STUDIES UPON ASIATIC CHOLERA

F. D'HERELLE

Everyone who has written upon the subject of cholera has pointed out that however familiar we may be with respect to the characters of the pathogenic organism discovered by Koch in 1883, with regard to its pathogenicity the single fact actually known is that the presence of this vibrio in the intestinal mucosa causes the disease. But why do the vibrios undergo modifications in the body? Why do they lose their virulence? Why do they disappear? Why does the patient recover? These questions have remained unanswered. There is the same obscurity with regard to the epidemiology of the disease, and there is no explanation for the sometimes bizarre progress of epidemics. Some towns and villages remain free of the disease despite the fact that infected persons are brought in, while in a neighboring town or village the arrival of a single cholera case is sufficient to start a violent epidemic. Similarly, no one has yet explained why an epidemic may terminate permanently, and often very suddenly, after having caused hundreds of thousands of deaths.

Much has been published upon the subject of cholera, but although purely laboratory studies are numerous, very rare have been those studies made within the devastated regions at the bedside of the patient. Of what value can pure laboratory studies be in such a subject? None at all. When the bacteriologist has wished to study the characteristics of the cholera vibrio he has turned to old laboratory cultures, vibrios which have been maintained for years outside of their natural environment. He may be able to add a new culture medium to the innumerable ones which have already been described, and this may indeed be useful although there is no organism more readily cultivable. And what shall we say of the results obtained by those who have undertaken to study the pathogenesis of cholera by animal inoculation, by attempting to infect with old strains of the vibrio, guinea pigs and rabbits, animals which are completely refractory? A recent and voluminous work of San-

From the Department of Protobiology, Yale University School of Medicine.

arelli, published after years of labor, affords an example of the type of conclusion to which this method of attack must lead. What shall we say of his results, when we compare them with the results of Ferran and of Haffkine who, between them, have inoculated for prophylactic purposes more than half a million men with *living* cultures of the cholera vibrio without causing a single case of cholera? Comparing these results with those of Sanarelli but one conclusion is permissible: the naturally refractory guinea pig or the rabbit, contracts experimental cholera, while man, naturally susceptible, is experimentally refractory. One can only repeat what Fraenkel wrote more than forty years ago;—"In the actual state of our knowledge, we cannot satisfy ourselves that the hecatomb of guinea pigs and the enormous amount of effort and intelligence expended up to the present time have accomplished anything". The only results have been those frightful errors from which science has been unable to free itself, and these errors will continue to multiply until that time when investigators comprehend that a given disease can not be studied logically in a naturally refractory animal. It is certainly true that it is easier and less dangerous to remain comfortably seated in a laboratory than to go to India or China to study the disease, travelling among the devastated villages, under a tropical sun, but this method is the only one which can solve the problems presented by the etiology and the epidemiology of infectious disease.

Early in 1927 the Government of India asked me to go to that country to study cholera, a disease with which I was already familiar from having observed it in Indo-China. They provided me with an assistant, Dr. Malone, a graduate of McGill University and a Major in the Indian Medical Service, and as technician they gave me Dr. Lahiri, a Hindoo physician.

I. THE CHOLERA VIBRIO

Let me describe first the results of our examinations of the many strains of the vibrio isolated in our studies. Two hundred and eighty-eight of these cultures of the vibrio, isolated from patients during the disease, were agglutinable to a titer of at least 1:1000 by a specific serum. All of the strains presented identical characteristics, which we must, then, recognize as the attributes of the typical cholera vibrio. The bacilli were slightly curved, of the

so-called comma form, 3 to 4μ in length. They were actively motile, possessing a single polar flagellum, and they were Gram-negative. These characteristics are entirely typical. All of the strains produced acid, but did not produce gas, in media containing dextrose, mannite, saccharose, and maltose. None of the cultures attacked lactose, although it has been generally stated that this sugar is utilized by the cholera vibrio. All of the strains formed indol in peptone media and reduced nitrates to nitrites, that is, they gave the so-called nitroso-indol reaction. In this connection it may be mentioned that Zlatogoroff in Russia has found that 10 per cent of cultures of the cholera vibrio do not give this reaction. Strains of this type we have not encountered; the reaction has been constant.

All of the 288 strains caused a vigorous hemolysis of *human* red blood cells. This observation is contrary to the statements found in text-books, and, indeed, as we know, Kraus has even suggested the test for hemolysin production as a means of differentiating the true cholera strains from the pseudo-cholera vibrios. According to him hemolysins are not produced by the true vibrios, although they are by the pseudo forms, but it is significant that in his studies Kraus utilized the red blood cells of the sheep. Our tests were made with human red cells, simply because for our purpose it was of little importance to know whether the cholera vibrio would or would not hemolyze the red blood cells of animals, while the demonstration of an hemolysin for human cells, on the contrary, was of the greatest importance, as we shall see later.

With our strains we found that the proteolytic power of the vibrio was inconstant and some of the cultures derived directly from patients were unable to cause the liquefaction of gelatin. Thus, for example, 44 strains isolated from 19 patients upon different days of the disease were all proteolytic, 11 strains isolated from the stools of six patients were non-proteolytic, and 26 strains isolated from the stools of eight patients were either proteolytic (15 strains), or non-proteolytic (11 strains), according to the day of the disease upon which they were isolated.

The experiments made upon the viability of the cholera vibrio in water revealed an apparently paradoxical fact. Many studies of this subject have been made since 1883, and many competent bacteriologists, working outside of India, have found that the vib-

rio retains its viability for a considerable period. Pfeiffer has stated that they live seven months; Hochsteter says 12 months; Wernicke reports that they lived for three months in an aquarium; Krylov records that they lived in water of the Volga for 568 days, while Smidowich noted that in Russia the wells remained contaminated for several months. In Japan, Yano found that the vibrio remained alive in sterilized water for 245 days. But in India all of the bacteriologists who have dealt with the question have observed that the viability of the vibrio in water was very slight. In water from the Jumna, and in that from the Ganges, Hankin noted a complete destruction after three hours, and Greig, who performed many experiments of this type, never found the vibrios to survive for more than four days. Our experiments completely confirm the latter. In raw well-water either recently drawn or preserved in flasks for several days, sterilized either by heat at 120°C. or by filtration, the vibrio remained alive, as a rule, for less than 48 hours. In many experiments it remained viable for even less than 24 hours. The longest period of survival was 4 days for agglutinable strains of the vibrio, and 7 days for inagglutinable strains, in well-water containing an abundance of organic matter. From these results we can only conclude that in countries where cholera occurs in the form of limited epidemics and at infrequent intervals the vibrio retains its viability in water for a long time, while in India, the classic soil of cholera, it can not survive in water for more than 4 days at a maximum.

Let us recall that all of our observations bearing upon the characteristics of the vibrio and our experiments as to its behavior have been made with strains which have not undergone repeated passage through artificial media. The cultures studied have been those derived directly from the plates of isolation. The characters which we have mentioned are those possessed by the cholera vibrio when it leaves the body.

We will consider the question "virulence" when we deal with the subject pathogenesis, but we may say here, however, that the virulence of the cholera vibrio is lost very quickly when it is grown upon artificial media. Apparently virulence is lost during the first passages. Many bacteriologists have ingested, either accidentally or voluntarily, cultures of the vibrio without experiencing any disturbance, and we have already mentioned the half-mil-

lion injections of living cultures made by Ferran and Haffkine which provide, indeed, absolute proof of the lack of virulence.

It should be emphasized that many authors have confused "toxicity" for the guinea pig with "virulence" for man. These two properties are entirely distinct, as is very well shown by the fact that the so-called "pseudo-cholera vibrios" are completely avirulent for man but toxic for the guinea pig, while typical cholera vibrios have little or even no toxicity.

II. BACTERIOPHAGY OF THE VIBRIO

Let us first consider the characteristics of bacteriophagy *in vitro* as it takes place in artificial media.

Following the usual method for the isolation of very active races of bacteriophage, let us suspend in peptone-water a specimen of stool derived from a cholera convalescent. Let us filter this through an L^5 Chamberland candle and let us introduce a drop of this filtrate into a very young culture of the vibrio. Incidentally, it may be said that the cholera vibrio develops very rapidly and a four-hour culture is suited to this purpose. Within from 2½ hours to 3 hours after the addition of the filtrate the medium becomes perfectly clear, all of the vibrios having been dissolved. Solid media inoculated with a drop of the vibrio culture to which a trace of the filtrate has been added shows, after incubation, the characteristic plaques.

In brief, bacteriophagy of the cholera vibrio takes place in exactly the same fashion as does that of all other bacteria. The races of bacteriophage virulent for the vibrio belong to the species *Protobios bacteriophagus*, for the races of bacteriophage isolated from the stools of cholera convalescents are at the same time virulent for *B. coli.*

As in the case of all other bacteria we observe all gradations of virulence on the part of the bacteriophage, from a virulence so weak as to produce only a slight delay in bacterial growth up to one sufficiently high to cause a complete and permanent dissolution of the culture within 2½ hours.

Studies of the virulence of the bacteriophage as it exists within the intestine of the patient, that is, as it appears in the stool filtrates, show some very interesting facts. Here are, for example, the results of such a study carried out on a patient who entered the hospi-

tal on the first of May and who was discharged cured on May 5. Vibrios were isolated from the stools on the first, second, and third of May; after that date they were not present. The filtrates of the stools obtained on the first, second, third, fourth and fifth days of May were tested with these three cultures of the vibrio. The following table summarizes the results. The degree of bacterial dissolution is indicated; "c.s." represents the development of a secondary culture.

TABLE I

Filtrate of the stool made on	Action upon vibrio isolated on	Bacteriophagy after 2½ hours	5 hours	24 hours	Virulence of Bacteriophage
May 1	May 1	—	+	c.s.	1
"	May 2	—	++	c.s.	2
"	May 3	—	+++	c.s.	6
May 2	May 1	—	+++	c.s.	6
"	May 2	+++	+++	c.s.	7
"	May 3	++	+++	c.s.	6
May 3	May 1	++	++	c.s.	5
"	May 2	++	++	c.s.	5
"	May 3	+	++	c.s.	4
May 4	May 1	++	++	c.s.	5
"	May 2	+	++	c.s.	4
"	May 3	+++	+++	+++	10
May 5	May 1	—	+	c.s.	1
"	May 2	—	++	c.s.	2
"	May 3	—	+++	c.s.	6

As is obvious, the virulence of the bacteriophage varies continually in the intestine of the patient and clearly it is not the same for the different cultures isolated upon different days of the disease. It was, for example, on May 4, moderate for the vibrio isolated upon the first day of the disease, weak for that isolated upon the second day, and maximal for the vibrio isolated upon the third day. This is not, indeed, of merely theoretical interest, for from the point of view of pathogenesis, as we shall see shortly, the single factor of importance is the virulence of the bacteriophage in the in-

testine for the vibrios which are there at the same time. Of what importance could it be if the virulence of the bacteriophage present on the first day were maximal for vibrios which are not to be found in the intestine until some 3 or 4 days later? The important thing is the virulence and susceptibility relationships for bacteriophage and vibrio as they coexist.

These differences in virulence, however, quickly disappear outside of the body. It is only necessary to make two or three passages *in vitro* with these races of bacteriophage for the virulence to become the same for all typical vibrios. For example, the bacteriophage isolated upon the 4th day of May manifested after but two passages a virulence of "9" for all cholera vibrios. The same phenomenon is exhibited by the vibrios, for at the time of isolation each strain may be attacked in a different manner by a single bacteriophage, but after two or three subcultures *in vitro* these differences disappear and all strains become equally susceptible to a given bacteriophage. For example, a bacteriophage whose "stabilized" virulence has a value of "8" with a vibrio likewise "stabilized" may have a virulence of only "4" with a vibrio freshly isolated but it will become "8" with a vibrio after two or three cultures *in vitro*.

These facts possess, moreover, an additional interest in that they show that within the body each vibrio and each bacteriophage have their own individual characteristics, and that these attributes quickly become modified by growth outside of the body. There is always a tendency toward uniformity.

A study of the secondary cultures produced through bacteriophagy is extremely interesting and explains the variations which take place in the vibrio and which have so perplexed bacteriologists for the past 40 years.

First let us consider experimental mutations. This experiment was performed with a vibrio isolated from the stool of a cholera patient on the first day of the disease. This vibrio was then maintained under artificial cultivation during the period of a year, and its characters remained throughout those of a typical cholera vibrio. Obviously it is a strain which does not undergo spontaneous mutation. Through the action of a potent bacteriophage a culture of this vibrio, when freshly isolated from the stool, was dissolved perfectly after three hours at 37°C., but a secondary culture appeared after five days. When 0.1 cc. of this secondary culture was inoc-

ulated into a tube of peptone-water the medium remained sterile, but when 0.1 cc. of this same secondary culture was spread over a petri dish there developed, after incubation, about 20 small colonies, varying in size from those approaching the limit of visibility to those having a diameter of about 2 mm. Six of these colonies were cultured and studied. Here are their characteristics.

TABLE II

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentation						Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose / Salicin	
1	slight	—	—	—	+	+	—	—	+	+	+ +	Bacilli and vibrios
2	+	+	—	1:50	—	+	—	—	+	+	+ +	" " "
3	slight	—	—	1:50	—	+	—	—	+	+	+ +	" " "
4	+	+	+	1:1000	+	+	—	—	+	+	+ +	Vibrios, cocci, and bacilli
5	+	+	+	1:250	—	+	—	—	+	+	+ +	Bacilli and cocci
6	+	+	+	1:1000	—	+	—	—	+	+	+ +	" " "

For purposes of study several tubes of bacteriophage were prepared, that is, tubes containing cultures of this vibrio dissolved through the action of a powerful bacteriophage. These were filtered through a candle. The strains of the vibrio were maintained in culture, and as repeated subculture has proved, the characters remained fixed. These vibrio strains were, therefore, not spontaneously mutating. But in the course of our studies some of the filtrates developed a turbidity after variable lengths of time. One of them showed a slight clouding 17 days after having been sealed, which excluded the possibility of the clouding being due to a contamination. In other tubes the clouding appeared six weeks after the tubes had been sealed. The characters of the organisms thus developing are certainly not those of a contaminating bacterium. Such secondary cultures represent, undoubtedly, the passage through the filter of "protobacterial" filterable forms and these "protovibrios" then developed into bacterial forms. Thus, when we inoculate 0.1 cc. into a tube of peptone-water there is no growth even after an incubation period of 15 days. When 0.1 cc. is spread upon the agar a few small colonies will appear, but only after incubation for four

days. These colonies will vary in size from the limit of visibility to the naked eye to colonies a fraction of a millimeter in diameter. Six of these colonies were studied, with the results here indicated.

TABLE III

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentation							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
7	slight	—	—	—	+	—	—	—	—	—	—	—	Bacilli
8	—	+	—	—	+	—	—	—	—	—	—	—	Small vibrios
9	—	—	—	—	—	—	—	—	—	—	—	—	Cocci and short vibrios
10	—	+	—	—	+	+	—	—	—	+	+	—	Bacilli
11	—	—	—	—	—	—	—	—	—	—	—	—	Vibrios and cocci
12	—	—	—	—	—	—	—	—	—	—	—	—	Cocci

These observations clearly show that *in vitro*, through the influence of bacteriophage, the cholera vibrio undergoes changes involving all of its attributes,—morphological, biochemical, and antigenic.

It may be mentioned that in the course of other studies of similar cultures we have found, upon three different occasions, cocci developing a yellowish pigmentation.

Let us now turn to mutation occurring within the body. In all of the cases of cholera studied we prepared a filtrate of the stool upon each day of the disease. These filtrates, distributed in tubes, were immediately sealed for purposes of possible future study. Several of these filtrates developed a turbidity, due, as microscopic examination has shown, to bacterial growth, and it is noteworthy that all of the tubes behaving in this fashion were derived from the stools of patients in whom we had demonstrated the simultaneous presence of the vibrio and the bacteriophage. We have studied many of these secondary cultures, and the results obtained with one of them are here presented. When 0.1 cc. was inoculated into a tube of peptone-water it remained sterile even after incubation at 37°C. for eight days, but when 0.1 cc. was spread upon agar there developed after four days a few very small colonies. Study of six of these colonies showed:

TABLE IV

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentation							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
13	—	+	—	—	—	—	—	—	—	—	—	—	Cocci and short vibrios
14	—	—	—	—	—	—	—	—	—	—	—	—	Cocci and bacilli
15	—	—	—	—	—	—	—	—	—	—	—	—	" " "
16	—	—	—	—	—	—	—	—	—	—	—	—	Cocci and short vibrios
17	—	+	—	—	—	+	—	—	—	+	+	—	Bacilli
18	—	—	—	—	—	—	—	—	—	—	—	—	Bacilli and small vibrios

Here again we find in the cultures variations comparable in all respects to those induced experimentally.

And, finally, for purposes of comparison here are the characters of eight strains of the vibrio isolated from chronic carriers by Colonel Stewart, Professor of Hygiene at the School of Tropical Medicine at Calcutta. These vibrios had undergone many subcultures in the laboratory. It should be stated that No. 22 at first, at the time of isolation, had the vibrio form.

TABLE V

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentation							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
19	+	—	—	—	+	—	—	—	—	—	—	—	Long vibrios and cocci
20	+	+	+	—	—	+	—	—	+	+	+	—	Short vibrios and bacilli
21	+	+	+	—	—	+	—	—	+	+	+	—	Short vibrios and bacilli
22	+	—	—	—	+	—	—	—	—	—	—	—	Bacilli
23	slight	—	—	—	+	+	—	—	—	—	—	—	Short vibrios and bacilli
24	—	—	—	—	—	—	—	—	—	—	—	—	Short vibrios and bacilli
25	—	—	—	—	+	—	—	—	—	—	—	—	Long vibrios
26	+	+	+	—	—	+	—	—	+	+	+	—	Long vibrios and bacilli

It is easy to understand that the extreme forms found under experimental conditions will not be isolated from the chronic carrier, for one will pick up on the isolation plates only colonies composed throughout, or in part, of vibrio forms. But we have sought and always found colonies formed of bacilli and cocci analogous to those which have been isolated from secondary cultures when we have studied the isolation plates inoculated with stools of carriers.

As may be seen by a comparison of these tables, the mutations undergone by the vibrios within the intestinal tract of chronic carriers (in whom we have always been able to demonstrate a bacteriophage virulent for the cholera vibrio) are of the same order as those produced experimentally through the action of the bacteriophage.

It is quite obvious that the successive mutations take place through the loss of characters, but each character varies independently of the others. Let us take the character of "vibrio-form" for example. In the case of colony 24 vibrio forms were still to be found, even though all of the other characters had been lost, while in colony 6 the vibrio form was no longer found, although the other characters of the cholera vibrio, with the exception of sensitivity to the bacteriophage, were retained. Even agglutinability to the titer of the serum persisted.

As for the nitroso-indol reaction, which indicates the simultaneous secretion by the organism both of indol and of a reductase transforming nitrates into nitrites; this may be lost in organisms which retain all of the other characters (see colony 3), and may be conserved in others which have lost almost all of their other distinctive attributes (see colony 13).

In general, it may be said that the character of agglutinability is the one most readily lost, although observations made upon patients and during the course of epidemics show that the character of virulence is still more fragile. Next in lack of stability is the character of secretion of hemolysin for the red blood cells of man. In so far as fermentative characters are concerned, the most stable is the fermentation of dextrose. *But each character is an entity which is lost or retained in an independent fashion...* This observation indicates that these transformations represent *mutations* in the true sense of the word, for in all of the mutations studied up to

the present time, this is precisely what has been observed,—characters undergo modification in an independent manner.

Various authors, Hadley among others, have sought to explain the transformations undergone by bacteria in terms of a "life cycle". Such an explanation can not be correct, for a "life cycle", such as is known with the protozoa, consists of an orderly sequence of definite transformations. The term "bacterial dissociation" gives a false conception in that it implies a spontaneity of the phenomena, such as would result from the existence of a life cycle. This is not shown by experiment. There are two phenomena in nature leading to the transformation of living beings; the life cycle and mutation. In the first phenomenon the transformations are orderly; in the second they occur in a disorderly way. When we observe that beings undergo transformation, if we would determine whether the changes encountered are expressions of a life cycle or evidences of mutation, we must show whether they occur in a regular or in an irregular manner. Bacterial transformations are certainly the most disorderly ever observed. They result from mutation. The transformations undergone by bacteria are, as the experiments described demonstrate, bacterial mutations, and a bacterial strain in which these transformations take place without an *apparent* cause is a mutating strain. The many studies which I have made during the last 12 years have convinced me that such bacterial mutations are produced exclusively through the action of bacteriophage. It is, however, obvious that there must be bacterial diseases other than bacteriophagy and that these may also be the cause of mutation. But in any case, a mutating bacterial strain is never a normal strain. It is a strain affected by a chronic disease due to the presence within it of some foreign parasite. Perhaps it is desirable to mention that I do not include as mutations those transitory transformations in morphology which take place as the result of a modification of the medium; through the action, for example, of certain antiseptic substances. Here the phenomenon is of an entirely different nature.

III. THE PATHOGENESIS OF CHOLERA

We must at once grant that the specific agent of cholera is the vibrio discovered by Koch, for the abundant evidence which has been accumulated will not permit of any doubt in this connection.

But this does not solve the entire question. Indeed the question is so obscure that Ernest Hart has stated, with some appearance of truth, "you can eat cholera, you can drink cholera, but you can not catch it". This confusion is referable to the fact that the virulence of the vibrio, even though the vibrio be entirely typical, is extremely transitory and is quickly lost outside of the human body. Furthermore, when present, the degree of virulence is variable. When it is at its maximum the incubation period of the disease may not be more than two or three hours and death may occur in less than eight hours after the time of infection. In other instances the virulence may be so weak that the attack of the vibrio may be evidenced only by a simple diarrhea which may persist for six or seven days, the patient exhibiting none of the symptoms of cholera, even though vibrios are present in almost pure culture in the intestine. Only when the virulence is increased does the attack commence. Virulence may be still more attenuated and in such cases it is necessary that an accessory factor become operative in order to build up the virulence. The most common contributory cause appears to be a catarrhal diarrhea, caused either by the ingestion of green fruits or of indigestible foodstuffs, a frequent case during famine, or by exposure to cold at night, or even by fear. How does virulence become enhanced in the intestine?

Contrary to what many authors seem to think, the mere presence of vibrios in the intestine does not necessarily lead to any of the symptoms of cholera. The fact that these simple diarrheal conditions associated with the vibrios often disappear spontaneously without the individual exhibiting any of the symptoms of cholera is ample proof of this. For true cholera to develop it is necessary that the vibrios invade and multiply in the intestinal mucosa in the region of the glands of Lieberkühn and in the interstitial glandular tissue. It is essential, then, that the vibrio be virulent, that is, capable of growing *in vivo*. In the last analysis virulence is the property of secreting enzymes capable of breaking down the protein of certain cells of the host. It is this which permits the organisms to assimilate the products of cleavage and to multiply *in situ*. One might, indeed, speak of "virulent enzymes". When, as the result of disuse, the faculty of secreting these enzymes is diminished or lost, the virulence of the organism is attenuated or lost. But we can readily believe that a vibrio in which this property is diminished

may be able to regain it in the intestine when the mucosa is irritated by any cause whatever. Finding in its environment the proteins liberated through some fortuitous destruction of the cells of the mucosa, the vibrio utilizes them, the production of virulent enzymes increases and the organism thus becomes able to break down the proteins of living cells *in situ*.

Without entering into details we may say that all of the symptoms, as well as the local manifestations, of cholera (rice-water stools, and vomiting) may be explained purely upon the basis of the production by the vibrio of virulent enzymes which damage the mucosa of the small intestine. As for the general symptoms of the disease, some, such as dehydration of the tissues and aphonia, are due to the enormous loss of water through the stools; while others, including anuria, are referable to the action of the powerful hemolysin secreted by the vibrio. It is probable that the vibrio does not secrete a toxin in the true sense of the word.

At the beginning of epidemics cholera is fatal in between 80 and 90 per cent of the cases. Then recoveries become more and more frequent and near the end of the epidemic the mortality amounts to only between 30 and 40 per cent.

Why do the vibrios undergo transformations within the body? Why do they disappear? Why do certain individuals die, while others recover? These are the problems which we have considered.

We have studied in detail 33 patients affected with cholera; 23 of these were in the Campbell Hospital at Calcutta and of them 7 died; 10 were in villages in the Punjab where they received no treatment, and of them 5 died. The mortality is always much higher in epidemics, as in the Punjab, than in Bengal where cholera is endemic.

The charts presented herewith effectively show the results obtained. These charts have been constructed in the following manner. We have devised a coefficient for each of the symptoms, the coefficient varying according to the intensity of the symptom. Each of the symptoms, such as the number of rice-water stools, the vomiting, the degree of collapse, the anuria, the density of the blood (which was determined each day), the condition of the pulse, and the general state of the patient were expressed in this way. The sum of these coefficients affords an index of the severity of the attack upon each day of the disease. Ten is the maximum, where all of

the symptoms are present to the highest degree. Zero represents the absence of symptoms at the time when convalescence takes place. Upon the charts the line expressing the symptoms is continuous.

In a similar way we expressed the virulence of the intestinal bacteriophage upon a scale of 10, where 10 represents a maximum virulence and 0 represents none. Virulences between 1 and 5 are weak, the lysis at no time being complete. Virulences 6 and 7 are moderate, lysis being complete, but secondary cultures developing quickly. Eight and 9 represent high virulences, where lysis is complete and secondary cultures develop late. Ten means that lysis is prompt, complete and permanent; secondary cultures do not develop. Upon the charts the virulence of the intestinal bacteriophage is expressed by the dotted line.

Let us repeat that the virulence of the intestinal bacteriophage is based upon the action of stool filtrates prepared each day upon the vibrio isolated from the same specimen of stool.

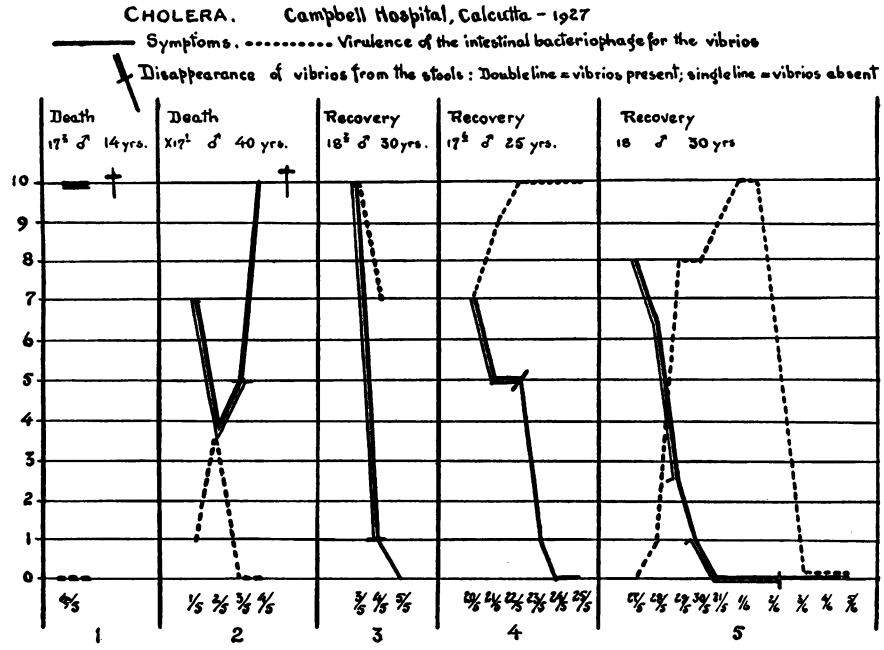

CHART 1.

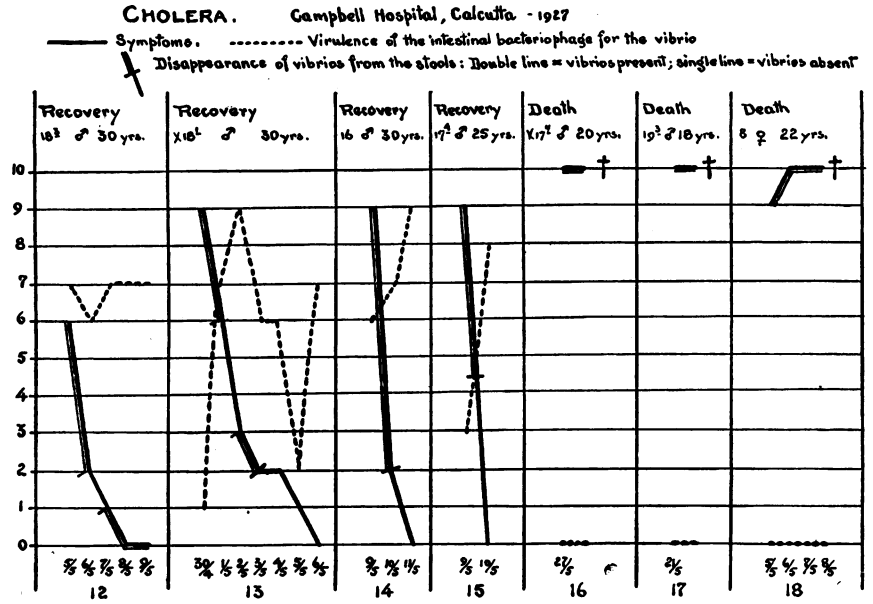

CHART 2.

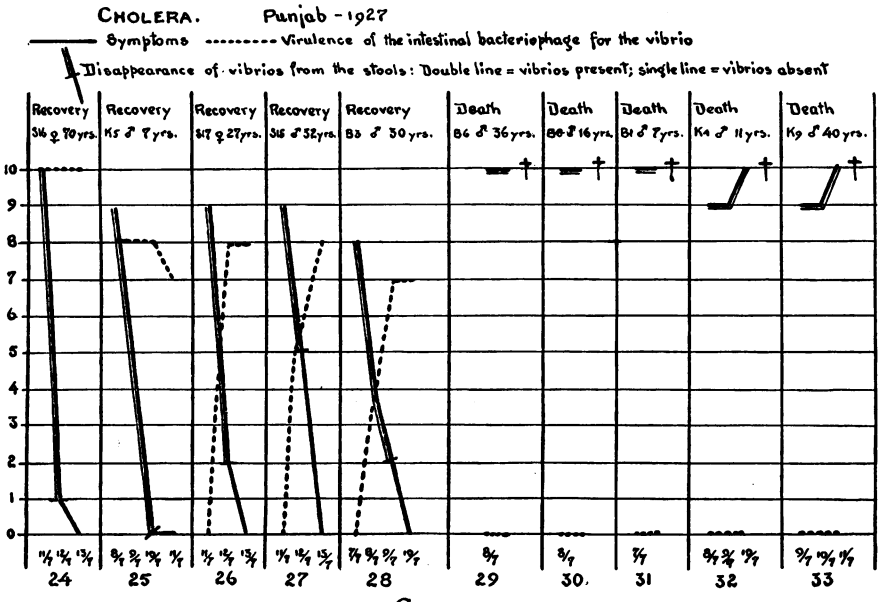

CHART 3.

Examination of the charts permits the following conclusions.

1. In those cases where the intestinal bacteriophage (which develops in the normal intestine at the expense of *B. coli*) does not adapt itself to bacteriophagy of the invading vibrio, the patient quickly succumbs without any improvement, even transitory, being apparent. This is what happened in cases 1, 16, 17, 18, 29, 30, 31, 32, and 33.

2. After an initial start of adaptation in which the virulence of the bacteriophage at no time goes above 5 the virulence becomes attenuated and disappears. Following a transitory improvement in the symptoms, corresponding to the weak virulence acquired, the condition again becomes aggravated and the patient dies within 24 hours after the disappearance of the virulence. This was the situation in cases 2, 19 and 20. Let us recall that in the scale used a virulence up to 5 indicates only a partial lysis.

3. When the virulence of the bacteriophage increases suddenly and reaches a virulence equal to or greater than 8, the symptoms disappear with like rapidity, and the patient immediately becomes convalescent whatever may have been the severity of the disease at the beginning. Examples of this are provided by cases 3, 14, 15, 24, 25, 26 and 27.

4. When the virulence increases more slowly the disease is of longer duration and the variations in the symptoms follow a course which parallels the virulence of the bacteriophage. The condition improves permanently, just as soon as the virulence reaches a value of 7, as a minimum.

Upon several occasions we have demonstrated that at a given time the bacteriophage manifests a virulence for both *B. coli* and the cholera vibrio and that this is not due to an admixture of two races of bacteriophage. It is due to two virulences of a single bacteriophage, as can readily be proved by successive passages *in vitro* at the expense of one of the two organisms. Since the double virulence is retained it can only mean that but a single bacteriophage is present.

On the charts the line expressing the symptoms appears as a double line during that period when the vibrios were present in the stools; as a single line when the vibrio was absent. As may be readily seen, when the disappearance of the vibrios coincides with a bacteriophage virulence of 9 or 10 the disappearance is permanent; a complete bacteriophagy *in vivo* has taken place. This is the situa-

tion in cases 3, 4, 10, 14, 21 and 31. When the disappearance of vibrios corresponds to a virulence of 8 the disappearance is usually permanent, as in cases 15, 22, 23, 24, 32 and 33; but in a few cases, as in 5 and 7, after a transitory disappearance the vibrios reappear; and, finally, when the disappearance corresponds to a virulence of 7, reappearance after a transitory absence is the rule.

This return of vibrios can only be due to the fact that after bacteriophagy a secondary culture develops. A comparison of what takes place *in vitro* with what occurs *in vivo* shows that the method adopted for evaluating virulence is legitimate and is certainly better than are other methods.

As a rule, when a secondary culture develops, the bacteriophage continues to increase its virulence and the secondary culture is in turn destroyed as soon as the virulence reaches a value of 9 or 10.

Let us observe that the presence of a secondary culture in the intestine in no way modifies the course of the disease; whether such a secondary culture develops or whether it does not, convalescence is not delayed. The first step in the mutations which develop under the action of the bacteriophage is the loss of virulence. Just as soon as bacteriophagy *in vivo* takes place the convalescent is no longer infectious, as has been demonstrated by the epidemiological study of cholera. Before bacteriophagy, the patient disseminates virulent vibrios, after bacteriophagy he disseminates avirulent vibrios and bacteriophage.

In brief, in cholera, as in other intestinal diseases, recovery is due to the bacteriophage which induces bacteriophagy *in vivo*. By way of comparison the two accompanying charts may be presented, the first referring to cases of bacillary dysentery, the second, to a case of typhoid fever. These curves have been taken at random from among many others which show the same things.

To these conclusions the objection may be raised that bacteriophagy *in vivo* may be merely an accessory phenomenon, and that the recovery in reality takes place through the acquisition of a true immunity. But first, may we ask if such an immunity occurs in dysentery and in cholera? All of the earlier authors who have described epidemics of cholera in Europe have stated that cholera is not an immunizing disease, for they have noted that a single individual may contract cholera several times during the course of a single epidemic. This same fact has frequently been observed in Asia. In dysentery it

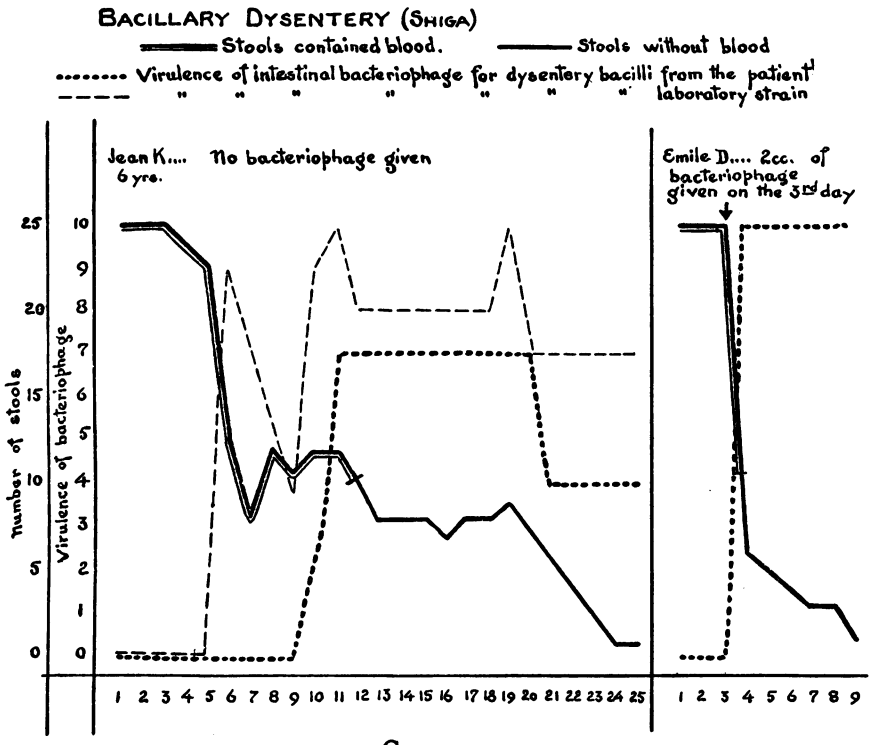

CHART 4.

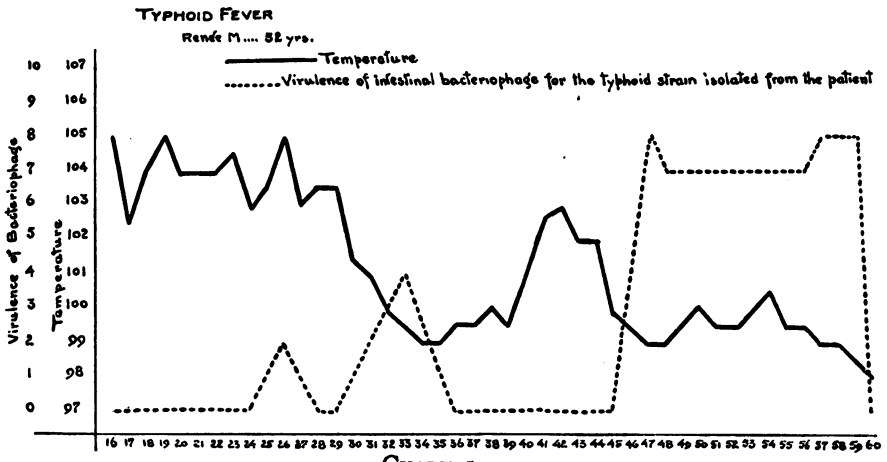

CHART 5.

is the same. Another evident, but important fact is that immunity, in all cases, even in typhoid fever, is not yet established at the time of recovery, for in all of these diseases relapses are relatively frequent during the course of convalescence. Obviously there can be no immunity at that time. Still another fact is significant. In cholera, especially, one may frequently see very severe cases in which the symptoms disappear very suddenly within a few hours after the onset of the disease. In other patients death may occur only after the disease has persisted for 10 to 12 days. If the immunity is able to develop so quickly in cases of the first type, how is it that it is not yet established in cases of the latter type, even after 12 days? As a matter of fact, everything that we know concerning acquired immunity shows that it is not such an inconstant process. Indeed, everything indicates that immunity is established only after recovery; that it is a result and not the cause of recovery.

It is, however, possible to demonstrate the action of the bacteriophage by a crucial experiment. We know how to cultivate bacteriophage, and if our deduction is correct it should be possible to bring about bacteriophagy *in vivo,* with recovery, by simply causing the patient, early in the disease, to ingest a bacteriophage of high virulence. In this way it should be possible to avoid the failures in the natural adaptation of the intestinal bacteriophage, and to reproduce at will the natural processes of recovery. This method has been applied in some tens of thousands of cases of bacillary dysentery, and recovery always takes place within 24 hours.

We first made some studies on cholera patients in the Campbell Hospital in Calcutta, and the curves here given show the results in patients to whom we administered 2 cc. of the virulent bacteriophage. The transverse line on that expressing the symptoms indicates the time of bacteriophage administration.

We next attempted to apply the method in the Punjab upon cases cared for in their own homes and receiving no other treatment. In these attempts we administered bacteriophage to all of the patients visited, for whom the parents accepted this mode of treatment exclusively. Two cubic centimeters of bacteriophage diluted in a little water were ingested in the presence of one of us, and 4 cc. diluted in half a glass of water were left with the family, with instructions to give it to the patient by the spoonful during the three following hours. Those patients who refused the treatment served as controls.

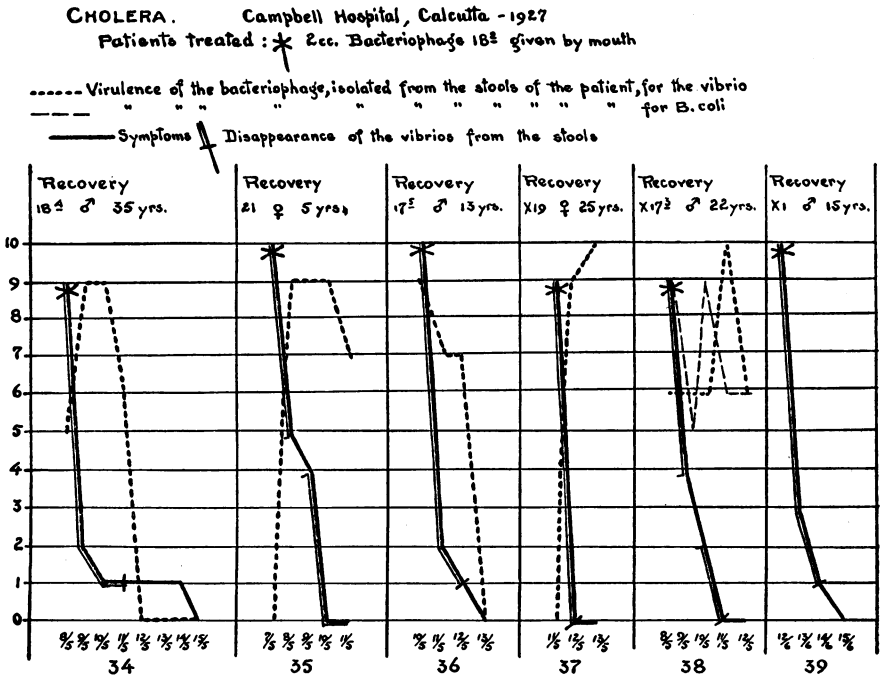

CHART 6.

Among the 74 cases treated in this way in the Punjab (those cases treated in Calcutta are not included in this tabulation) 6 died, that is, a mortality of 8.1 per cent. Among the 124 controls 78 died, a mortality of 62.9 per cent.

Of the treated cases 26 received the bacteriophage within the first 6 hours following the appearance of the initial symptoms. Of these none died. In 41 cases the administration of bacteriophage took place between 6 and 24 hours after the onset of the disease. In this group 5, or 12.2 per cent, died. There were 7 cases in which the bacteriophage was given later and of these 1, or 14.3 per cent, died.

The bacteriophage should be administered as early in the infection as possible. It is competent to induce bacteriophagy, but it can do nothing with lesions already formed.

IV. EPIDEMIOLOGY

Epidemics of cholera assume a distinctive character. The disease persists in endemic form exclusively in Bengal, in the delta of the Ganges, and in Indo-China. Elsewhere when an epidemic starts the pathogenic organism is imported from these regions. Even in India when cholera breaks out, outside of Bengal, it will be found that the first case has come either directly from Bengal or has recently been in contact with the inhabitants of this region. This latter is, naturally, common in the course of the religious pilgrimages so frequent in India.

This aspect of the disease would itself suffice to demonstrate that there are no carriers of the virulent vibrios, that is, infectious carriers. Drawing an analogy with typhoid fever, a number of authors have expressed the opinion that carriers of the vibrio may be infectious, but manifestly they have not considered the fundamental differences between the two diseases. An epidemic of typhoid fever is in reality a local outbreak of a general and perpetual pandemic and this pandemic character is due to the fact that there are infectious carriers of typhoid bacilli. In cholera, on the contrary, there are no cases of cholera until the vibrio is imported and then an epidemic, often very violent, develops, persisting for one, two or even three years. Then the disease permanently disappears, despite the fact that after an epidemic carriers of the vibrio are abundant, sometimes involving as many as 20 to 30 per cent of the population. If the carriers remained contagious how could the disease permanently disappear? Furthermore, the experience at Tor has the value of an experiment involving some hundreds of thousands of individuals. Tor is a quarantine station associated with the International Sanitary Council of Egypt, and here all of the Mohammedan pilgrims dwelling in the countries of the Mediterranean basin are quarantined on their return from Mecca. It frequently happens that epidemics develop among the pilgrims in the Hedjaz, the disease being imported by the pilgrims from India. In these cases the cholera continues to spread among the pilgrims during their return voyage from the Hedjaz to Tor. At Tor they undergo a quarantine which lasts for nine days after the last case of cholera has developed. But the disease quickly disappears at Tor, thanks to the rigorous control of the pilgrims and to the immediate isolation of patients during the very earliest stage of the disease. Never has any atten-

tion been paid to the carrier, and we know from the studies of Crendiropoulo that they are numerous. I have observed the same thing, but the segregation of the sick is sufficient to stop the outbreaks. The carriers invariably become distributed throughout all of the Mohammedan countries of the Mediterranean basin and never has a case of cholera broken out among them after their passage from Tor; never have they been the origin of an epidemic. Certainly we may conclude that they are not infectious carriers of the vibrio.

The carrier of the vibrio is not infectious, and our experiments have further shown that all carriers of the vibrio are likewise carriers of the bacteriophage virulent for the pathogenic organism. Within the carrier of the vibrio the same phenomena take place as those occurring in the normal individual with reference to *B. coli.* There is within the intestine a perpetual secondary culture, that is to say, a bacteriophage-vibrio symbiosis, and the carrier of the vibrio instead of being infectious, propagates the recovery, as we shall see shortly.

An explanation for the permanency of pathogenic vibrios in Bengal is still lacking. Why is not cholera completely eradicated there just as it is elsewhere? Why has the disease been present there continuously from the most remote times when in other places it disappears so quickly? We have not undertaken to investigate this subject for it would certainly require many months of study. We may, however, formulate a deduction and an hypothesis. The deduction is this. In order that the epidemic be persistent in Bengal and in Indo-China it is essential that there exist a local cause which induces in the avirulent vibrios a return of virulence. Upon the basis of elimination, this local cause can hardly be other than the presence in the artificial ponds, which are present in each village, of some aquatic animal,—worm, mollusk, or crustacean,—within the body of which reacquisition of virulence is effected. Obviously this is only a working hypothesis.

In the Punjab cholera develops in the form of epidemics. We have carried out experiments in the villages of this province and the conclusions to which we have been led, based upon more than a thousand examinations of stools, water, and flies, are these.

1. Before the epidemic begins in a region, neither cholera vibrios nor races of bacteriophage active for these vibrios are present in the environment.

2. The pathogenic vibrio is imported by a patient having the disease or by a person in the incubation period of the disease. This incubation period is usually but a few hours, but in cases where the attack of the disease is preceded by a diarrhea it may extend for a period of seven days. Indeed, the most dangerous persons are those who are affected with diarrhea, for the vibrios are extremely abundant in their stools, sometimes in practically pure culture, and since they present none of the symptoms, they move about freely and pass undetected. As a matter of fact bacteriophagy frequently takes place in them before the disease becomes manifest, but as long as bacteriophagy does not occur, and I have observed a case where it did not take place until after seven days, the individual disseminates virulent vibrios. The pathogenic vibrios are disseminated from those individuals who simply have diarrhea but are infected with the vibrio, by those in the incubation period of cholera, and by those actually having the disease. Contamination takes place by direct contact, by polluted water, and by flies which transport the vibrios to foodstuffs, to fruits in particular. Cases of the disease multiply in proportion to the degree of this dissemination.

3. The first cases are usually fatal, but in some the intestinal bacteriophage enhances its virulence quickly and these patients recover. Being distributed from these convalescents, the adapted bacteriophage becomes disseminated through the same agencies that disseminate the vibrio itself,—by contact, by water, and by flies. Recoveries become more and more frequent as the diffusion of the adapted bacteriophage increases and the epidemic stops when all of the exposed individuals are inoculated with the adapted bacteriophage.

4. In a region where an epidemic is occurring, towns and villages may be divided into three categories with regard to cholera:—

A. "Susceptible" towns and villages, in which cholera may be implanted and in whose environment will be found neither atypical vibrios nor bacteriophage.

B. "Recovered" towns and villages, in which cholera has occurred in the course of this same epidemic. Here will be found atypical vibrios and adapted bacteriophage.

C. "Non-infected but refractory" towns and villages, that is, districts free of cholera, which have not become contaminated in

spite of the fact that they are surrounded by other districts in which the epidemic progresses and from which cases may be imported. One finds disseminated in these towns and villages atypical vibrios and adapted bacteriophage, and these can only have been introduced by recovered carriers or healthy carriers.

Each time that we have observed the refractory state, either in an individual or in a community, we have recovered a bacteriophage adapted to bacteriophagy of the pathogenic vibrio in association with atypical, non-virulent vibrios. These permit the development of the bacteriophage and through their persistence in the intestine guarantee their dissemination into the environment.

We have made the crucial experiment which confirms these observations. We have introduced into villages at the beginning of the epidemic races of bacteriophage already adapted to the vibrio. The method which experience has shown to be most practical consists simply in pouring cultures of the bacteriophage into the wells which provide the potable water for the population.

In one village the number of cases quickly diminished, but still four more cases developed at the rate of one a day. In a second village the disappearance of the epidemic was equally prompt, from ten cases on the day of distributing the bacteriophage to six on the next day, and one upon the day following. The last case developed three days afterward. In three villages the last case developed upon the day following implantation of the bacteriophage, and in four villages the epidemic ceased upon the day of the implantation.

As the result of these experiments the Government of India has instituted at Patna a special laboratory for the preparation of virulent bacteriophage designed for the treatment and the prophylaxis of cholera and of bacillary dysentery. At the same time in this laboratory, experiments are being conducted with regard to bubonic plague. Upon my recommendation Dr. Asheshov has been selected as Director. This laboratory began to operate last September. The coming year will show what the method may accomplish when applied upon a large scale.