THE RÔLE OF THE TWORT-D'HERELLE PHENOMENON IN EPIDEMICS OF MOUSE-TYPHOID[1]

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(With 4 Charts.)

In a recent report (Topley, Ayrton and Lewis, 1924) we recorded an experimental epidemic of mouse-typhoid, during the course of which the faeces of each mouse were examined daily for the presence of *B. aertrycke*. It was noted that, during the earlier phases of the epidemic, there was a marked rise in the excretion-rate among the population at risk, followed by a rapid fall. During this fall a large proportion of the mice in the cage ceased to excrete this organism in detectable amount almost synchronously with one another. This suggested the possibility that some external factor might be involved, either acting simultaneously on all the mice, or capable of being passed from one mouse to another. The possible nature of such a factor was not discussed in the report referred to, but it was clearly conceivable that the lytic principle, described by Twort (1915), and studied independently and in more detail by d'Herelle (1917), might give rise to such a phenomenon as that recorded. A lytic principle of this type, active against *B. aertrycke*, had in fact been isolated from the faeces during the course of the epidemic.

The present report deals with experiments devised to investigate the rôle of this lytic principle in experimental epidemics of *B. aertrycke* infection among mice.

Altogether apart from the observations referred to above, it had been our intention to study this question at an early opportunity, since the conclusions reached by d'Herelle with regard to the rôle of the bacteriophage in epidemiology are clearly of fundamental importance, and have not, so far as we know, been confirmed or refuted by other workers.

For our present purpose we may quote from the English translation of d'Herelle's book on the bacteriophage (d'Herelle, 1922), which contains a summary of all the relevant experiments, and of the conclusions drawn from them.

For convenience, d'Herelle's results and conclusions may be summarised under the following heads:

[1] A Report to the Medical Research Council.

(1) *Does ingestion of the bacteriophage cause the disappearance of the homologous bacterium from the faeces of an infected host?*

There are few recorded results which are directly relevant to this particular issue. D'Herelle (1922, p. 266) records five cases of human dysentery treated by ingestion of the bacteriophage, in which *B. shiga* was isolated from the faeces before the lytic filtrate was administered, but was absent when the stools were examined 1 to 3 days later. He does not consider this point in his discussion of his results, where he deals mainly with the therapeutic effect, which is recorded as uniformly successful.

(2) *Does the presence of the bacteriophage in the alimentary tract, at the time when the homologous bacterium first gains access to it, prevent the proliferation of that bacterium in the intestine, and so prevent or limit its excretion in the faeces?*

This question is dealt with only inferentially in the actual records, but there would seem to be little doubt of the direction in which this inference leads, and the conclusion is clearly stated. D'Herelle (*ibid.* pp. 204–216 and 241–248) records and discusses observations and experiments on fowl typhoid, which will be referred to again and need not be detailed here. In his conclusions (*ibid.* p. 211) he states: "A weak or moderate activity of the intestinal bacteriophage is sufficient to render the animal resistant to infection. The pathogenic bacteria which are able to penetrate into the intestine are destroyed before they can multiply."

(3) *Does the presence of the bacteriophage in the intestinal tracts of the animal hosts, exposed to risk during the course of an epidemic, prevent the further spread of infection, and thus bring the epidemic to an early close?*

On this point the recorded observations and experiments are precise. Observations are recorded (*ibid.* p. 208) showing that an increase in the prevalence and activity of the specific bacteriophage, among the fowls exposed to risk during the course of an epidemic of fowl typhoid, coincided with the abrupt cessation of the epidemic. The experiments recorded (*ibid.* p. 211) on six fowls from a non-epidemic area indicate that fowls which have ingested, or have been inoculated with anti-gallinarum bacteriophage, are resistant to infection *per os* with this organism, while controls are susceptible, and that non-infected fowls, placed in contact with others carrying both *B. gallinarum* and the specific bacteriophage, become carriers of the bacteriophage, and are resistant to subsequent additional infections.

In later experiments (*ibid.* p. 240) an anti-gallinarum lysin was administered, by subcutaneous inoculation, to the survivors in 14 epidemics of fowl typhoid. The epidemics had lasted for varying times before the bacteriophage was administered. The total number of treated birds was 1333. In all cases the epidemic came to an abrupt end. Of the 1333 fowls, 53 were noted to be sick at the time of the inoculations; of these, three died shortly after the bacteriophage was administered; the rest recovered.

In two cases, in which the inoculation of the lytic filtrate produced no effect on the course of the epidemic, the administration of a mixed bacteriophage culture, active against *B. gallinarum*, *B. pullorum* and *B. pfaffi*, brought the epidemic to an abrupt close. Subsequent bacteriological investigations showed that *B. pfaffi* was probably the cause of the epidemics in these poultry-yards.

In another farmyard, in which about 100 fowls were exposed to infection with *B. gallinarum*, 20 were taken at random and inoculated with the homologous bacteriophage. One month later all these 20 birds were alive and in perfect condition, while of the rest only 20 survived.

In two other poultry-yards 150 survivors from epidemics caused by *B. gallinarum* were treated by administration of the bacteriophage *per os*. In each case the epidemic was immediately and completely checked.

D'Herelle draws the obvious conclusions, that the *immediate* cessation of the epidemic in all these cases indicates that it is the bacteriophage itself which confers protection in the first instance. He points out, however, that the inoculation of the lytic filtrate is followed by the appearance of a different type of immunity, after an incubation period, which he refers to as "organic" immunity. This point he deals with more fully in his experiments on barbone in cattle.

(4) *Does the administration of lytic filtrates lead to an immunity of the kind which is known to follow the parenteral administration of certain bacterial antigens?*

Since we cannot cultivate the bacteriophage apart from the living and multiplying bacteria, and cannot obtain it free from the products of bacterial lysis, we should expect, *a priori*, that the inoculation of lytic filtrates would lead to some degree of anti-bacterial immunity, assuming the bacterial protoplasm not to be so altered by the lytic process as no longer to act as a satisfactory bacterial antigen. No useful purpose would be served by a discussion of the various properties of an anti-bacteriophage serum, and we may confine ourselves to a brief consideration of d'Herelle's observations on barbone (*ibid.* pp. 217, 248).

This disease of cattle, studied by d'Herelle in Cochin-China, is due to infection with a *Pasteurella*. In his main series of experiments on immunisation, d'Herelle records the results obtained with 73 animals. The amount of bacteriophage culture inoculated varied between 20 c.c. and 0·04 c.c. The animals which received large doses, and were tested by the inoculation of living *Pasteurella* within 5 or 6 weeks of the immunising inoculation, died as rapidly as the controls. Animals tested after longer intervals showed themselves completely refractory. The smaller the dose the shorter was the interval between the immunising inoculation and the onset of the refractory state. With a dose of 0·25 c.c. 20 of the inoculated cattle became completely refractory to 1000 lethal doses of living *Pasteurella* between the 21st and 60th days. With a dose of 0·04 c.c. of lytic filtrate resistance was acquired after

4 days. This last experiment must be considered in somewhat more detail, since on it d'Herelle bases an important part of his conclusions. Eight steers were inoculated, each with 0·04 c.c. of lytic filtrate. Two animals were tested on the 1st day after the inoculation. Both proved resistant. One steer was tested on the 2nd day, and one on the 3rd. Both succumbed to an acute infection. Two animals were tested on the 4th day, one on the 5th and one on the 60th. All proved completely resistant.

On this series of experiments d'Herelle bases the conclusion that the immunity bestowed by the inoculation of the lytic filtrate has a dual character. There is an immediate protection, afforded by the presence of the bacteriophage in the tissues. This is exemplified by the first two animals in the last experiment. In passing, we may comment upon the fact that the necessary concentration of the lytic principle throughout the host's tissues must, in this case, have been attained by injecting 0·04 c.c. of the filtrate subcutaneously into the body of a young steer, under circumstances in which no multiplication of the lytic principle could have taken place before the test inoculation of the living *Pasteurella*, 24 hours later. This immediate protection is, d'Herelle believes, rapidly lost owing to the elimination of the bacteriophage, except in those cases in which the entry of the homologous bacterium into the host's body, while the bacteriophage is still present, allows the latter to perpetuate itself.

This primary protection, in a non-infected animal, gives place to a period of susceptibility, preceding the onset of that general immunity which results from the response of the host's tissues to the inoculation of the products of bacterial lysis. Once this response has taken place the animal is highly resistant, indeed almost completely refractory to infection, and remains so over long periods of time.

D'Herelle does not hesitate to apply his conclusions, rigorously and in their entirety, to the fundamental problems of epidemiology. He states (*ibid.* p. 277):

> Observation shows that in the last analysis the history of an epidemic registers the variations in the struggle between the two agents, the pathogenic bacterium and the bacteriophagous ultramicrobe. It is also clear that the latter is transmissible from individual to individual. The immunity is contagious in the same degree as the disease itself. The beginning of an epidemic is marked by the diffusion of a bacterium whose virulence is increased progressively by passages through susceptible individuals. Thus the epidemic extends. In its turn the ultramicrobial bacteriophage increases in virulence for the pathogenic bacterium, and extends equally. The epidemic ceases when all susceptible individuals have been infected with the virulent bacteriophage.

The procedure necessary to suppress any epidemic follows simply:

> Whatever may be the epidemic (provided, of course, the agent is known and cultivable) we have first the possibility of individual vaccination by means of a single injection of a small quantity of bacteriophage culture active for the causative bacterium. But we have seen that the presence in the intestine of active ultramicrobes assures the protection of a susceptible individual. We are then able to consider the possibility of collective immunisation

of the population, for it would be easy to mix cultures of the bacteriophage with the drinking water, especially in urban centres. One might then be assured of an active bacteriophage in the intestine of all susceptible individuals (*ibid.* p. 281).

Although reports have been published dealing with the therapeutic administration of lytic filtrates, the preventive and epidemiological issues, raised in so startling a manner by d'Herelle himself, have received comparatively little attention from other workers. The vast mass of literature which has accumulated around the Twort-d'Herelle phenomenon has been concerned almost entirely with the nature of the lytic principle.

We would, however, refer to a recent communication by Zdansky (1924), in which the claims for the effective action of the bacteriophage in the animal body are critically examined, in the light of our present knowledge of the conditions which limit the action of the lytic principle *in vitro*.

The Present Investigation.

The experiments to be described were devised to yield information on the four questions set out above, d'Herelle's conclusions on which have been outlined.

General Technique of Experiments.

It is not necessary to give in any detail the general technique employed, since, so far as it concerns the method of examining the faeces for the presence of *B. aertrycke*, or of conducting post-mortem examinations on the dead mice, it has been repeatedly described in previous reports. It is sufficient to state that all strains of *B. aertrycke* have been identified by agglutination against a high-titre specific serum.

The technique employed in the isolation of the original lytic principle, in the preparation of the lytic filtrates, and in testing specimens of faeces for the presence of the bacteriophage, follows closely the well recognised methods. In our original search for the bacteriophage we employed the method recommended by d'Herelle, and we have maintained our supply of lytic principle by frequent subcultures, and filtration through Chamberland candles. In the routine examination of large numbers of samples of faeces, or of cultures from the tissues, we have used the simpler heating method, which will be described below.

The Presence of the Lytic Principle in the Faeces of Normal and of Infected Mice.

We have never demonstrated the presence of a bacteriophage active against *B. aertrycke* in the faeces of normal mice, but our experience in this respect has been limited, since we have not made a special study of this question. We have, however, examined the faeces of large numbers of mice, which have been submitted to the risk of infection, or fed on cultures of *B. aertrycke*, but to which no lytic filtrate has been administered. In such mice we have never demonstrated the presence of the homologous lytic principle

during the early stages of a primary epidemic, nor during the early weeks after infection by feeding. It is, however, by no means uncommon for the lytic principle to make its appearance in mice which have passed through a preliminary period of infection, though many mice appear to pass through all stages, ending in death or recovery, without ever harbouring the specific bacteriophage.

It is clear therefore that, when we compare the observations made on infected mice to which we have administered the lytic principle, with observations made on infected mice which are included as controls, we are not justified in regarding the two series as representing, in the one case infection with the presence of the bacteriophage, and in the other in its absence. By examining adequate numbers of samples of faeces from the experimental mice, it is possible to assess with reasonable accuracy how far this disturbing factor does, in fact, interfere with the results; and in the experiments to be described an attempt has been made to do this. As will be realised, however, with regard to these particular experiments, the difficulty is not a serious one, since our main object has been to determine whether the administration of the lytic principle does, or does not, produce certain definite effects, and the fact that such an effect could be observed in certain of our control mice, owing to the spontaneous development of a lytic principle in their intestines, would not introduce any source of error, especially as it would be quite easy to detect such an occurrence.

The Bacteriophage Culture Employed.

The lytic filtrate we employed in these experiments was originally obtained from the faeces of a mouse, during the epidemic of *B. aertrycke* infection described in a previous report (Topley, Ayrton and Lewis, 1924). After many passages in peptone water cultures of a sensitive strain of *B. aertrycke*, it was active against this organism in dilutions of 1/10,000 to 1/100,000 when tested by the plate method described by Gildermeister and Hertzberg (1923). In broth or peptone water cultures this bacteriophage has never given rise to complete and permanent lysis. An 18 hrs. broth culture of *B. aertrycke*, in the presence of this lytic principle, is always markedly less turbid than a control culture. Sometimes it appears almost entirely clear. In samples taken from cultures at the time of their minimum turbidity it may be impossible to find any recognisable bacilli by examination with dark-ground illumination. The fluid in such cases appears to contain nothing but granular material. If, however, such cultures are returned to the incubator at 37° C. they always, sooner or later, show a secondary growth of *B. aertrycke*, and in our experience the bacilli which develop are not resistant to the lytic principle, but, when subcultured to agar, show all the appearances characteristic of its action.

On solid media we have only occasionally met with the typical isolated sterile areas described by d'Herelle and by others. The abnormal types of

colony recorded by many investigators, the bitten or nibbled forms, the small isolated colonies, and the amorphous *débris*, have been constantly found in agar subcultures from broth or peptone water cultures which contain the lytic principle. The surface growth from such cultures is always markedly less profuse than that from control tubes. Frequently it is very scanty, and interspersed with large irregular sterile areas. Sometimes only one or two distorted colonies develop.

The transmissibility of the lytic principle, through apparently unlimited series of successive subcultures, and the increased activity of a weak lysin by such a procedure, have been uniformly demonstrated.

We have not tested our cultures over a wide range of organisms other than *B. aertrycke*, since, for our purpose, it sufficed to demonstrate its activity against this organism. We have on a few occasions tested a lytic filtrate against *B. shiga* and against a strain of *B. coli* obtained from a mouse. In each case the results have been negative.

Experiment I.

In this experiment we wished to determine whether the administration of a lytic filtrate, *per os*, would cause the disappearance of *B. aertrycke* from the faeces of a mouse which was previously excreting this organism.

Our procedure was as follows. We fed a considerable number of mice, housed in separate cages, on broth cultures of *B. aertrycke*, using a dropping pipette. At each feeding each mouse received 0·02 c.c. of a 1/10 dilution of an 18 hrs. broth culture grown at 37° C., containing approximately 2,000,000 bacilli.

The faeces of each mouse were examined several times each week. As soon as *B. aertrycke* had been isolated on two occasions from one of the mice, this mouse was set apart for the purpose of the experiment, and a lytic filtrate was administered each day, *per os*. The second mouse which yielded two positive cultures from its faeces was set aside as a control. The third such mouse was fed on lytic filtrate, and so on alternately, until 10 mice were collected to be fed on lysin and 10 as controls.

At the commencement of the experiment, 0·02 c.c. of undiluted lytic filtrate was administered daily to each mouse of the test series, but after the first few days this practice was discontinued, and a drinking vessel, slightly modified from the type described by Ponselle (1920) was placed daily in each cage, containing about 10 c.c. of filtrate, diluted 1 in 10 with tap water. The mice of the control series were given a similar vessel containing a 1 in 10 dilution of peptone water.

Faeces from each mouse were examined at frequent intervals during the next 6 weeks for the presence of *B. aertrycke* according to the technique already described (Topley and Ayrton, 1924). Specimens of faeces from the mice of each series were also examined on two occasions for the presence of a bacteriophage, active against *B. aertrycke*, using the technique which will

be described below in discussing the isolation of the lytic principle from the tissues. On both occasions the samples from the control mice yielded consistently negative results. On the first occasion, when the faeces of the test series were examined, three out of six specimens showed the presence of the lytic principle: on the second occasion, four out of five samples gave a positive result.

The general results of this experiment are set out in Table I and in Chart I. The chart is constructed on the same general plan as those included in previous reports. On each base line, P 1, P 2, C 1, C 2, etc. are recorded the results obtained with an individual mouse. The short vertical lines placed below each base line indicate the days on which a specimen of faeces from that mouse was examined for *B. aertrycke*. The black area above each base line represents the degree of faecal excretion on the corresponding day. The height of the area is proportional to the logarithm of the number of *B. aertrycke* isolated from 1 c.c. of a faecal suspension of standard turbidity.

Table I.

Showing the results obtained by examining the faeces of mice, known to be excreting B. aertrycke *and subsequently fed on a lytic filtrate (Series P), and those obtained by examining the faeces of untreated excreting mice (Series C).*

Series P	
Number of mice fed	10
Number of specimens examined	142
Number of specimens yielding *B. aertrycke*	42
Percentage of specimens yielding *B. aertrycke*	29·6
Specific mortality per cent.	50
Number of survivors	5
Number of survivors with positive spleen cultures	4
Series C	
Number of mice fed	10
Number of specimens examined	177
Number of specimens yielding *B. aertrycke*	46
Percentage of specimens yielding *B. aertrycke*	26
Specific mortality per cent.	20
Number of survivors	7
Number of survivors with positive spleen cultures	5

The vertical line, cutting all the base lines, indicates for each series the day on which the test period commenced. The black areas to the left of this line indicate the degree of faecal excretion of *B. aertrycke* which had occurred before the mouse was first fed on lytic filtrate, or was set aside as a control.

The vertical arrows indicate the day on which each mouse died, or on which it was killed at the termination of the experiment. Where the circle beneath any arrow is black, *B. aertrycke* was isolated from the tissues of that mouse. Where the circle is left unshaded, the bacteriological findings at the post-mortem were negative. In almost every case those mice which died during the course of the experiment showed other evidence of active *B. aertrycke* infection, while those mice which were killed after 6 weeks, and gave positive spleen cultures, showed no other evidence of disease.

A glance at Chart I and Table I serves to answer the question at issue. The numerical results collected in Table I are curiously similar for the two series, as regards the measurements of excretion; indeed, we should not expect so close an agreement between the figures obtained with two random samples of so small a size. Quite apart from such figures, moreover, the charted results supply our answer. The results obtained with P 1, P 2, P 3, P 6 and P 10 make it quite clear that the daily ingestion of relatively enormous amounts of lytic filtrate does not prevent the occurrence of massive and persistent excretion leading to death, nor of intermittent or persistent excretion in the absence of serious illness.

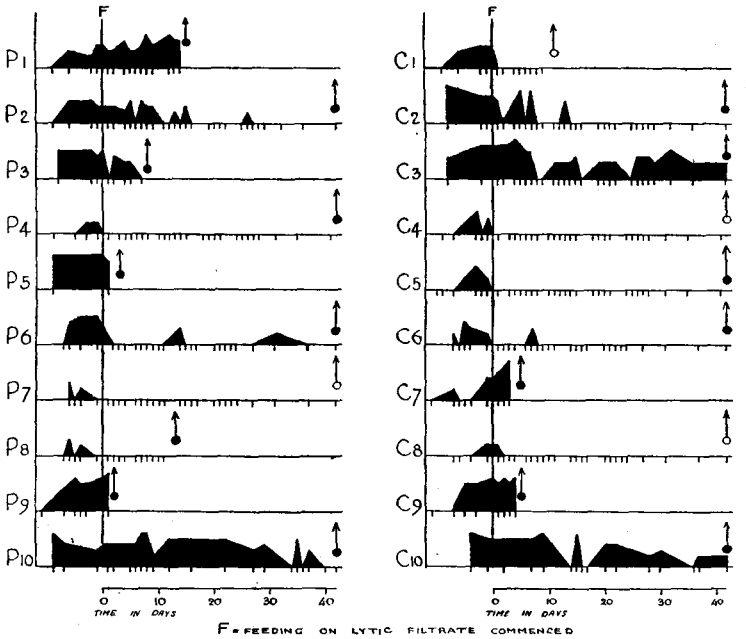

Chart. I. Showing the effect of administering a lytic filtrate to mice already excreting *B. aertrycke*.
P 1–10 = mice receiving filtrate.
C 1–10 = controls.

The deaths, during the course of the experiment, were slightly more numerous among the 10 mice fed on the lytic filtrate than among the controls, but the difference is of course not significant. The mice killed at the termination of the experiment showed, in each series, the high proportion of latent infections to which attention has been drawn elsewhere.

Experiment II.

This experiment was devised to test the effect, as regards subsequent excretion of *B. aertrycke*, of feeding mice on cultures of that organism mixed with an active lytic filtrate. It seemed possible, especially in the light of d'Herelle's results with fowl typhoid, that the presence of the lysin, from the

moment when the mice first ingested the bacterium, might prevent the establishment of an infection.

For this purpose 20 mice were taken, and fed on a mixture of an 18 hrs. broth culture of *B. aertrycke* and an active lytic filtrate. The broth culture was diluted ten times with a 50 per cent. dilution of lytic filtrate in nutrient broth, and 0·02 c.c. of this mixture was fed to each mouse with a dropping pipette on three occasions at weekly intervals. As controls, 20 mice were fed on the same dates and in the same manner, on a 1 in 10 dilution of the same bacterial culture in nutrient broth. As in the last experiment, each mouse was housed in a separate cage. The faeces of each mouse in both series were subsequently examined on three occasions during each of the first two weeks of the experiment, on two occasions during each of the following three weeks, and on the last two days of the period of observation, unless any mouse refused to yield a specimen for examination or died before the experiment was completed. On the 42nd day, or immediately after it, all survivors were killed. These mice, and those dying during the experimental period, were submitted to the usual post-mortem examination.

On three occasions during the course of the experiment the faeces of the surviving mice in each series were tested for the presence of the bacteriophage. Twenty days after the commencement of the experiment all the control mice gave negative results, while two of the 20 mice fed on the mixture of lytic filtrate and bacterial culture yielded an active bacteriophage from their faeces. On the 35th day all the controls were again negative, while one of 17 test mice gave a positive result. At the close of the experiment, on the 42nd day, five of 12 surviving controls showed the presence of the bacteriophage, while among the test mice the proportion was nine out of 15.

We meet here the difficulty referred to above. The administration of *B. aertrycke* to mice will be followed, in a proportion of cases, by the appearance of the lytic substance in the faeces, quite apart from the administration of a lytic filtrate. We cannot, therefore, assume that our controls will remain free from the interference of the lytic principle over any given period. In the present experiment it appears that the controls remained free from the bacteriophage for the first 35 days after the feeding commenced, while we know that a large excess of lytic principle was ingested by each of the test mice with each dose of bacterial culture.

It may be noted that the bacteriophage was, throughout, recovered from a smaller proportion of the test mice than in the last experiment, when a lytic filtrate was administered daily during the 6 weeks of observation.

The main results of this experiment are set out in Table II and Chart II, and need little comment. The collected and averaged figures give slightly higher results, as regards excretion, for the control as compared with the test mice, but the difference is well within the limits of chance variation. A glance at the chart serves to answer the main question at issue. The presence of an active lytic principle in the alimentary canal, at the moment when *B. aertrycke*

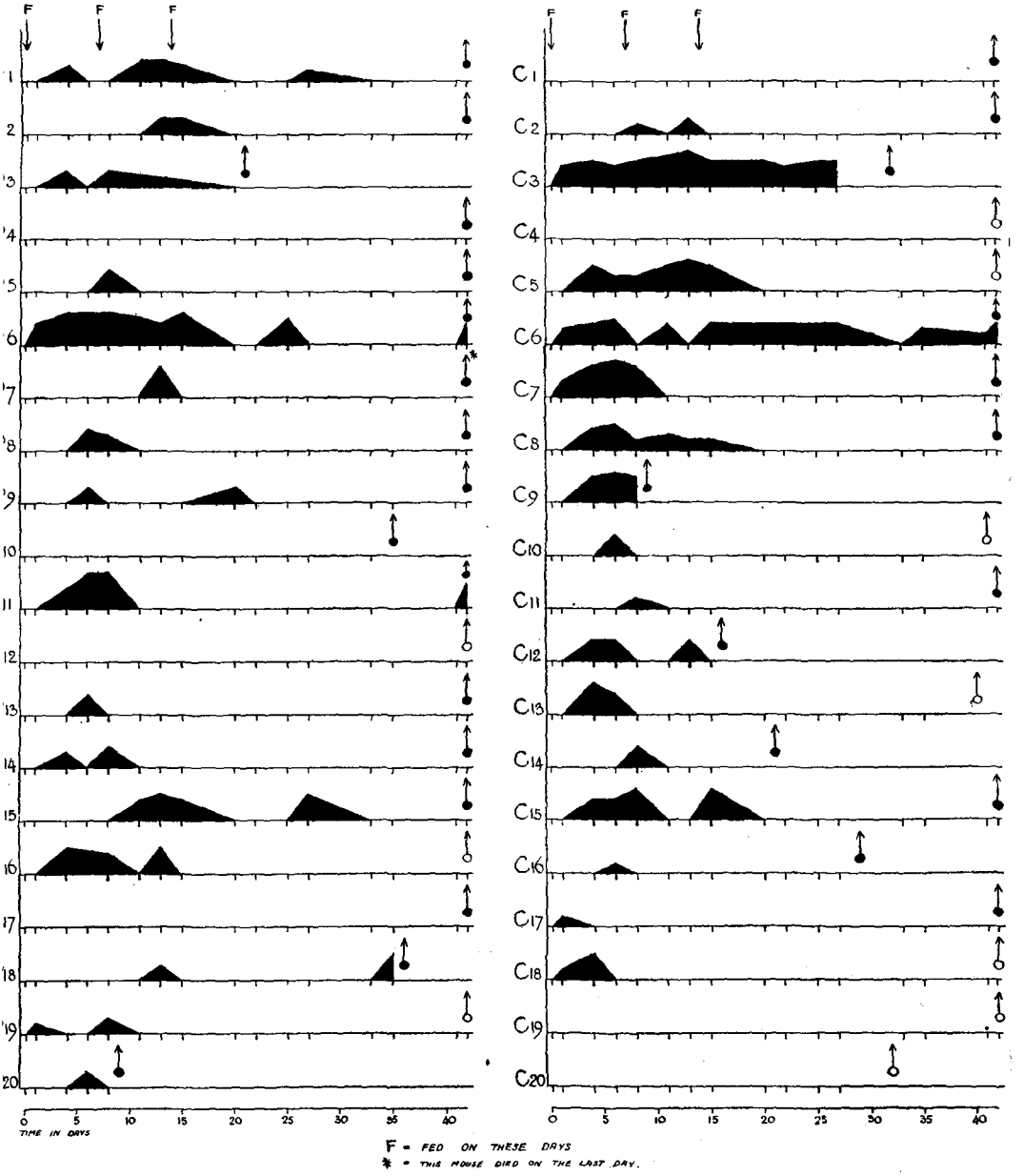

Chart II. Showing the effect of feeding mice on cultures of *B. aertrycke* with or without the addition of a lytic filtrate.

P 1–20 = mice receiving *B. aertrycke* and filtrate.
C 1–20 = mice receiving *B. aertrycke* alone.

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first gains access to it, does not appreciably reduce the probability that infection will take place.

Table II.

Showing the effect of feeding mice on cultures of B. aertrycke *mixed with a lytic filtrate (Series P), and on cultures of* B. aertrycke *alone (Series C).*

Series P	
Number of mice fed	20
Number of specimens examined	269
Number of specimens yielding *B. aertrycke*	43
Percentage of specimens yielding *B. aertrycke*	16
Specific mortality per cent.	25
Number of survivors	15
Number of survivors with positive spleen cultures	12
Series C	
Number of mice fed	20
Number of specimens examined	256
Number of specimens yielding *B. aertrycke*	60
Percentage of specimens yielding *B. aertrycke*	23·5
Specific mortality per cent.	25
Number of survivors	12
Number of survivors with positive spleen cultures	8

Experiment III.

This experiment was devised to test the effect of administering a lytic filtrate during the course of an experimental epidemic of *B. aertrycke* infection.

For the purpose of the first series of epidemics, we started a preliminary epidemic among a considerable number of mice. When this epidemic was well under way, we took 80 surviving mice and divided them into four groups of 20, the constitution of each group being similar as regards the number of mice of any given cage-age which it contained. Using these groups as our infecting material, we started four fresh epidemics by placing each group in an experimental cage of the usual type, and adding to it 80 normal mice, bringing the total population at risk to 100 in each case.

The mice in two of the cages (P 1 and P 2) were provided each day with drinking vessels containing a 1 in 10 dilution of an active lytic filtrate. The mice in the two other cages (C 1 and C 2) were given vessels containing a 1 in 10 dilution of peptone water.

The epidemics were observed for three months. All mice which died were submitted to the usual post-mortem examination, unless they were found wholly or partially eaten. As regards the general features of the epidemics, it is not necessary to give detailed figures. They pursued the course which we have learned to expect in epidemics of this kind, with certain modifications which are discussed below. In almost all cases the mice which died were proved to have succumbed to *B. aertrycke* infection. No other recognisable cause of death was met with.

The results as regards mortality are recorded in Chart III. This chart is constructed on the same principle as a chart included in a previous report (Topley, 1921) and records the percentage of survivors remaining in each cage for each day of the experiment.

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During the course of the experiment, specimens of faeces were obtained from small samples of mice on three occasions, and examined for the presence of the bacteriophage. At the end of the epidemic the faeces of all survivors were tested in the same way. The results obtained during the course of the epidemic can hardly be regarded as significant, since only 14 samples of faeces

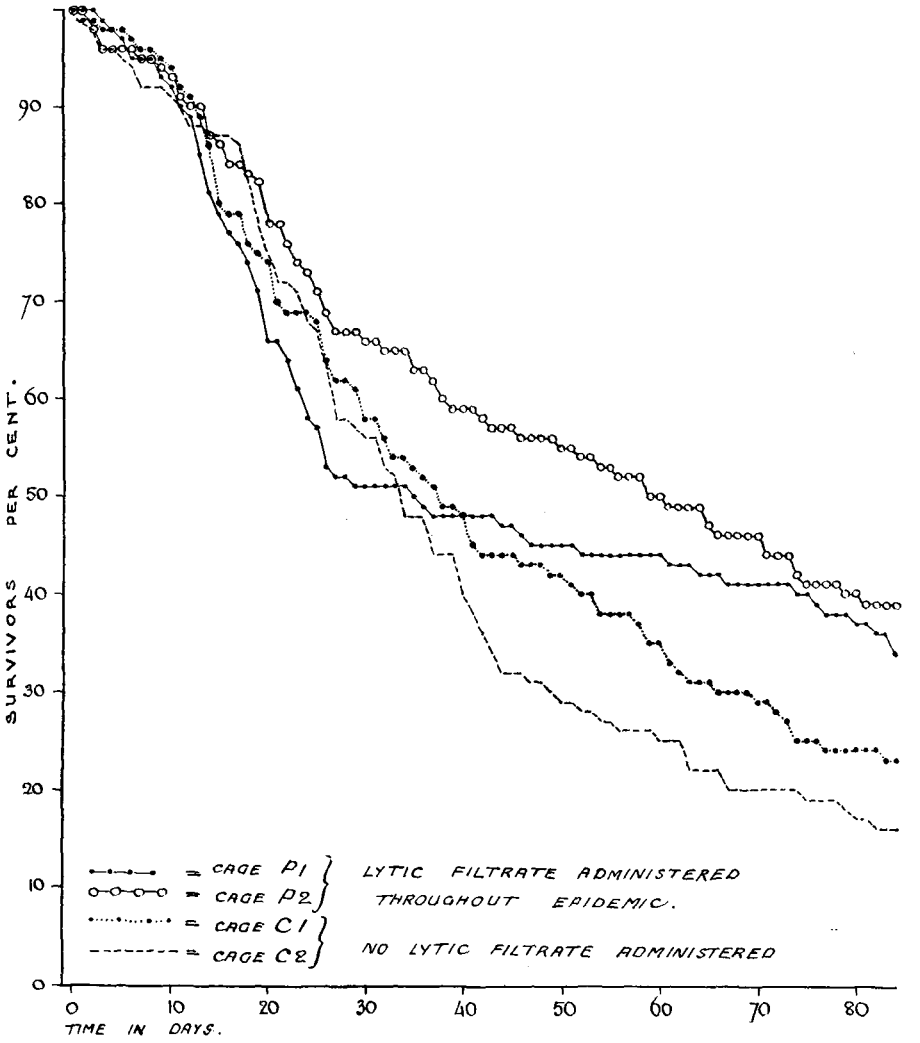

Chart III. Showing the daily percentage of survivors in four epidemics of *B. aertrycke* infection.

were examined from each cage. From cages P 1 and P 2, to which the lytic filtrate was administered, 25 of 28 samples showed the presence of the bacteriophage. From the control cages (C 1 and C 2) four of 28 samples gave positive results, and all these four specimens were obtained during the second half of the epidemic period. At the close of this first period the specific lysin was obtained from the survivors in the four cages in the following proportions:

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Cage P 1, lysin obtained from 32 out of 34 mice (94·1 per cent.)
„ P 2 „ „ 34 „ 39 „ (87·2 „)
„ C 1 „ „ 5 „ 23 „ (21·7 „)
„ C 2 „ „ 9 „ 16 „ (56·3 „)

Examining the main results in the light of the observations recorded on Chart III, the following comments seem justified.

Daily feeding on large amounts of an active lytic filtrate does not prevent, nor delay, the onset of an epidemic of *B. aertrycke* infection, when infected mice are mixed with susceptibles. During the first three or four weeks of the epidemic period there is no obvious difference between the course of events in the two cages in which the lytic filtrate was administered, and in the two control cages. About the 26th day of the experiment, however, a curious phenomenon is observable. While the two control epidemics continue their normal course, the two epidemics among mice receiving the lytic filtrate show a sudden slackening, so that the survival curves for cages P 1 and P 2 show a sudden flattening, and thenceforward run more nearly parallel to the base line. The curve for epidemic P 1, which had hitherto been running at the lowest level, now bends abruptly, and within a few weeks crosses the curves for the two control epidemics, which continue their downward course. At the close of the period of observation the survivors from the two epidemics, in which the bacteriophage was administered, numbered 68; the survivors from the two control epidemics numbered 39. In the light of further experiments, to be considered later, we cannot regard these results as certainly significant. At the same time we feel that it is a curious coincidence that the two test epidemics should show, at approximately the same moment, the same sudden slackening in mortality, not observable in either of the control epidemics. We would, however, point out that it is only in the case of epidemic P 1 that this abrupt slackening is strikingly apparent. If this phenomenon is to be regarded as other than the result of chance, it clearly suggests that the equilibrium has been shifted in favour of the hosts by some process involving an increase in herd-resistance, rather than from the direct action of the lytic principle. The three weeks' period at the commencement of the epidemic, during which the bacteriophage had every opportunity for direct action, showed no evidence of its interference.

Experiment III a.

In order to re-test the observations made in Exp. III, we added to each cage the requisite number of susceptible mice to make each population up to a total of 100. The four cages then had the following composition:

Cage P 1 *a* had 34 survivors from epidemic P 1 + 66 normal mice.
„ P 2 *a* „ 39 „ „ P 2 + 61 „
„ C 1 *a* „ 23 „ „ C 1 + 77 „
„ C 2 *a* „ 16 „ „ C 2 + 84 „

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To the new cages P 1 *a* and C 1 *a* lytic filtrate was administered daily throughout the following three months. To cages P 2 *a* and C 2 *a* only peptone water was given. The results are shown in Chart IV. They need little comment. There is no indication that the two cages P 1 *a* and C 1 *a*, which received the

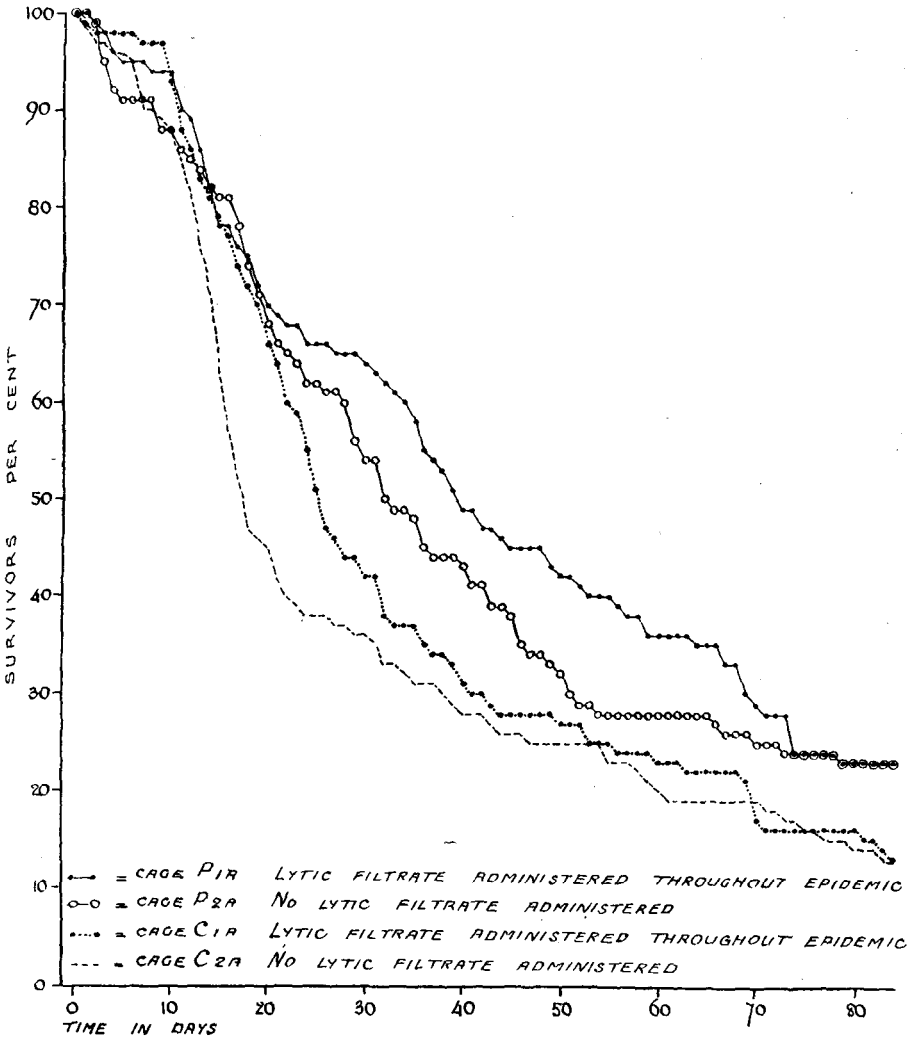

Chart IV. Showing the daily percentage of survivors in four epidemics of *B. aertrycke* infection.

bacteriophage during this second period, derived any benefit from it. Cages P 1 *a* and P 2 *a*, that is, those cages containing the survivors from the two earlier epidemics during which the bacteriophage had been administered, show throughout an apparent advantage over cages C 1 *a* and C 2 *a*. This is almost entirely owing to the fact that they contained a somewhat higher proportion of survivors as compared with fresh susceptibles. It has been

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clearly established, in earlier reports, that such survivors are relatively resistant.

It will be realised that there could be no question, in this experiment, of excluding the bacteriophage from the control cages, and that we had no data on which to base any *a priori* assumption as to the extent to which spread of the bacteriophage was likely to occur in cages P 2 *a* and C 2 *a*, neither of which received lytic filtrate during the present epidemic, but in both of which some of the survivors from the previous experiment were excreting it in their faeces. Specimens of faeces from all the survivors in each cage were tested on different dates for the presence of the lytic principle. The results are summarised in Table III. An examination of this table shows that, in cages P 1 *a* and C 1 *a*, in which lytic filtrate was administered throughout this second epidemic, the proportion of mice harbouring the lytic principle remained high throughout the period of observation. In cages P 2 *a* and C 2 *a*, on the other hand, many mice were excreting the bacteriophage during the early stages of the epidemic, but none at its close. In cage C 2 *a* the disappearance of the lytic principle is especially striking. These results would suggest that the bacteriophage tends to disappear from the excreta during the later stages of an epidemic, unless renewed from some external source. This conclusion is strengthened by the results obtained in examining the tissues of surviving mice for the presence of the lytic principle.

We may note in passing that a comparison of Table III with Chart IV will show that, in the case of epidemic C 2 *a*, the great majority of the host-population were harbouring the lytic principle during a fulminating epidemic in which over half the population at risk succumbed within 19 days.

Table III.

Showing the Results of Testing the Faeces of Mice, for the Presence of the Lytic Filtrate.

Period of epidemic	Cage P 1 *a*			Cage P 2 *a*			Cage C 1 *a*			Cage C 2 *a*		
	Number examined	Number positive	Per-centage positive	Number examined	Number positive	Per-centage positive	Number examined	Number positive	Per-centage positive	Number examined	Number positive	Per-centage positive
3rd to 5th week	41	36	87·8	64	23	35·9	46	27	58·7	46	40	87
6th to 8th week	—	—	—	33	17	51·5	28	21	75	27	0	0
9th week	—	—	—	—	—	—	—	—	—	23	0	0
End of 12th week	23	17	73·9	23	0	0	15	15	100	12	0	0

The Presence of the Lytic Principle in the Tissues.

We may refer briefly to some further observations made during the course of these two series of epidemics. Those mice dying during the latter half of the first series, and all those dying during the second series, on which it was possible to carry out a satisfactory post-mortem examination, were tested for the presence of the lytic principle in their tissues, by examining cultures of *B. aertrycke* obtained from their hearts or spleens. The technique employed was as follows. The direct broth cultures, obtained by placing a small portion

of the heart or of the spleen in tubes of nutrient broth, were incubated at 37° C. for 18 hours, and then killed by heating for 1 hour at 58° C. To each tube were then added 5 c.c. of peptone water, and a drop (0·02 c.c.) of an 18 hrs. broth culture of a sensitive strain of *B. aertrycke*. All tubes were replaced in the incubator for 18 hours, and then subcultured to agar slopes. After incubation for 24 hours the slopes were examined for the typical appearances of bacteriophage action. In all cases control platings from the heart and spleen on McConkey's medium gave an abundant growth of *B. aertrycke*, almost always in pure culture, but occasionally mixed with a few lactose-fermenting organisms.

The results obtained are summarised in Table IV. It appears that, during the first series of epidemics, the lytic principle was present in the tissues of a considerable proportion of the mice dying in those cages to which a lytic filtrate was administered, while it was absent in the mice dying in the control cages. During the second series of epidemics, the proportion of mice yielding the bacteriophage from their tissues was higher in those cages which received the lytic filtrate than in those which did not, but in the case of cage C 2 *a*, in which the majority of deaths occurred during the early phases of the epidemic and before the bacteriophage had disappeared from the excreta, a considerable number of the mice which died were harbouring the lytic principle in their tissues.

Table IV.

Showing the Results of Testing the Tissues of Mice for the Presence of the Lytic Filtrate.

Epidemic	Administration of lytic filtrate	No. of mice tested for lysin in tissues	No. of mice giving positive results	Percentage positive
P 1	+	25	14	56·0
P 2	+	21	5	23·8
C 1	0	40	0	0
C 2	0	37	0	0
P 1 *a*	+	60	23	38·3
P 2 *a*	0	67	6	9·0
C 1 *a*	+	65	17	26·2
C 2 *a*	0	67	14	20·9

It appears also that mice may die from acute or subacute *B. aertrycke* infection, with sensitive strains of that organism, which are actually carrying the lytic principle at the time when they give rise to the fatal infection.

At the end of the second epidemic period, the survivors in all cages were killed, and the cultures made from their spleens were examined for the presence of *B. aertrycke* and of the bacteriophage. The results obtained were as follows:

Of 23 survivors from cage P 1 *a*, 18 yielded *B. aertrycke* and 1 lysin
„ 23 „ P 2 *a*, 20 „ 0 „
„ 13 „ C 1 *a*, 10 „ 1 „
„ 13 „ C 2 *a*, 12 „ 0 „

It would appear that only a small proportion of those survivors, which are harbouring *B. aertrycke* in their spleen, are also harbouring a bacteriophage

active against this organism, and that its presence is confined to the survivors coming from those cages in which a lytic filtrate was administered throughout the epidemic period.

We may note that there would appear to be some degree of localisation of the lytic principle within the tissues. In 71 cases, in which cultures from the tissues showed the presence of the bacteriophage, we examined cultures from both heart and spleen of the same mouse. In 22 cases both cultures gave a positive result. In 9 cases the heart culture was positive, but the spleen culture negative. In 40 cases the lytic principle was present in the culture from the spleen, but absent in the culture from the heart.

The Presence of Bacteriophage-resistant Strains during the Course of the Epidemic.

During the second epidemic period a considerable number of strains, isolated from the tissues of mice which died from *B. aertrycke* infection, were tested as regards their sensitiveness or their resistance to the action of a lytic filtrate. The technique employed was as follows. One or more colonies from the plate cultures from the heart or spleen were subcultured to a tube containing 5 c.c. of peptone water. Ten drops of an undiluted lytic filtrate were added, the tubes were incubated for 24 hours at 37° C. and subcultured to an agar slope, which was incubated and examined in the usual way.

By this method 125 strains derived from the tissues of 73 mice were examined for sensitiveness or resistance. The strains were derived in about equal number from the four cages. Of these strains 124 proved fully sensitive. One only was resistant. This was derived from the heart of a mouse dying in cage C 2 *a* during the later stages of the epidemic. The strain from the spleen of the same mouse was fully sensitive. It seems clear that the epidemics here studied have pursued their normal course, associated with a high rate of mortality, without any appreciable tendency for the appearance of bacteriophage-resistant strains.

Experiment IV.

This experiment was devised to test the efficacy of a lytic filtrate as an immunising agent, when administered *per os* or parenterally. Five series, each of 20 mice, were employed. To the mice of Series A, undiluted lytic filtrate was administered by intraperitoneal inoculation on three occasions at weekly intervals. At each inoculation each mouse received 0·25 c.c. of an active filtrate. These mice were then set aside for another 14 days. There was no mortality associated with this procedure. Several of the mice showed symptoms of acute anaphylactic shock on the second or third injection, but they all recovered completely within a few minutes. To the mice of Series B, lytic filtrate was administered intraperitoneally, in the same dose as before, on one occasion 24 hours before the test inoculation. To the mice of Series C, undiluted lytic filtrate was administered on 6 days in each week, for a total period of 14 days, by means of the drinking vessels already described. These

mice were then kept for another 14 days without receiving any treatment. To the mice of Series D lytic filtrate was administered in drinking vessels on the day before the test inoculation was given. The mice of Series E served as normal controls.

Each of the 100 mice now received 0·00025 c.c. of a broth culture of *B. aertrycke* grown for 18 hours at 22° C. This dose contained approximately 200,000 bacilli. On the day of the inoculation a specimen of faeces from each mouse was examined for the presence of the bacteriophage. The mice of Series A gave positive results in 4 cases out of 20, those of Series B in 4 out of 20, those of Series C in 2 out of 20, those of Series D in 18 out of 20.

The results of the test inoculations are shown in Table V. Series C and D show that feeding on a lytic filtrate confers no increased resistance, whether the feeding is performed shortly before the test injection, or is followed by an interval of 14 days before the test is made. It would appear that, when administered *per os*, the filtrate neither affords any protection *per se*, nor causes any immunising response.

Table V.

Showing the Results of Immunising Mice by the Administration of a Lytic Filtrate. All mice tested by intraperitoneal injection of 250,000 B. aertrycke.

Series	Number of mice	Immunising dose given	Time between last immunising dose and test dose in days	Day of death	Average time to death	Number of survivors	Percentage of survivors
A	20	I.P.	14	7, 7, 7, 9, 9, 10, 10, 10, 11, 13, 13, 14, 14, 20, 21	11·7	5	25
B	20	I.P.	1	1, 2, 2, 4, 5, 5, 5, 5, 6, 6, 6, 6, 6, 7, 9, 9, 9, 10, 10	6·0	0	0
C	20	P.O.	14	1, 1, 1, 1, 1, 1, 3, 4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 6, 7, 7	3·7	0	0
D	20	P.O.	1	1, 1, 1, 1, 1, 2, 2, 2, 3, 4, 4, 4, 4, 5, 5, 5, 6, 6, 7, 14	3·9	0	0
E	20	Controls	—	1, 2, 2, 2, 2, 2, 2, 3, 3, 3, 4, 4, 5, 5, 6, 6, 7, 8, 8, 10	4·3	0	0

I.P. = Intraperitoneally. P.O. = *per os*.

Series B indicates that the intraperitoneal injection of lytic filtrate, given 24 hours before the test inoculation, affords no obvious protection. The slight delay in the average time to death in this series would be expected in any series of mice which had received an intraperitoneal injection of broth or peptone water 24 hours before an inoculation of living bacteria into the same body cavity. It would appear that the presence of the bacteriophage, awaiting the introduction of the homologous bacterium into the same serous cavity, hinders little, if at all, the process of multiplication and invasion.

Series A shows that the lytic filtrate acts as an adequate immunising agent, in the sense that its injection is followed, after a period of incubation, by a very definite immunising response. It seems possible, in the light of the somewhat extensive data now available with regard to the immunisation of mice

against the bacteria of this group, that it may prove an exceptionally favourable form of bacterial antigen for this purpose.

Summary and Conclusions.

Summarising our results, and considering their probable significance, we should draw the following conclusions.

Our observations do not suggest that the dissemination of a bacteriophage is likely to have been the cause of the sudden decrease in the excretion rate of *B. aertrycke*, which was observed among the population at risk during the epidemic previously recorded (Topley, Ayrton and Lewis, 1924).

Our results are completely at variance with those recorded by d'Herelle in the case of fowl typhoid, a disease which shows many points of similarity to enteric infection in mice. The observations we have recorded do not suggest that the presence of the bacteriophage will, in itself, prevent the epidemic spread of infection, check an epidemic when it has once started, or appreciably reduce the mortality among the population at risk. In the light of these results we cannot share d'Herelle's optimism as to the probable results of the wholesale administration of the lytic principle in a public water supply. We do not suggest that the Twort-d'Herelle phenomenon has no significance in the epidemic spread of disease, but it does not appear to us that the problem is the simple and straightforward one envisaged by d'Herelle.

The results of the last experiment of this series are in substantial agreement with d'Herelle's experiments on barbone in cattle, so far as they concern the action of the lytic filtrate in causing an immunising response, though we have not obtained such strikingly uniform and satisfactory results.

We would note, in conclusion, that we have not yet tested the effect of the administration of the lytic principle, *by injection*, during the course of an experimental epidemic.

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