THE SPREAD OF BACTERIAL INFECTION.

SOME CHARACTERISTICS OF THE PRE-EPIDEMIC PHASE.

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(With 5 Charts.)

In previous communications (Topley, 1919, 1921 *a*, 1921 *b*) experiments have been described, which were designed to throw light on the processes under-lying the spread of bacterial infection among mice. The present report is concerned with certain phenomena, which may be observed during the early or pre-epidemic phase of the spread of infection among a population exposed to risk, both when it is receiving regular or irregular increments, and when it is segregated from other susceptible individuals of the same species.

THE METHOD OF EXAMINATION OF THOSE MICE WHICH SUCCUMB TO INFECTION.

In order correctly to appreciate the meaning of the results obtained, it is necessary to realise the limitations imposed by the routine technique adopted, and the extent to which this may be relied upon to give a true picture of the facts. Every animal which is found dead, or which is killed when *in extremis*, is submitted to post-mortem examination, unless it is in such a condition that no useful purpose is likely to be served by such a procedure. A certain pro-portion of the dead mice are wholly or partially eaten by their companions, so that no information, beyond the fact of their death, can be obtained. The deaths are recorded daily, the interval chosen being between 5 p.m. on one day and the same hour on the next. From 9 a.m. to 5 p.m. the cages are looked over at intervals of a few hours, so that mice dying during this period are examined within an hour or so of their death. Any animal found in an obviously dying condition at one of the cage-examinations during the latter part of the day is immediately killed. Where a mouse is found obviously ill, but not moribund, it is transferred to a separate cage overnight, in order to eliminate the risk of its being found eaten next day. It will be realised that, under these circumstances, those mice which die during the day-time present the most favourable material for examination. Unfortunately there is a definite tendency for deaths to occur more frequently during the night hours. When relatively few deaths are taking place in the cage the greater part of

them occur at night, and the risk of partial or complete consumption of the dead by the living is at a maximum. When, however, an epidemic is well under way, a considerable proportion of the numerous deaths will always be found to occur in the day-time, and the degree of cannibalism is much reduced. Thus it happens that the phase of the epidemic, which precedes the rise of a definite wave of mortality, affords material far less favourable for study than that obtained during the rise and crest of the wave. That these difficulties have not rendered the results obtained during the pre-epidemic period value-less will, I think, be demonstrated by a study of the charts in which they are recorded, and in which the failure to carry out a satisfactory examination of many of the mice is clearly indicated. At the same time it is important to remember this possible source of fallacy.

The examination carried out on each dead mouse follows a definite routine. The thorax is opened, after searing the subcutaneous tissues, the condition of the pleural and pericardial cavities and of the lungs is noted, and film-preparations are made from the pleural and pericardial fluids. If these be scanty, a film is also prepared from the heart's blood. A drop of the latter is, at the same time, spread over the surface of an agar plate and of a plate of MacConkey's medium. The abdomen is then opened, and a record is made of the condition of the liver, spleen and intestines. The presence of any excess of peritoneal fluid is also noted and a film-preparation is made of that which is present. If the quantity be minimal, the loop is rubbed over the surface of the omentum. The spleen is then cut across and cultures are prepared from it, in the same way as in the case of the heart's blood. The film-preparations are then stained and examined.

The further treatment of the plate-cultures is varied to some extent according to the information, which has been obtained as a result of the post-mortem examination. If the film-preparations have demonstrated beyond question that death has been caused by infection with the bacterial parasite which is being studied, the presence on the plates of typical colonies is, in certain cases, taken as sufficient confirmatory evidence, a proportion of the cultures being submitted to further examination as a further control. This method has, for example, been followed in several epidemics due to infection with *Pasteurella muris*, and with *B. murisepticus*. With either of these organisms the lesions produced, taken in conjunction with the morphology and arrangement of the bacteria in the film-preparations, are so typical as to leave no doubt as to their nature. In the case of epidemics of enteric infection in mice more detailed examination is clearly necessary. The routine procedure in such cases consists in preparing broth subcultures from three colonies from the plate-culture obtained from the heart, and three from that obtained from the spleen. After 24 hours' growth these are killed by heating after the addition of formalin, as described in an earlier report (Topley, Weir and Wilson, 1921), and are then tested against high-titre agglutinating sera. The occurrence of agglutination at a dilution nearly approaching the full titre of the serum has,

· in the majority of cases, been accepted as proof of the nature of the organism concerned, a minority of strains being tested as regards their fermentation reactions as a control for the whole series. Where the agglutination results have been negative with all six strains from one mouse, these strains have been further examined.

The examination of several thousand mice has convinced us that such a technique suffices for the recognition of a fatal infection with any of the organisms which have so far been studied. It leaves a certain proportion of the deaths unaccounted for, but this fact is due to our almost complete ignorance of the infections which are natural to mice, and of such causes of death as are unconnected with any infective process; so that no elaboration of the routine procedure would at present serve to eliminate these undiagnosed cases. An attempt is being made to obtain more information on these matters, and all mice dying among the normal stock are examined post-mortem. In a considerable proportion of all cases full post-mortem examinations are made, including the examination of sections of the more important tissues, but for routine purposes it is regarded as sufficient to establish or exclude the presence of the infections actually being studied.

THE COURSE OF EVENTS DURING THE PRE-EPIDEMIC STAGE OF THE SPREAD OF INFECTION.

With these preliminary points in mind, we may proceed to the consideration of the earlier stages of the experimental epidemics which have been so far observed. The experiments cited in this report are in no way selected, but include the earlier phases of all the more considerable epidemics, which have been studied during the course of this investigation.

In order to appreciate the points of immediate interest, it is sufficient to be able to trace the deaths as they occur in time, and to differentiate those cases, in which the death was proved to have been due to the infection under investigation, from those in which a post-mortem examination failed to yield such proof, and from those in which no such examination was possible. As all these points are clearly recorded on the charts, it is unnecessary to enter into any detailed description in the text, and the experiments may be briefly considered in groups.

Experiments 1, 2, *and* 3 are recorded in Chart I. They were all commenced by feeding three mice on cultures of *B. enteritidis* (Gaertner), and thereafter adding normal mice to the experimental cage. In Exps. 1 and 2, three normal mice were added daily. In Exp. 3, the batches of normal mice were added irregularly. Each square corresponds to the death of a mouse. The deaths of those mice which were infected by feeding are not charted. A black square indicates that *B. enteritidis* (Gaertner) or *B. enteritidis* (aertrycke) was isolated from the tissues post-mortem. A white square indicates that a post-mortem examination was carried out with a negative bacteriological result. A square

with a diagonal cross indicates that no post-mortem examination was made. The vertical line preceding the horizontal arrow at the end of each chart indicates that, although the record is discontinued, the epidemic actually lasted well beyond the period covered by the chart.
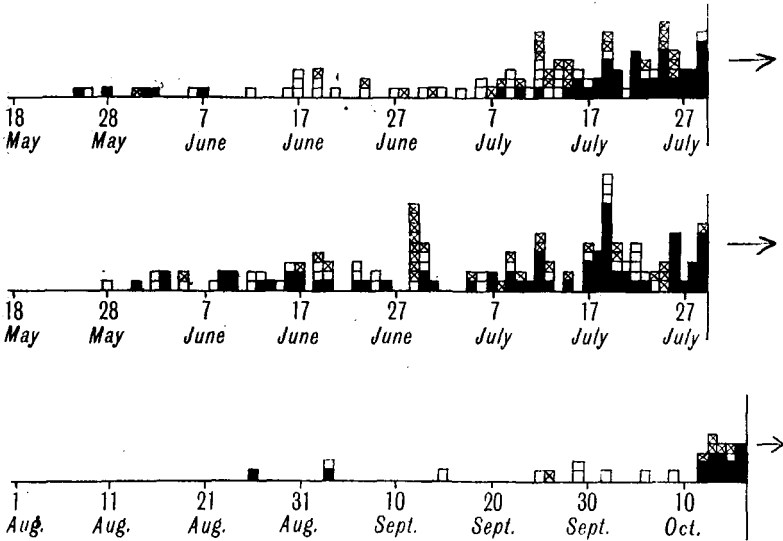

Chart I.

Experiments 4 and 5 are recorded in Chart II. At the date on which the record commences a certain number of mice, which had passed through an earlier experimental epidemic of enteric infection and had remained in apparent
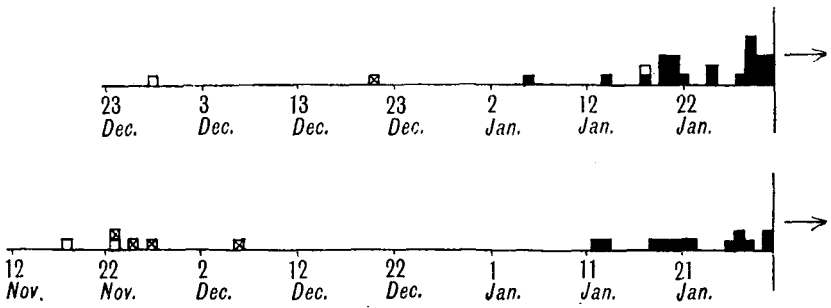

Chart II.

health over many weeks, were placed in each of the two experimental cages, and to them were added four times their number of normal mice. The subsequent events are indicated in the chart in the same way as for the preceding experiments.

Experiment 6 is recorded in Chart III. It shows the course of events in an experiment, in which an epidemic due to infection with *B. murisepticus* occurred in a cage, to which mice were being added in the hope of inducing an epidemic of enteric infection. The chart is constructed in exactly the same

way as the previous one, but the black squares indicate that the examination of blood-films, and of films of serous effusions, showed the presence of the bacillus whose typical appearance and arrangement were described by Koch (1878) more than forty years ago. Attention may perhaps be drawn to the
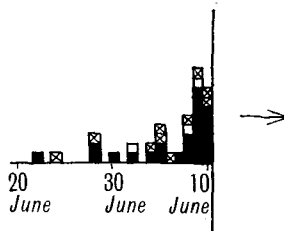

Chart III.

admirable description which he gives of the clinical picture of this infection of the mouse. In almost every case the bacillus was recovered in pure culture from the blood and spleen, and the further study of many strains showed them to possess all the characteristics of this organism. Throughout the period covered by this chart, three normal mice were daily added to the cage.

Experiment 7 is recorded in Chart IV. It shows the course of events in a large cage, in which mice were being collected for another experiment. This experiment was prevented by the spontaneous occurrence of an epidemic caused by an organism of the pasteurella group. Some 120 mice had been collected together before the occurrence of the first death recorded in the chart.
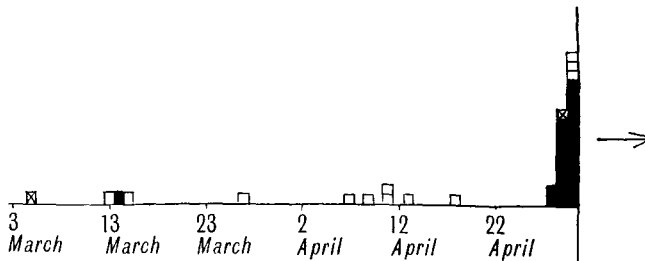

Chart IV.

Other mice were added at irregular intervals during March and the early part of April. The black squares indicate the deaths of mice, which showed the typical lesions of a pasteurella infection, and from whose tissues the organism was isolated post-mortem. The other symbols have the meanings already indicated.

An examination of these charts will establish the points to which it is desired to call attention. In every case the onset of the main epidemic is preceded at a considerable interval by the occurrence of an isolated death, or small group of deaths, due to the infection in question. In many cases this interval is so considerable that these early deaths cannot possibly be regarded as forming part of the rise of the main epidemic wave. In almost every case the pre-epidemic period is marked by the occurrence of a varying number of

deaths, with regard to which it is possible to state that they were not caused by the infection which constituted the main epidemic. In some cases (Exps. 1, 3 and 7, see Charts I and IV) a series of such deaths from other causes were interposed between the early deaths or groups of deaths, due to the infection concerned, and the main epidemic wave.

It may here be recalled that in certain experiments, in which only the early stages of the spread of infection from mice, infected by feeding, to their healthy fellows were studied (Topley, 1919), it was noted that, although the course of events in certain experiments seemed to leave little doubt that such a spread had taken place, yet it was often impossible to prove that the later deaths were due to the same infection as the earlier ones, which occurred in the mice infected by feeding.

It is not easy completely to satisfy oneself that these deaths from unknown causes are really an essential phenomenon of the pre-epidemic process. A certain number of deaths will always occur among a considerable mouse-population kept together in a single cage. Some of these will be due to fighting, and although, in these experiments, all deaths which were clearly due to this cause have been excluded from consideration, yet it seems possible that a certain number of deaths may be caused by violence which does not involve the infliction of easily recognisable wounds. Another difficulty is introduced by the cannibal habits of the mice. A glance at the charts will show that the picture presented would differ considerably, according as the crossed squares were coloured black or white. Yet the experience, gained by studying large mouse-populations during the past three years, leaves little doubt that the occurrence of those deaths which are indicated by unshaded squares, both from their number and still more from their time-relations, cannot be regarded as due to the normal death-rate; and the course of events recorded in Exps. 1, 3 and 7 suggests very strongly that these undiagnosed deaths are a regular feature of the earlier stages of that spread of bacterial infection, which eventually leads up to a definite and considerable epidemic.

Among those experiments, which have been carried out by adding susceptible individuals to an infected cage at a constant rate, one has now been continued uninterruptedly for over sixteen months. In this instance, in which advantage has been taken of a spontaneously occurring epidemic due to a mouse-pasteurella, a long-period epidemic wave has developed, with a sufficiently long inter-epidemic period to allow a comparison to be made between the course of events during the pre-epidemic phase of an initial wave, and during that period which elapses between the decline of such a wave and the rise of the wave that follows.

Chart V shows the course of events during the inter-epidemic period of this experiment. It is constructed in the same manner as the preceding charts. During the whole period three normal mice were daily added to the cage. It will be seen that there is a close similarity between the events which led up to the secondary wave, and those which we have already noted in the case

of an initial pre-epidemic period. During the first wave, the last stage of which
is recorded on the chart, almost every mouse submitted to post-mortem exami-
nation gave definite evidence of having succumbed to a pasteurella infection.
After the decline of the wave a decreasing proportion of the deaths which
occurred in the cage were due to this infection, and as the inter-epidemic period
progressed the isolation of this organism from the mice which died became
more and more infrequent; so that, on two occasions, a period of 14 days
elapsed without a single death occurring which could be referred to this
infection. The chance of error involved by mice being eaten by their com-
panions was at a maximum during this period, as is indicated in the chart.
It will, however, be seen that, while 47 mice were examined between the end
of July and October 3rd, in only nine of them was any evidence obtained of
pasteurella infection, a result contrasting so strongly with that obtained during
the epidemic period, that we may feel confident that the inability to examine
a considerable proportion of the mice has not sufficed to obscure the actual
facts. It will also be noted that the rise of the second wave of mortality from
pasteurella infection is preceded by that rise in the mortality from all causes,
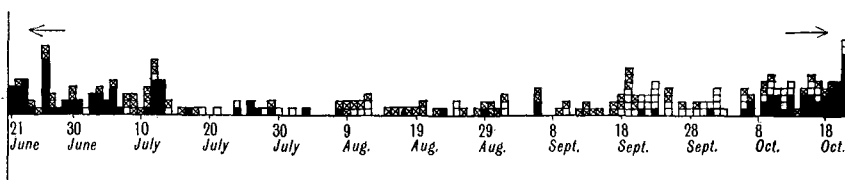

Chart V.

which we have noted in previous experiments. Between September 17th and
October 3rd there was an unmistakable rise in the daily death-rate. During
this period 29 mice were examined post-mortem, but in three instances only
was *Pasteurella muris* demonstrated in the tissues. On October 6th the new
epidemic wave was clearly established, and it will be seen that the rise in
mortality was now mainly due to pasteurella infection, and that the proportion
of total deaths, which could be definitely assigned to this cause, increased as
the new wave progressed.

It would seem, therefore, so far as we may judge from this solitary experi-
ment, that there is a close similarity between the events which precede the
rise of a secondary wave, and those which occur during the initial pre-epidemic
period. In both cases we note the occurrence of a few deaths from a specific
disease, which are relatively isolated in time; so that it seems as though the
infection could, in this stage, produce the death of certain susceptible mice,
without being able to spread to their companions in such a manner as to
produce a similarly fatal result. In both we note that the actual rise of the
wave of specific mortality is preceded by an increase in the daily death-rate
from all causes.

It is of interest to enquire how far these experimental results are paralleled
by observations carried out on naturally occurring epidemics among human

or animal populations. Epidemics affecting mankind can, indeed, seldom be studied under conditions which are in any way favourable for the acquisition of the information desired. Nor can we expect that medical records will contain the details we require, unless an especially minute study has been made of the course of events in relatively small and isolated groups of the general population. It is possible that a careful survey of the records of infectious diseases among aggregates of human beings, who are by some especial circumstances isolated for a considerable period from other members of their species, might afford instances which would tell for or against the conclusions drawn from experimental data. It is impossible, at the present time, to consider this aspect of the question in any detail, but reference may be made to one series of records, which are peculiarly applicable to the problem before us, because of the detailed information which they contain. Peters (1911), in a study of the incidence of epidemic diarrhoea in Mansfield during the summer and autumn of 1908, found that the incidence of this disease was not distributed uniformly, nor at random, among the population at risk, but tended to be concentrated upon small groups formed by neighbouring houses or streets, these houses frequently having some special feature, such as a yard-in-common, which tended to increase the chances of social intercourse between the families inhabiting them. He found also that situation within or without such a localised focus of infection was one of the principal factors determining the incidence of disease among the members of a given family, while the relative dirtiness or cleanliness of a household, situated in such an area, had a minimal effect upon its risk of infection. He gives detailed figures of the daily incidence of diarrhoea in a considerable number of such localised centres of infection, and his records show clearly that in any such centre a single case, or small group of cases, may precede by several weeks the main outbreak of disease, no new cases being recorded in the interval. A study of the charts included in his monograph shows many striking points of similarity with those contained in this report.

If we turn to the question of an increase in mortality or morbidity from all causes, preceding an outbreak of some specific infective disease, it is still more difficult to discover instances, which carry any conviction. It is tempting to refer to the records of "sickly seasons" preceding great outbreaks of plague, such as that of 1665 in London, while the undoubted rise in deaths from all causes associated with major outbreaks of influenza may perhaps be a significant phenomenon from this point of view; but the factors concerned in such large outbreaks are so complex and so little understood, that there is no justification for appealing to such evidence as confirmation of experimental results, obtained under conditions which are relatively simple.

It seemed possible that observations had been recorded in connection with epidemic diseases among live-stock, which would be more closely comparable with the experimental results reported here. For this reason, and in view of certain results which are referred to in a further report, I have tried to obtain

information bearing on the points at issue. As regards outbreaks in this country, I am indebted to Sir John M'Fadyean and to Sir Stewart Stockman for giving me the benefit of their experience in these matters. In America, Dr Simon Flexner very kindly forwarded some questions regarding the course of events during the pre-epidemic period to Dr Theobald Smith, who has been good enough to give me his opinion on these points. To all the above I should wish to express my thanks. In each case the opinions expressed are essentially the same. There is apparently little evidence that an outbreak of a specific infection among live-stock is usually preceded by a single case, or small group of cases, occurring at a considerable interval before the main epidemic; or that such an outbreak is preceded by a rise in mortality or morbidity from other causes. There is general agreement that the observations necessary to form an opinion on this point, one way or the other, are not at present available.

Discussion.

With regard to the experimental results, we should naturally wish to know what are the processes underlying the pre-epidemic phenomena observed. Such knowledge can be obtained only when we have more detailed information as to the distribution, during this period, of the parasites concerned among the population at risk. Accurate data on this point are at present wanting. Certain investigations along these lines have been carried out, and it is possible to state that, at the time when the first warning death occurs, the specific parasite in question can be recovered from the tissues of many of the other inhabitants of the cage, although these are apparently in normal health. Until, however, a more adequate supply of observed facts is available, it is of little use discussing this side of the question at greater length. It would appear almost impossible to avoid the conclusion that an essential factor is a series of passages, leading to a variation in the infective powers of the parasite, and to simultaneous variation in the resisting powers of the animal hosts through whom the passages occur.

It will be useful to have some convenient expression to denote that accumulation of circumstances, which appears to be necessary before an epidemic wave can be propagated. The term "Epidemic Potential," employed by Peters in discussing the factors concerned in the spread of epidemic diarrhoea, seems particularly well fitted for this purpose. Using the term in this general way, we may conclude that the pre-epidemic phase is occupied by the gradual development of epidemic potential, and that, when this potential has reached some critical value, a wave of specific disease will be propagated among the population concerned; while the occurrence of single deaths, or small groups of deaths, due to this specific infection, and perhaps a rise in mortality from all causes, are evidences of a rising potential, which has not yet reached the height necessary for the propagation of an epidemic wave.

The term "pre-epidemic phase," employed in this report, might suggest that every epidemic would be preceded by a stage characterised by the features which have been considered. Such a supposition would clearly be unwarranted.

Those observations, which have been based on epidemics purposely initiated, have, in reality, been concerned with the events which follow the admixture of a population, which has passed through one epidemic wave, with another, composed of relatively large numbers of susceptible individuals of the same species, or with the events which are brought about by continuously adding susceptible individuals to others, which have been infected by feeding.

Even were we to conclude that such an admixture formed the basis of all epidemics of bacterial infections, we should still expect that a pre-epidemic phase, of the type described above, would occur only when the state of equilibrium among the potentially infective unit of population was such as to necessitate a somewhat prolonged process, before that degree of epidemic potential could be obtained which would lead to a well-marked wave of sickness or mortality. If the result of the mixture of populations were the introduction of a fully infective and virulent virus among a large number of susceptible hosts, we should expect an entirely different course of events.

It appears, then, that the foundation required for further enquiry is a more precise knowledge of the effects produced by forming aggregates, in which the number of infective and susceptible individuals vary relatively to one another, and the infectivity and susceptibility of these two categories themselves vary from high to low orders of value. If the technical difficulties could be satisfactorily overcome, it might reasonably be expected that results of some interest and value would be obtained from such studies.

CONCLUSIONS.

1. During the pre-epidemic stage of the spread of bacterial infection among mice, single deaths, or small groups of deaths, due to the specific infection being studied, are found to occur at considerable intervals before the rise of the main epidemic wave, and to afford a warning that such a wave is at hand.

2. In many cases there may be observed, during the same period, an increase in the daily mortality from all causes.

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