85	481	649	29	0
Michigan	17	14	17	28	1,173	1,294	2	2
Wisconsin	3	3	38	390	466	1,007	1	1
West North Central States:								
Minnesota	6	12	----	5	1,297	61	0	0
Iowa	10	3	----	----	4	3	4	2
Missouri	21	15	9	34	259	60	3	1
North Dakota	2	----	----	----	84	52	0	0
South Dakota	2	----	----	2	12	14	1	0
Nebraska	9	2	35	----	27	1	0	0
Kansas	7	10	6	12	349	270	4	2
South Atlantic States:								
Delaware	3	----	1	7	4	2	0	0
Maryland [3]	9	10	18	303	28	46	1	3
District of Columbia	4	7	----	3	6	9	0	2
Virginia	12	----	----	----	274	----	3	4
West Virginia	20	16	14	367	294	419	0	5
North Carolina	24	22	22	168	636	428	1	1
South Carolina	8	6	352	2,262	229	118	0	0
Georgia [4]	10	15	102	209	84	33	1	1
Florida	10	6	1	5	58	6	0	2
East South Central States:								
Kentucky	10	12	35	469	58	58	2	0
Tennessee	13	8	66	739	35	209	2	3
Alabama [4]	11	18	43	294	51	10	0	1
Mississippi	11	6	----	----	----	----	0	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended April 8, 1933, and April 9, 1932—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932
West South Central States:								
Arkansas	8	3	12	198	464	----	0	0
Louisiana	10	28	15	37	29	27	0	1
Oklahoma [5]	1	9	71	231	89	29	10	3
Texas [4]	67	39	186	625	1,139	57	3	0
Mountain States:								
Montana	1	5	23	13	44	138	0	0
Idaho	1	1	----	3	36	----	0	0
Wyoming [6]	----	----	----	----	6	4	0	0
Colorado	3	4	29	----	4	139	1	0
New Mexico	4	10	1	1	8	50	0	0
Arizona	----	1	1	9	32	2	1	0
Utah [3]	1	2	2	----	12	2	1	0
Pacific States:								
Washington	10	1	1	----	45	513	0	0
Oregon	1	4	29	65	47	332	0	0
California	45	62	47	62	1,219	534	4	5
Total	659	804	1,435	7,000	18,600	13,721	95	69

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932
New England States:								
Maine	0	0	23	21	0	0	1	0
New Hampshire	0	0	35	32	0	0	0	0
Vermont	0	0	12	7	1	3	0	0
Massachusetts	0	0	450	500	0	0	2	1
Rhode Island	0	0	27	71	0	0	0	0
Connecticut	0	1	167	85	0	0	0	1
Middle Atlantic States:								
New York	0	1	1,116	1,442	0	0	6	6
New Jersey	0	1	380	282	0	0	3	2
Pennsylvania	1	3	990	578	0	0	7	7
East North Central States:								
Ohio	1	1	764	351	33	45	6	5
Indiana	0	1	190	178	4	12	1	0
Illinois	0	1	507	439	5	10	8	5
Michigan	3	1	665	436	2	13	2	11
Wisconsin	0	1	160	103	1	3	10	1
West North Central States:								
Minnesota	0	0	101	124	1	1	0	0
Iowa	0	0	55	36	26	27	0	3
Missouri	0	0	108	62	14	18	1	1
North Dakota	0	0	9	26	1	3	0	0
South Dakota	0	1	18	4	2	2	5	0
Nebraska	0	0	33	31	2	11	5	0
Kansas	0	0	67	70	1	6	1	0
South Atlantic States:								
Delaware	0	1	17	11	0	0	0	0
Maryland [3]	0	0	120	155	0	0	3	6
District of Columbia	0	1	12	23	0	0	0	0
Virginia	0	----	61	----	1	----	7	----
West Virginia	0	1	25	26	0	3	4	1
North Carolina	0	0	53	44	0	1	0	6
South Carolina	0	0	10	9	2	0	7	7
Georgia [4]	0	0	6	7	1	0	4	11
Florida	1	0	3	6	0	0	2	15
East South Central States:								
Kentucky	0	1	64	63	0	9	10	8
Tennessee	0	1	25	32	2	14	4	7
Alabama [4]	0	0	5	14	2	11	2	6
Mississippi	0	0	16	13	0	23	3	2

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended April 8, 1933, and April 9, 1932—Continued

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932	Week ended Apr. 8, 1933	Week ended Apr. 9, 1932
West South Central States:								
Arkansas	0	0	3	5	53	6	0	2
Louisiana	0	0	10	15	0	6	23	16
Oklahoma [5]	0	0	13	33	5	11	0	1
Texas [4]	0	0	73	62	33	113	11	3
Mountain States:								
Montana	0	0	18	10	2	0	0	1
Idaho	0	3	2	3	8	0	0	0
Wyoming [6]	0	0	11	6	0	0	2	2
Colorado	0	0	31	30	10	3	1	1
New Mexico	0	0	12	18	2	0	2	4
Arizona	0	0	10	11	0	0	0	1
Utah [3]	0	0	9	8	0	0	0	0
Pacific States:								
Washington	0	0	62	38	9	29	1	1
Oregon	0	0	16	20	4	8	3	3
California	3	0	161	161	43	7	7	10
Total	9	20	6,725	5,701	270	398	154	157

[1] Includes delayed reports.
[2] New York City only.
[3] Week ended Friday.
[4] Typhus fever, week ended April 8, 1933, 9 cases: 3 cases in Georgia, 3 cases in Alabama, and 3 cases in Texas.
[5] Figures for 1933 are exclusive of Oklahoma City and Tulsa.
[6] Rocky Mountain Spotted fever, week ended April 8, 1933, 2 cases in Wyoming.

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week.

State	Meningococcus meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
February 1933										
Mississippi	4	28	5,989	950	1,536	238	0	40	7	17
Nevada	-------	1	53	-------	3	-------	0	16	1	0
March 1933										
Alabama	4	55	502	16	114	16	1	55	17	10
Connecticut	5	21	72	-------	1,044	-------	0	631	5	2
Indiana	24	122	334	-------	389	-------	1	695	9	7
Iowa	10	41	1	-------	42	-------	1	186	174	1
Massachusetts	2	90	34	1	1,547	-------	1	1,913	0	9
Missouri	31	128	70	-------	1,063	-------	1	416	36	10
Nebraska	2	50	27	-------	95	-------	0	178	5	0
Pennsylvania	37	314	-------	-------	6,319	-------	3	4,892	0	33
Tennessee	16	36	383	76	205	9	2	191	5	25
Vermont	-------	3	-------	-------	154	-------	-------	88	1	1

February, 1933

	Cases
Mississippi:	
Chicken pox	425
Dengue	3
Dysentery (amebic)	34
Hookworm disease	268
Mumps	274
Ophthalmia neonatorum	3
Puerperal septicemia	19
Rabies in animals	3
Trachoma	2
Tularaemia	1
Whooping cough	757
Nevada:	
Chicken pox	15

March, 1933

Actinomycosis:	
Massachusetts	2
Pennsylvania	1
Anthrax:	
Massachusetts	2
Pennsylvania	1
Chicken pox:	
Alabama	80
Connecticut	591
Indiana	673
Iowa	204
Massachusetts	1,505
Missouri	264
Nebraska	313
Pennsylvania	5,250
Tennessee	394
Vermont	112
Conjunctivitis, infectious:	
Connecticut	1
Dysentery:	
Massachusetts	1
Missouri	2
Pennsylvania	1
Tennessee	2
German measles:	
Connecticut	24
German measles—Contd.	
Iowa	5
Massachusetts	46
Pennsylvania	99
Tennessee	150
Impetigo contagiosa:	
Tennessee	6
Lead poisoning:	
Massachusetts	3
Lethargic encephalitis:	
Alabama	9
Nebraska	1
Pennsylvania	6
Tennessee	1
Mumps:	
Alabama	165
Connecticut	448
Indiana	266
Iowa	292
Massachusetts	1,098
Missouri	288
Nebraska	242
Pennsylvania	2,579
Tennessee	172
Vermont	237
Ophthalmia neonatorum:	
Indiana	1
Iowa	2
Massachusetts	103
Pennsylvania	19
Tennessee	1
Paratyphoid fever:	
Massachusetts	1
Tennessee	1
Puerperal septicemia:	
Pennsylvania	11
Tennessee	2
Rabies in animals:	
Connecticut	7
Missouri	24
Tennessee	56
Scabies:	
Tennessee	24
Septic sore throat:	
Connecticut	14
Massachusetts	28
Septic sore throat—Contd.	
Missouri	7
Nebraska	6
Tennessee	41
Tetanus:	
Alabama	1
Pennsylvania	2
Trachoma:	
Connecticut	1
Indiana	1
Massachusetts	2
Missouri	111
Tennessee	15
Trichinosis:	
Connecticut	10
Massachusetts	1
Pennsylvania	3
Tularaemia:	
Missouri	3
Tennessee	7
Typhus fever:	
Alabama	15
Tennessee	25
Undulant fever:	
Connecticut	4
Iowa	8
Missouri	20
Pennsylvania	1
Tennessee	1
Vermont	4
Vincent's angina:	
Indiana	4
Tennessee	18
Whooping cough:	
Alabama	140
Connecticut	467
Indiana	135
Iowa	65
Massachusetts	1,138
Missouri	48
Nebraska	49
Pennsylvania	1,139
Tennessee	129
Vermont	62

WEEKLY REPORTS FROM CITIES

City reports for week ended April 1, 1933

State and city	Diphtheria cases	Influenza cases	Influenza deaths	Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
Maine:											
Portland	0	----	0	0	3	3	0	0	0	12	25
New Hampshire:											
Concord	0	----	0	0	0	1	0	0	0	0	10
Manchester	0	----	0	0	0	4	0	0	0	0	10
Nashua	0	----	0	0	0	0	0	0	0	0	----
Vermont:											
Barre	0	----	0	1	0	0	0	0	0	17	1
Burlington	0	----	0	0	0	3	0	0	0	0	18
Massachusetts:											
Boston	2	3	1	96	12	85	0	6	0	78	223
Fall River	1	1	1	0	2	14	0	2	0	4	30
Springfield	0	----	0	1	0	8	0	5	1	14	47
Worcester	2	----	1	7	3	25	0	6	0	12	41
Rhode Island:											
Pawtucket	----	----	----	----	----	----	----	----	----	----	----
Providence	1	----	0	0	5	15	0	1	0	25	68
Connecticut:											
Bridgeport	0	----	1	25	4	16	0	1	1	0	42
Hartford	0	1	0	16	6	25	0	1	0	5	27
New Haven	0	----	0	2	4	11	0	1	0	8	41
New York:											
Buffalo	9	----	0	46	19	70	0	7	0	36	131
New York	43	37	10	2,703	148	395	0	97	1	145	1,504
Rochester	1	----	1	3	2	35	0	2	0	11	81
Syracuse	0	----	1	1	4	32	0	0	1	13	56

City reports for week ended April 1, 1933—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
New Jersey:											
Camden	1	2	2	3	1	12	1	0	0	0	28
Newark	6	5	0	699	9	37	0	2	0	31	85
Trenton	2		0	47	8	21	0	3	1	4	40
Pennsylvania:											
Philadelphia	3	9	3	141	35	149	0	25	1	9	482
Pittsburgh	4	3	2	5	10	56	0	11	1	19	142
Reading	0		0	63	2	16	0	0	0	3	17
Scranton	0			1		38	0		0	0	
Ohio:											
Cincinnati	3	2	1	8	8	32	0	9	0	2	130
Cleveland	6	79	0	4	12	305	0	9	0	37	190
Columbus	4	1	1	44	6	12	0	4		0	67
Toledo	1		0	352	2	142	0	2		10	61
Indiana:											
Fort Wayne	4		0	0	4	7	0	2		0	32
Indianapolis											
South Bend	0		0	4	2	10	0	1	0	4	25
Terre Haute	0		1	0	3	9	0	1	1	1	21
Illinois:											
Chicago	13	2	0	480	50	358	0	42	1	22	644
Springfield	0		0	2	5	1	0	0	1	0	27
Michigan:											
Detroit	7	10	3	696	9	208	0	27	2	167	285
Flint	0	5	0	306	5	8	0	1	0	3	23
Grand Rapids	0		0	5	2	5	0	1	0	22	37
Wisconsin:											
Kenosha	0		0	0	0	4	1	0	0	6	4
Madison	0			165		3	0		0	3	
Milwaukee	1		0	3	3	24	0	1	0	42	90
Racine	0		0	1	0	3	0	0	0	10	16
Superior	0		0	0	0	0	0	0	0	10	5
Minnesota:											
Duluth	0		0	1	2	0	0	2	0	62	19
Minneapolis	4		2	317	8	51	0	2	0	30	92
St. Paul	0		0	678	2	12	0	3	0	79	49
Iowa:											
Des Moines	6			0		4	0		0	0	24
Sioux City											
Waterloo	0			0		0	1		0	0	
Missouri:											
Kansas City	2		1	132	11	21	0	3	0	2	106
St. Joseph	0		0	41	8	1	0	0	0	1	33
St. Louis	16	2	2	22	7	15	0	14	1	3	210
North Dakota:											
Fargo	0		0	0	1	0	0	0	0	0	8
Grand Forks	0		0	0	0	8	0	0	0	0	
Nebraska:											
Omaha	5		0	15	5	5	0	1	0	2	52
Kansas:											
Topeka	0		0	117	3	1	0	0	0	0	13
Wichita	0		1	0	2	0	0	3	0	8	27
Delaware:											
Wilmington	1		0	12	8	4	0	2	0	0	38
Maryland:											
Baltimore	4		0	1	28	78	0	14	0	15	206
Cumberland	0		0	0	1	2	0	0	0	0	9
Frederick	0		0	0	1	0	0	0	0	0	4
District of Col.:											
Washington	3	2	2	4	9	17	0	13	0	2	153
Virginia:											
Lynchburg	2		0	0	0	0	0	0	0	2	12
Norfolk	0		0	2	4	4	0	2	0	1	32
Richmond	0		0	6	2	9	0	1	0	0	45
Roanoke	0		0	107	0	2	0	0	0	0	17
West Virginia:											
Charleston	0	2	1	2	1	1	0	1	0	0	27
Huntington	1			3		1	0		0	0	
Wheeling	0		0	8	2	0	0	0	0	3	11
North Carolina:											
Raleigh	0		0	0	0	0	0	0	0	1	11
Wilmington	0		0	178	1	0	0	0	0	0	10
Winston-Salem	2	1	0	8	1	5	0	5	1	4	21
South Carolina:											
Charleston	0	22	0	0	0	0	0	2	0	1	24
Columbia											
Greenville	0		0	27	1	0	0	0	0	0	5

City reports for week ended April 1, 1933—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Georgia:											
Atlanta	1	22	3	14	8	4	0	8	1	18	85
Brunswick	0	-------	0	1	1	0	0	0	0	0	5
Savannah	0	38	0	0	1	1	0	2	0	0	21
Florida:											
Miami	3	4	1	1	0	0	0	2	2	37	24
Tampa	1	1	1	0	3	0	0	0	2	3	28
Kentucky:											
Ashland	1	-------	0	36	0	0	0	0	0	1	0
Lexington	0	3	0	5	2	1	0	2	0	1	13
Tennessee:											
Memphis	2	-------	2	16	6	4	0	7	1	4	73
Nashville	1	-------	0	0	2	1	0	5	0	0	58
Alabama:											
Birmingham	1	4	7	3	3	10	0	3	0	1	58
Mobile	0	-------	1	4	1	1	0	1	0	0	23
Montgomery	0	-------	-------	1	-------	0	0	-------	0	0	--------
Arkansas:											
Fort Smith	0	-------	-------	3	-------	0	0	-------	0	2	--------
Little Rock	1	-------	0	14	1	1	1	0	0	0	1
Louisiana:											
New Orleans	5	10	8	9	12	9	1	10	2	7	139
Shreveport	0	-------	0	0	8	1	0	2	0	0	43
Oklahoma:											
Oklahoma City	1	20	0	6	5	2	1	0	0	0	42
Tulsa	0	-------	-------	44	-------	2	9	-------	0	3	--------
Texas:											
Dallas	13	1	1	-------	4	0	0	2	1	3	53
Fort Worth	1	-------	0	89	5	1	0	0	0	0	29
Galveston	1	-------	0	1	1	2	0	1	1	0	14
Houston	8	-------	0	24	10	2	0	4	0	0	63
San Antonio	1	-------	5	20	7	1	0	5	0	0	54
Montana:											
Billings	0	-------	0	0	0	0	0	0	0	0	7
Great Falls	0	-------	0	1	0	0	0	0	0	3	8
Helena	0	-------	0	0	0	0	0	0	0	0	1
Missoula	0	-------	0	0	0	2	0	0	0	0	3
Idaho:											
Boise	0	-------	0	16	0	0	2	0	0	0	6
Colorado:											
Denver	2	29	1	2	12	13	0	5	0	2	101
Pueblo	1	-------	0	0	0	3	0	0	0	5	4
New Mexico:											
Albuquerque	0	-------	0	8	0	1	0	7	0	0	10
Arizona:											
Phoenix	0	-------	0	-------	1	2	0	2	0	0	--------
Utah:											
Salt Lake City	0	-------	0	0	2	5	0	0	0	16	31
Nevada:											
Reno	0	-------	0	0	0	1	0	0	0	0	5
Washington:											
Seattle	0	-------	-------	7	-------	9	1	-------	0	9	--------
Spokane	0	-------	-------	0	-------	1	0	-------	0	0	--------
Tacoma	1	-------	0	0	3	1	1	1	0	0	23
Oregon:											
Portland	0	-------	1	5	3	10	2	4	0	2	77
Salem	0	-------	0	22	0	0	0	0	0	0	--------
California:											
Los Angeles	16	12	1	521	13	42	40	23	0	57	303
Sacramento	0	1	0	0	4	0	0	7	0	62	31
San Francisco	1	8	0	4	9	19	0	6	0	66	157

City reports for week ended April 1, 1933—Continued

State and city	Meningococcus meningitis		Poliomyelitis cases	State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths			Cases	Deaths	
Massachusetts:				Georgia:			
Springfield	0	1	0	Atlanta	2	1	0
New York:				Tennessee:			
New York	1	0	0	Memphis	1	0	0
Pennsylvania:				Louisiana:			
Philadelphia	1	0	0	New Orleans	1	0	0
Pittsburgh	2	2	0	Texas:			
Ohio:				Fort Worth	1	0	0
Toledo	1	1	0	Houston	0	1	0
Indiana:				Utah:			
Fort Wayne	2	0	0	Salt Lake City	1	1	0
Illinois:				Washington:			
Chicago	15	2	0	Seattle	1	0	0
Michigan:				Spokane	0	0	1
Detroit	0	0	1				
Missouri:							
Kansas City	0	1	0				
Nebraska:							
Omaha	1	1	0				

Lethargic encephalitis.—Cases: New York, 2; Pittsburgh, 1; Chicago, 2; Fargo, 1; Baltimore, 1.
Pellagra.—Cases: Charleston, S.C., 3; Savannah, 1; Birmingham 1; Dallas, 2.
Typhus fever.—Cases: Savannah, 1.

FOREIGN AND INSULAR

BRITISH HONDURAS

Vital statistics—1931.—The following table shows birth and death rates in British Honduras during the year 1931:

Birth rate per 1,000 population	36. 78
Death rate per 1,000 population	[1] 36. 72
Infant mortality rate per 100 births	[1] 15. 17
Population (estimated)	52, 139

CANADA

Provinces—Communicable diseases—Two weeks ended March 25, 1933.—The Department of Pensions and National Health of Canada reports cases of certain communicable diseases for the two weeks ended March 25, 1933, as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Cerebrospinal meningitis	1	1		1	1	1	1			6
Chicken pox		6	1	243	611	45	30	15	144	1,095
Diphtheria		1	2	43	17	14	4			81
Erysipelas				21	1	6		1	1	30
Influenza		20		6	18	9	18		9	80
Measles	5	40	5	182	450	3		10	58	753
Mumps					425	77	33		44	579
Paratyphoid fever					3					3
Pneumonia (all forms)		3			17		23		14	57
Poliomyelitis			1	1	1				1	4
Scarlet fever		13	43	122	141	57	37	12	14	439
Smallpox					2		10			12
Trachoma					1		1		4	6
Tuberculosis	2	5	5	153	104	28	6	11	64	378
Typhoid fever			4	25	11	3			2	45
Undulant fever					2					2
Whooping cough		2	1	234	253	76	12		46	624

[1] The death rate for 1931 was almost twice as high as for 1930, and the infant mortality rate was also much higher (9.22 for 1930), due to the deaths caused by the hurricane of Sept. 10, 1931.

ITALY

Milan—Deaths from certain diseases—Years 1900, 1926, 1931, and 1932.—The following table shows the death rates per 10,000 population in the city of Milan, Italy, from certain causes during the years 1900, 1926, 1931, and 1932:

Cause of death	Death rate per 10,000 population			
	1900	1926	1931	1932
Apoplexy	13.50	7.43	9.54	10.78
Arteriosclerosis	3.16	7.23	4.42	4.60
Bronchitis and pneumonia	44.74	22.09	22.60	21.60
Cancer	8.75	10.18	10.64	10.88
Enteritis	15.41	8.29	4.52	4.05
Heart diseases	13.92	13.12	12.85	14.83
Influenza	3.97	2.46	2.02	1.37
Old age	9.95	4.83	3.37	3.51
Typhoid fever	4.73	3.57	0.57	0.56
Other causes	106.87	55.40	47.17	47.52
Total	225.00	134.60	117.70	119.70

MEXICO

Tampico—Communicable diseases—March, 1933.—During the month of March, 1933, certain communicable diseases were reported in Tampico, Mexico, as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Diphtheria	2	1	Paratyphoid fever	2	1
Enteritis (various)	42	47	Tuberculosis	----	42
Influenza	35	----	Typhoid fever	6	3
Leprosy	1	----	Whooping cough	7	----
Malaria	221	4			

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

(NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for March 31, 1933, pp. 334-345. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued April 28, 1933, and thereafter, at least for the time being, in the issue published on the last Friday of each month.)

Cholera

Philippine Islands.—During the week ended April 8, 1933, no case of cholera was reported in the Philippine Islands.

Plague

Argentina.—During the month of March, 1933, 2 cases of plague, with 1 death, were reported at Rosario, Argentina, and 5 cases, with 2 deaths, in Cordoba Province.

On vessel.—The steamship *Kingsborough* was reported at an Argentina port during March, 1933, with a case of plague aboard.

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