THE SPREAD OF BACTERIAL INFECTION.

FURTHER STUDIES ON AN EXPERIMENTAL EPIDEMIC OF MOUSE-TYPHOID[1].

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(With 2 Charts.)

In previous reports (Topley and Ayrton, 1924 *a*, *b* and *c*) we have recorded the results obtained by feeding mice on cultures of *B. aertrycke*, and studying the subsequent excretion of that organism in the faeces. The present report deals with the application of the same technique to the study of an experimental epidemic of mouse-typhoid.

Procedure employed.

The method adopted was as follows. Broth cultures of *B. aertrycke* were administered, *per os*, to a number of mice on three successive days. Their faeces were examined daily, and on the fifth day five mice were selected from whose faeces *B. aertrycke* had been recovered. These mice are referred to as F 1, F 2, etc. To initiate the epidemic these five mice were placed in an experimental cage of the type already described (Topley, 1923) and to them were added 20 normal mice, referred to as A 1, A 2, etc. On the following day, and on each subsequent day throughout the course of the experiment, one normal mouse was added to the cage. These mice were entered in the records as E 1, E 2, etc. All such normal mice had, before entry to the cage, been examined for the presence of *B. aertrycke* in their faeces with negative results.

A specimen of faeces was collected daily from each mouse, except on Sundays, or on those occasions when one or more of the mice refused to yield a specimen. These were examined according to the technique already described (Topley and Ayrton, 1924 *a*). All mice which died were submitted to the routine post-mortem examination, including the preparation of cultures from the tissues, the subculture of 20 or more colonies from plates yielding non-lactose fermentors, and the testing of these broth cultures against type and group agglutinating sera.

Three days after the termination of the experiment all surviving mice were killed. A specimen of blood was collected from each mouse for agglu-

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tination tests, and the serum was tested, in a long series of dilutions commencing at 1/20 against type and group suspensions of *B. aertrycke*. Each mouse was submitted to autopsy, and a tube of nutrient broth was inoculated with a portion of spleen tissue. From those spleen cultures which showed growth within 48 hours, plates of McConkey's medium were inoculated, and these plates were further examined as described above.

Method of recording results.

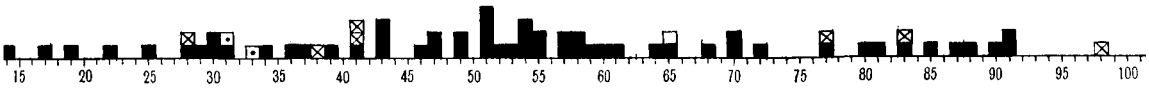
The significant results are recorded in Charts I and II and it would serve no useful purpose to include a detailed description in the text. It would, indeed, be difficult to prevent such a description becoming tedious and involved.

Chart I records the deaths, the daily excretion-rate, expressed as the percentage of the cage-population from whose faeces *B. aertrycke* was recovered on that day, and the excretion coefficient for that day's cage-population. This latter figure is the sum of the scores obtained by the excreting mice, allotted on the logarithmic scale already described (Topley and Ayrton, 1924 *a*), multiplied by 100 and divided by the number of specimens examined. It differs from the excretion-rate in that it allows for the relative copiousness of excretion of *B. aertrycke* by the individual mice. As will be seen, it appears to add no useful information to that obtained by a record of the excretion-rate.

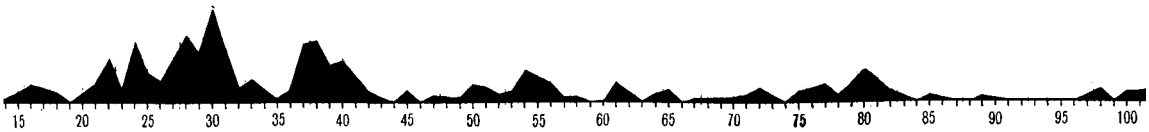
In this chart are also included the results of the agglutination tests with the strains of *B. aertrycke* isolated from the faeces, or from the tissues after death. They are charted as percentages of type or group strains isolated on any given day. The percentages for the faecal and tissue strains are recorded separately in diagrammatic form.

In recording the results of these agglutination tests certain corrections have been made, in the light of our present knowledge of the errors introduced by factors which have recently been investigated (Topley and Ayrton, 1924 *c*). This investigation has shown that the very great majority of strains of *B. aertrycke*, recently isolated from the faeces or tissues of mice, can be sharply differentiated into Type or Group varieties by agglutination tests. There is a high probability that those strains which were found, in an earlier investigation, to agglutinate with both type and group sera were in fact altered type strains. During the first 39 days of the present experiment we were in ignorance of these facts. In this period 781 strains were isolated from the faeces and tested by agglutination. Of these, 330 reacted as type, 305 as group and 146 as mixed. During the remaining 78 days 591 faecal strains were examined, with adequate precautions. Of these, 533 reacted as type, 52 as group and 6 as mixed. It was clearly necessary to allow in some way for the errors introduced by faulty technique. There was, *a priori*, every reason for believing that all, or almost all, those strains which had reacted as mixed should be regarded as altered type strains. This probability was much strengthened by a consideration of our records. We may divide the first

Chart I.



IE OF MICE EXCRETING B.AERTRYCKE



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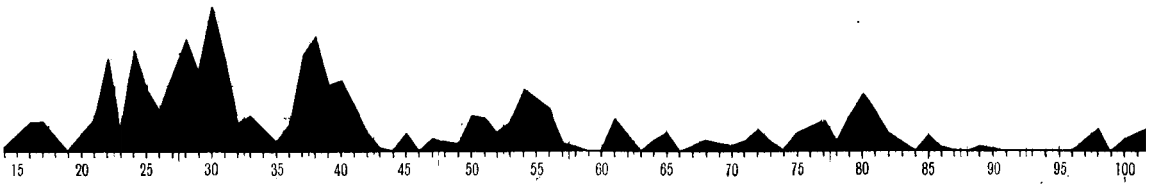
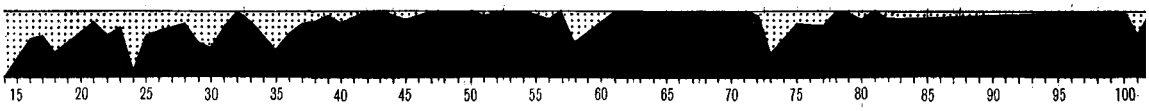
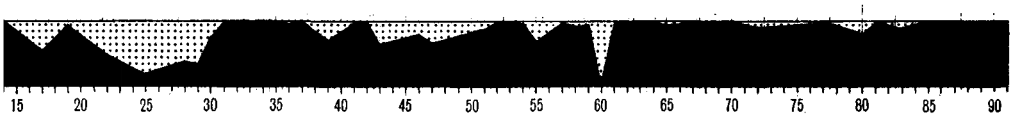


E OF STRAINS FROM FAECES REACTING AS TYPE OR GROUP



ENTAGE OF STRAINS FROM TISSUES REACTING AS TYPE OR GROUP



ISE DEAD — *B.AERTRYCKE ISOLATED P-M.*
" — *NO EVIDENCE OF B.AERTRYCKE INFECTION.*
" — *NOT EXAMINED P-M*
MISSING OR ACCIDENTALLY KILLED

BLACK AREA = TYPE
DOTTED AREA = GROUP

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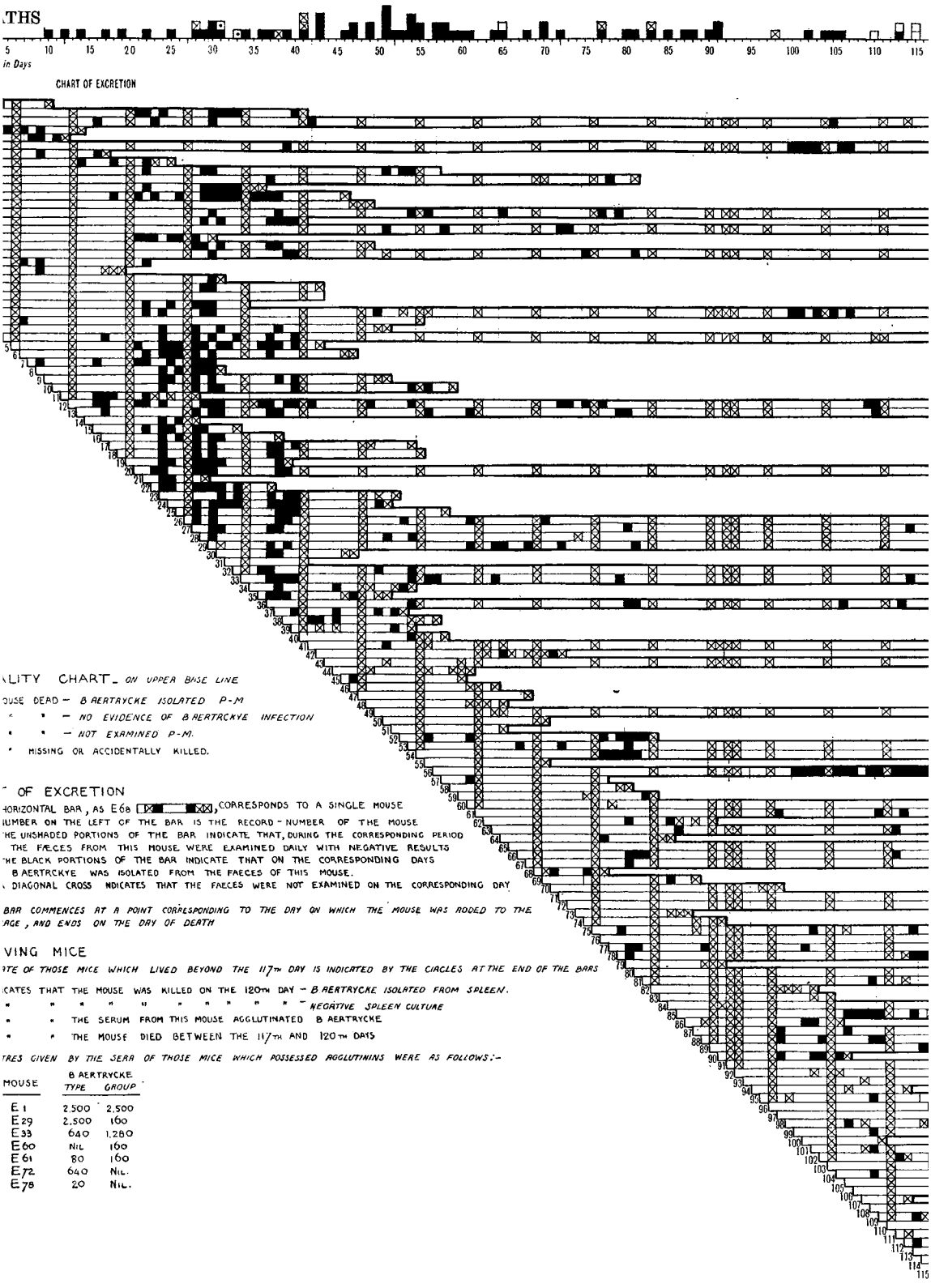
39 days of the experiment into three periods. During the first 14 days, 61 faecal strains were tested. Of these, 9 reacted as type, 47 as group and 5 as mixed. Thus, during a period when group strains were certainly being excreted in far larger numbers than type strains, mixed strains were relatively infrequent. Between the 14th and 25th days we examined 209 strains. Of these 60 reacted as type, 110 as group and 39 as mixed. Thus, during a period when the frequency of the type strains was increasing relatively to that of the group strains, the proportion of mixed strains was also increasing. Between the 25th and 39th days we examined 511 strains. Of these, 261 reacted as type, 148 as group and 102 as mixed. Thus, during the period in which the frequency of type strains exceeded the frequency of group strains, the frequency of mixed strains was at its maximum. Closely similar results were found to obtain in connection with the strains isolated from the tissues. It appears that the nearest approximation to truth will be obtained by regarding all the mixed strains as being, in reality, altered type strains; and this is what we have done. There would be no change in the general form of the diagrams, if we neglected all strains recorded as mixed, or introduced a separate shaded area to indicate those strains which were originally recorded under this head.

Two minor adjustments of another nature have also been made. It will be clear that the percentages charted on any date must be based on a very varying number of observations, according as many or few mice were excreting on the day in question. Where the number of colonies of *B. aertrycke* in cultures from any one specimen exceeded 5, that number of colonies were subcultured for agglutination tests. In many cases less than five colonies of *B. aertrycke* were obtained from a given specimen. The number of strains examined on a single day actually varied between 1 and 101. The significance attaching to the percentages recorded for the two serological types will clearly vary accordingly. On two occasions, once on the 11th day and once on the 89th day, a single strain was isolated during an interval in which the results were otherwise negative over several days. These two strains differed in serological reaction, from the strains which were isolated shortly before or shortly after them. To include these results as representing 100 per cent. of the serological variety in question, would introduce two large areas on the diagram suggesting a high frequency of these varieties over the corresponding periods. Since this is clearly unjustifiable, on the slender basis of a single agglutination test, these two results have been neglected in constructing the chart.

Chart II records the deaths, as in Chart I, and below this mortality base-line are recorded the results of the daily examination of faeces for each individual mouse. Each mouse is allotted a horizontal bar, extending from the day on which it entered the cage to the day on which it died, or on which it was killed at the termination of the experiment. These bars are shaded, or otherwise marked, to indicate the results of the daily examinations of the faeces. The signs employed to express these results, and also to record certain other observations, are fully explained on the chart.

Chart II.



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Discussion.

Serological results.

Dealing briefly with the serological results, Chart I shows clearly that, during the first phase of the spread of infection, group strains were far more frequently excreted than were type strains. In this connection it may be noted that the five infecting mice had been fed on strains of each variety. During the four days preceding the commencement of the main experiment, 29 strains of *B. aertrycke* had been isolated from the faeces of these mice, and of these 8 reacted as type and 21 as group. After about the 14th day, however, type strains rapidly increased in frequency, while the group strains diminished both relatively and absolutely. This phase corresponded to a period of increase in the percentage excretion-rate. The replacement of group by type strains continued thereafter, with few interruptions, throughout the whole of the experiment. There are some indications that periods, immediately preceding rises in the excretion-rate, are marked by an increased frequency of group strains; but we should not attach any great significance to this point, since the instances observed are neither striking nor numerous.

Of the replacement of group by type strains, during and subsequent to the epidemic period, there is no doubt. We know from previous experience that group strains, when fed to mice, give rise to faecal excretion somewhat more readily than do type strains; while type strains show a well-marked tendency to replace group strains in the tissues in sub-acute or chronic infections, and, to a less extent, to replace them in the faeces if excretion be long continued.

It is of some interest to find that a similar replacement of group by type strains occurs during an epidemic period; so that the one serological variety comes to predominate, among the population at risk, almost to the exclusion of the other. One can hardly avoid recalling the striking predominance of certain serological types in the epidemic spread of such diseases as pneumonia or cerebro-spinal fever in the human subject; but whether this apparent analogy has any real significance it is impossible at the moment to decide.

From the tissues of mice, which were killed at the termination of the experiment, 278 strains of *B. aertrycke* were isolated. Of these, 266 reacted as type, when tested by agglutination. Thus the results of the present experiment confirm our previous observations, with regard to the predominant rôle of the type variety in persistent tissue-infection.

The results of the agglutination tests, with the sera of those mice which survived beyond the 117th day, will be considered later. Reference to Chart II will show that, where agglutinins are produced, they may indicate a response to infection with either serological variety alone, or with both.

Fluctuations in the excretion-rate.

The daily determination of the percentage of excretors of *B. aertrycke*, among the population at risk, has enabled us to construct a continuous curve, showing the fluctuations in the excretion-rate during the entire course of the experiment. A consideration of this curve raises points of considerable interest. As will be seen in Chart I the excretion-rate begins to rise from about the 19th day of the experiment and attains a maximum on the 30th day, with minor fluctuations during its rise. It then falls sharply, commences to rise again about the 36th day, reaches a second peak on the 38th day, and then falls sharply again, maintaining a very low level between the 42nd and 49th days. Thereafter there are at least two minor but distinct fluctuations, with maxima on the 54th and 80th days, but the curve never again reaches the height obtained at the first or second peak. Towards the end of the epidemic there are indications of the rise of a fifth wave.

The first death is recorded on the 10th day, and thereafter sporadic deaths occur at frequent intervals, but the epidemic does not begin until about the 28th day, and does not reach its maximum until about the 51st day, after which it slowly declines, although occasional deaths continue to occur throughout the whole course of the experiment. The rise in the excretion-rate, therefore, precedes the rise in the mortality-curve, reaches its maximum during the early phase of the epidemic, and declines while the epidemic is still under way. The likeness to the course of events during the epidemic spread of cerebro-spinal fever is obvious and we may refer particularly to the observations recorded by Bruns and Höhn (1908).

The results obtained in this phase of the experimental epidemic afford an example of a generalisation which we believe to be of wide application: that, during the pre-epidemic phase of the spread of bacterial infection, there is a wide diffusion of the causative parasite among the population at risk, and that the proportion of hosts who are harbouring the parasite reaches a high level before the conditions requisite for an epidemic wave are attained.

The subsequent course of events raises another problem. The sudden decrease in the excretion-rate after the 30th, and after the 38th day, and the somewhat more gradual decreases after the 54th and 80th days are clearly shown in Chart I. How can we account for the fact that such a decrease may occur in a mouse population containing numerous excretors of *B. aertrycke*, when non-infected mice are present in the cage and fresh susceptibles are daily gaining admittance?

An examination of Chart II serves at least to narrow the problem. If the fate of individual mice be followed in this chart, it will be seen that we cannot explain the decrease in the excretion-rate by ascribing it to the removal, by death, of the excreting mice. It is clear that the rise of the excretion-rate to its first peak on the 30th day is due to the spread of the bacteria to hitherto uninfected mice. The chart shows, equally clearly, that the fall in the ex-

cretion-rate is due to the fact that the great majority of these mice cease to excrete at about the same time.

A careful study of the chart yields a clear demonstration that the fluctuations in the excretion-rate are due largely to intermittent excretion on the part of individual mice: A 1, A 9, A 14, E 12, E 13, E 26, E 29, E 33, E 36, E 52, E 53, and E 54 will serve as examples. Some factor seems to determine the approximately synchronous onset of excretion, and its synchronous cessation, in a high proportion of the population at risk; and individual mice respond to this factor by excreting, ceasing to excrete and excreting again in tune with their companions.

It is of some interest to consider in a little more detail the happenings during a single wave of excretion. If we take the period from the 20th to the 33rd day inclusive, covering the rise and fall of the first wave, we find that 52 mice were exposed to risk during some part of this interval. Of these, 42 mice excreted *B. aertrycke*. Of the 10 mice which did not excrete during this period, 4 were added on the last four days, so that their exposure to risk was of very short duration. There were 6 mice only which failed to excrete, though resident in the cage for the whole of the 14 days. Yet, on the 35th day, two days after the end of this period, only one mouse out of a cage-population of 43 was excreting *B. aertrycke* in detectable amount.

The data obtained in the course of earlier experiments, in which mice were fed on a single dose of *B. aertrycke*, throw some light on the question at issue. The observations recorded in the case of 219 mice are set out in Table I. The dose of culture fed to these mice varied widely in amount, but was in all cases relatively large, compared to the number of viable *B. aertrycke* likely to be ingested in a single dose during the natural spread of infection. The mice were observed for six weeks after feeding, or until death occurred at some earlier date, and 16 to 19 specimens of faeces were examined from each mouse which survived through the whole period.

When a mouse excreted *B. aertrycke* on more than one occasion, this excretion was often markedly intermittent; so that the table affords no data with regard to the actual duration of excretion. It does, however, show clearly that persistent excretion of *B. aertrycke*, after the ingestion of a single dose of that organism, is a rare event. It shows also that excretion is usually accompanied by tissue infection, and this is especially true with regard to those mice which excrete on more than one occasion.

It would seem to follow that a saprophytic multiplication of *B. aertrycke* rarely occurs in the intestinal tract of mice. Even when tissue infection is established it would appear to be unusual for *B. aertrycke* to secure a hold among the bacterial flora of the intestine.

It is not surprising then to find that a spread of *B. aertrycke* among the population at risk is followed by a phase of diminishing excretion. The rapidity of the fall in the excretion-rate, involving an almost simultaneous cessation of excretion on the part of all the infected mice, is not, however, easily ex-

plicable on the basis of our results with mice which have been infected by feeding. In such mice excretion is so irregular and intermittent that any curve, constructed by superposing the results obtained with 40 excreting mice, would show a slow and very irregular fall in the excretion-rate. It is difficult, in the light of our present knowledge, to account for the rapid and regular fall in the percentage of excreting mice, after each wave has reached its maximal point, on the basis of the reaction between the individual hosts and the parasites which they harbour.

An alternative hypothesis might be based on the assumed existence of some external factor, which affected all the mice in the cage simultaneously,

Table I.

Showing the frequency distribution of 219 *mice, classed according to the number of times* B. aertrycke *was isolated from their faeces, with the percentage of tissue infection observed in different classes.*

No. of isolations of *B. aertrycke*	No. of mice	No. of mice dying of *B. aertrycke* infection	No. of survivors with positive spleen cultures	Total No. of mice showing evidence of tissue infection	Percentage of mice showing evidence of tissue infection
0	127	22	24	46	36·2
1	33	14	8	22	66·7
2	18	9	9	18	100
3	11	5	2	7	63·6
4	11	7	3	10	90·9
5	6	4	2	6	94·7
6	2	0	1	1	
7	3	0	3	3	
8	1	0	1*	1	
9	1	1	0	1	
10	1	0	1	1	
11	1	0	1	1	
12	2	0	2*	2	
14	1	0	1*	1	
17	1	0	1*	1	

* Including one mouse kept alive for more than 42 days.

or in rapid succession; but we have, at the moment, no evidence that any such factor is involved. The solution of the problem must clearly await further investigation.

Fluctuations in the Excretion Coefficient.

In attempting to study the excretion of *B. aertrycke* in the faeces of mice, by a method which would yield quantitative results, having a relative significance in spite of a wide margin of error in the absolute values recorded, one of our objectives was to obtain data on the part played by dosage in the epidemic spread of enteric infection. It has already been suggested (Topley and Ayrton, 1924 *a* and *b*), that there is no simple conception of dosage, as a quantity which we can define and measure, under the conditions obtaining in an epidemic. The distribution of *B. aertrycke* in the total excreta of the mouse-population will determine not only the chance of some mouse ingesting a number of *B. aertrycke* falling within any given limits, but also the probable proportion of the population at risk which will ingest any *B. aertrycke* within

a given time-interval. If a few mice are excreting *B. aertrycke* in very large numbers, then it is likely that some mouse will ingest a large dose, while many mice will receive none. If a large proportion of the mice are excreting *B. aertrycke*, but none in great amount, then a high proportion of the total population will probably ingest some *B. aertrycke* within a given period, though none will receive a large dose. An extended experience of the actual course of events in individual mice shows conclusively that the variations in copiousness of excretion are in fact enormous. Is it possible to decide from the data before us whether mice which are excreting copiously play a preponderating rôle in the spread of infection?

The excretion coefficient differs from the excretion-rate in allowing for this factor of relative copiousness of excretion. If, in Chart I, we compare the curve showing the percentage carrier or excretion-rate with the curve showing the daily coefficient of excretion, it is clear that there is no possibility of differentiating between them, when attempting to correlate fluctuations in either with fluctuations in the curve of mortality. Their general form and the position in time of their maximal and minimal points are identical.

From the detailed records, not included in this report, we have noted the days on which specimens of faeces have been obtained giving a particularly high count of *B. aertrycke*, and have observed the fluctuations in the excretion-rate in the period immediately following. The results do not suggest that copious excretion on the part of individual mice is followed by a rise in the excretion-rate of the population in general. The highest count obtained in any one individual mouse, during the course of the experiment, was observed on the 22nd day, during the rise of the first wave of excretion, but after its commencement. The second highest count was observed on the 51st day, at a similar position on the third wave of excretion; but counts not insensibly inferior, coming indeed well within the wide limits of experimental error inherent in our technique, were observed on the 38th, 39th and 40th days, that is, during the early part of a fall in the wave of excretion, and preceding a prolonged interval marked by a low excretion-rate. Counts only slightly lower were observed on days 52, 53, 68, 81, 82, 85, 102, 103, and 105, a distribution which does not suggest any significant correlation with the other events observed.

There are many reasons for believing that repetition of relatively small doses may be a factor of crucial importance, and we may call attention in particular to some highly interesting observations recently reported by Lange (1924) and referred to in a recent report.

It appears to us that the whole question of dosage is too complex, and involves too many different factors, to permit of a useful discussion of its rôle in the epidemic spread of infection in the light of our present knowledge. A more extensive study of the individual factors concerned, under more strictly controlled conditions, may yield data which can be applied to the interpretation of such results as those here reported.

The ratio of infected mice to the whole population at risk.

Excluding the five mice infected by feeding, the population at risk during some part of the experimental period numbered 135. Of these, 122 yielded some evidence of infection.

There remained 13 mice, in which no evidence of infection was obtained by any of the methods of examination employed. Of these, 6 resided in the experimental cage for less than 14 days. One mouse, whose sojourn in the cage was 27 days, was eaten by his companions and could not be examined post-mortem. Thus, of 135 mice, which were exposed to risk for 14 days or more, only 6 failed to react in any way to the presence of the parasite, so far as could be determined by the methods of examination employed.

It appears that, under the conditions of this experiment, almost the whole of the population at risk played some part in the spread of infection.

The fate of individual mice.

In Chart II, it is possible to follow the fate of each individual mouse, from the time of its entry into the cage until its death, and to study the ways in which it reacted to the presence of the parasite.

The technique employed allows us to recognise four events which may be regarded as reactions to infection, using this term in a broad sense. A mouse may excrete *B. aertrycke* in its faeces. It may die, with or without the typical lesions of enteric infection, and yield cultures of *B. aertrycke* from its tissues. It may survive throughout the experimental period, but, when killed and submitted to post-mortem examination, yield a growth of *B. aertrycke* from its spleen. It may develop agglutinins to *B. aertrycke*.

A study of Chart II will show that the mice exposed to risk during the course of this experiment, afforded examples of most of the possible combinations of these criteria of infection.

The mode of reaction of those mice which died during the course of the experiment.

Excluding those mice which died from causes other than enteric infection, we may recognise certain well-defined modes of reaction, which have previously been noted in mice fed on cultures of *B. aertrycke*. A mouse may excrete this organism on several occasions, shortly before the fatal termination. Examples of this type of reaction are afforded by A 6, A 10, A 12, A 20, E 5, E 7, E 8, E 15, E 22, E 24, E 37, E 52 and E 90 (see Chart II).

Other mice may die of typical enteric infection without that organism ever having been isolated from their faeces, although specimens have been examined on many occasions. Examples are afforded by A 19, E 44, E 46, E 47, E 48, E 50, E 55, E 58, E 73, E 74, E 79, E 82, E 83, and E 94.

Among the former class are included certain mice, for example A 6 and A 10, which passed several weeks in the cage, during which they never

excreted *B. aertrycke* in detectable amounts, and then passed through a period of persistent excretion leading up to death. Mouse E 56, which died on the 119th day, affords a good example of this type of reaction. To the latter class might be added such mice as E 38, E 45 and E 57, which, after excretion on one or more occasions, passed through a long period during which they failed to excrete, before they succumbed to infection.

Other mice excreted intermittently over long periods, so that they might have been regarded as chronic carriers, but eventually died with the typical lesions of enteric infection. Examples are afforded by A 4, A 5, A 13, E 3, and E 10.

A study of Chart II will show that there is some grouping of the first two classes, according to the time of their entry into the cage. The mice added between the 44th and 50th days, for example, include a notably high proportion of animals which succumbed to enteric infection without ever yielding cultures of *B. aertrycke* from their faeces. These mice were added subsequent to the two earlier and more marked waves of excretion, which affected the cage-population in general, and before the third wave had risen to any considerable height.

We cannot, it would seem, regard a definite period of faecal excretion as an essential, or even as a modal phenomenon in mouse-typhoid. There are no grounds for believing that the mice at risk become infected with *B. aertrycke*, pass through a definite incubation period, and then suffer from an attack of disease, conforming closely to a particular type, and ending in recovery or death. The fact that a mouse has excreted over a certain period, and then survived for many days or weeks without yielding positive cultures from its faeces, does not render it improbable that that mouse will later die with the typical lesions of the disease. A mouse which might be judged, on the evidence of prolonged but intermittent excretion, to have become a chronic carrier may come to a similar end.

Under the conditions of this experiment, that is under conditions which allow unhindered transference of the parasite to and fro from host to host, an acute attack of disease ending in death, or in recovery with acquired immunity, seems to play a minor part. The equilibrium between parasites and hosts, which must fluctuate as an epidemic wave progresses, appears to depend on many other factors.

The mode of reaction of those mice which survived throughout the experimental period.

The results to be considered under this head raise questions of considerable interest. On the 117th day, when the experiment terminated, 62 mice were living in the cage. Of these, seven died before the survivors were killed three days later, and these seven mice are not further considered.

The history of the remaining 55 survivors can be traced in Chart II, but the more important facts may be summarised as follows. From the spleen of

each of these mice a culture was made in nutrient broth and examined in the manner already described. From the spleens of 28 of the 55 survivors, cultures of *B. aertrycke* were obtained. In the remaining 27 cases the cultures were negative. The correlation between excretion of *B. aertrycke* during life and the isolation of *B. aertrycke* from the spleen after death is very low. Eighteen of the 55 survivors had never excreted *B. aertrycke* in detectable amount. Of these, 8 gave positive spleen cultures and 10 negative. Of the latter, one mouse had been in the cage for two days only, so that this result is without significance. Of the 37 mice which had excreted *B. aertrycke* on one or more occasions during life, 20 gave positive and 17 negative spleen cultures. Among the 20 mice, which excreted *B. aertrycke* and gave positive spleen cultures, the interval between the last occasion on which excretion occurred and the termination of the experiment varied between 0 and 63 days, with a mean interval of 16·25 days. For the 17 mice with negative spleen cultures this interval varied between 0 and 78 days, with a mean value of 21·5 days.

A specimen of blood was collected from each mouse immediately before it was killed, and was tested for the presence of agglutinins against *B. aertrycke*. Seven of the 55 survivors gave positive results, the titres varying from 1/20 to 1/2500. The sera of the remaining 48 mice gave no agglutination at a dilution of 1/20. Of the seven mice with agglutinins in the blood serum, three had positive spleen cultures and four gave negative results in this respect. Two of the seven mice had never excreted *B. aertrycke* during life and these had negative spleen cultures.

The Factors concerned in Survival.

We have summarised above the findings in 55 mice, which were living in the cage at the termination of the experiment, but we are mainly concerned with the condition of survivors. The duration of the exposure to risk, on that date, had varied between 2 and 117 days. Clearly the mouse which had been in the cage for two days cannot be regarded as a survivor. There are, indeed, no adequate grounds for selecting any particular period as significant in this sense; such a choice must be an arbitrary one. For the purpose of discussion we have taken an exposure to risk of 14 days or over as the point at which survival begins. The choice of any other adjacent limit would give essentially similar results.

Taking as attributes, the presence of *B. aertrycke* in the spleen tissue, the excretion of *B. aertrycke* during life, and the presence of agglutinins for this organism in the blood serum, we can construct 8 possible classes, and assign to each a proportion of the 46 surviving mice, which had lived in the cage for 14 days or more. The results are shown in Table II.

It will be seen that six mice had apparently failed to react in any way to the presence of the parasite, and reference to Chart II will show that these six mice were among the more recent entrants to the cage.

Of 40 mice which showed some evidence of infection, seven had agglutinins

in their serum. These results are in accord with those recorded in the preceding report, for mice fed on a single dose of *B. aertrycke* (Topley and Ayrton, 1924 *c*). We might be led to the conclusion that the formation of agglutinins is an unimportant mechanism in the survival of mice during the epidemic spread of enteric infection, but we do not think that such a view would be justified.

Webster (1922) was able to demonstrate the presence of agglutinins, active in a dilution of 1/200, or over, in 8 of 11 mice which had survived 30 feedings with heated cultures of mouse-typhoid bacilli, followed by a single intrastomachal injection of living culture. All 11 mice survived a subsequent intrapleural injection of many minimal lethal doses of living culture. It should be noted that the treatment by heat had apparently not sufficed to kill the culture used for the preliminary feedings, since 12 of 24 mice succumbed to infection with the bacillus employed.

Table II.

Summarising the findings in 46 *survivors which had lived in the experimental cage for* 14 *days or more.*

Class	Spleen culture positive	Agglutinins positive	Excretion positive	No. of mice
1	+	+	+	3
2	+	+	−	0
3	+	−	+	17
4	+	−	−	5
5	−	+	+	2
6	−	+	−	2
7	−	−	+	11
8	−	−	−	6

In a later experiment, in which he fed 18 mice on living cultures, 7 survived the preparatory period. These 7 mice were then given an intrastomachal injection of living culture. One of them died, without showing any lesions post-mortem. The other six survived, while 7 controls died with typical lesions. Of the 6 survivors, one contained agglutinins in the blood serum, active at a dilution of 1/200. All six mice survived a subsequent intraperitoneal injection with many times the minimal lethal dose of living culture.

In another experiment, Webster fed 50 mice for four weeks on a killed culture of mouse-typhoid bacilli. All agglutination tests were negative at the commencement of the experiment, and again one week after the feeding had ceased. The immunity of these mice was then tested, in 16 animals by intraperitoneal inoculation, and in 17 by intrastomachal injection. The results suggested some degree of immunity, but were far less clear-cut than in the preceding experiments.

Another series of 25 mice were fed daily on living cultures of mouse-typhoid bacilli. At the end of 30 days there were 18 survivors. Agglutination tests were negative at the commencement of the experiment, and again on the 13th day, but on the 36th day the sera of 7 of the 18 survivors gave agglutination at 1/200. All 18 mice were now inoculated intraperitoneally

with a living culture. Of the 18 mice, only four succumbed, and none of these had shown the presence of agglutinins.

Amoss (1922), in an experiment in which batches of susceptible mice were added at irregular intervals to an infected population, tested the blood of 56 survivors from one epidemic wave. During this wave a mortality of 69 per cent. had occurred among a population of 300 mice. He found that 37, or 66 per cent., showed the presence of agglutinins in their blood serum, giving some degree of flocculation at a dilution of 1/40. He records the further observation that 20 of these 56 mice agglutinated the bacilli completely at 1/40, while 36 agglutinated it partially or not at all in this dilution. Of the former one mouse died during the subsequent epidemic wave, while of the latter 8 succumbed. The protocols given do not show how these 8 mice were divided, as between those which gave partial agglutination at 1/40, and those which were completely negative.

Further data on this question are afforded by many experiments recorded by Webster in later reports (Webster, 1923, 1924) in which the agglutination tests were set up in progressive dilutions starting at 1/20. Webster himself summarises the results of his very thorough investigations by stating that: "In any series infected *per os* with a fixed dose, 20 to 30 per cent. show no sign of infection, no positive blood cultures, and no agglutinins; 5 or 10 per cent. present symptoms of disease, positive blood cultures, and then recover with or without homologous agglutinins; 70 or 80 per cent. develop positive blood cultures and succumb in a more or less constant ratio relative to time." Or, again: "The agglutination phenomenon is no criterion of immunity; a surviving mouse may or may not give a positive reaction."

A survey of the experimental results would seem to indicate that the more severe the immunising treatment to which any group of mice has been subjected, and the smaller the ratio of survivors to the whole population which has passed through a given experience, the higher will be the frequency of occurrence of agglutinins among the surviving mice. It is, however, clear from Webster's experiments that a well-marked resistance to infection may exist in the absence of agglutinins.

The small proportion of the surviving mice, in the experiment under discussion, which showed the presence of agglutinins in the blood, may be due to the fact that they were not survivors in the strict sense; that is, the epidemic had not been allowed to run its complete course. Had the addition of susceptibles been stopped, and the experiment been continued until deaths had ceased to occur, the proportion of mice showing the presence of agglutinins might have been very different.

Webster's observations yield powerful support for the view that, when an immunity reaction does occur, it is of that general type which is frequently associated with the formation of antibodies, and with their presence in the circulating blood. This conclusion is strongly supported by Neufeld (1924) in a recent critical review of the fundamental questions of active immunity,

which deals very fully with the available evidence in the case of mouse-typhoid infection.

Finally, we may consider the significance of our findings with regard to the presence of *B. aertrycke* in the spleen. This question has already been discussed in a preceding report (Topley and Ayrton, 1924 *c*) and it is of some interest to find that the results obtained, when mice are fed on cultures of *B. aertrycke*, are closely paralleled when they are exposed to the risk of natural infection. We can hardly doubt that the phenomenon of latent infection is of fundamental importance in the epidemic spread of disease; and its relation to the problem of superinfection, recently studied by Lange (1921) in the case of mouse-typhoid, has already been referred to.

We are not, however, in a position to discuss in any detail its real significance. It has been demonstrated that many mice, when infected by feeding or by exposure to risk during the course of an epidemic, contract a latent infection which may persist for many weeks at least, and probably for much longer. Our results give no indication as to whether such mice are more or less susceptible than their companions to the ingestion of further doses of living *B. aertrycke*.

The question has recently been considered by Webster (1924), but, in the absence of more detailed protocols, it is impossible to assess the real significance of his results. If we have not misread his account of the experiments in question, the demonstration that a mouse was suffering from chronic infection, at the time when it received a second dose of living culture, depended upon the isolation of two serological varieties of bacilli from the tissues after death. If this were so, it would clearly be impossible to detect the presence of latent infection when the bacilli employed for the preliminary treatment and for the subsequent infecting dose were serologically indistinguishable, and the results recorded suggest that this was frequently the case. Webster describes chronic infection in those cases where the preliminary treatment was carried out with his M.T. I bacillus or with *B. enteritidis*, strains which are serologically distinct from the M.T. II strain used in the subsequent test dose. The other strains employed for the preliminary treatment, with the exception of *B. paratyphosus* B, are, so far as we know, serologically indistinguishable from Webster's M.T. II strain, and with these no cases of chronic infection are recorded. On the evidence recorded it appears impossible to determine the proportion of mice which, at the time of the test inoculation, were harbouring living bacteria in their tissues. In the absence of this knowledge, we cannot compare the resistance of such mice with that of others, from whose tissues the bacillus was absent.

This problem is, indeed, beset with technical difficulties and until they are overcome we cannot arrive at any just conclusion.

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