EXPERIMENTAL EPIDEMIOLOGY.

By

M. Greenwood etc.

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PREFACE

It would be difficult to imagine a subject of study more important for human welfare than the spread of epidemic diseases among mankind. To this a noteworthy contribution has been made by Professor W. W. C. Topley and Professor M. Greenwood, with the assistance of colleagues, in experimental researches at the London School of Hygiene and Tropical Medicine. The cost of these necessarily prolonged investigations has been provided during a period of eighteen years by the Medical Research Council, who are now glad to issue this report dealing comprehensively with the whole scheme of work; many of the results on particular points have of course already been made known by preliminary publications in scientific journals.

The experimental method which the report describes was devised to solve a particular type of problem that has proved refractory to other forms of study. On the one hand, laboratory experiments, performed under strictly controlled conditions, have yielded a mass of information with regard to the response of the individual host to artificial infection, and as to methods by which that response may be modified. On the other hand, the data collected by the epidemiologist have taught much in regard to the behaviour of naturally infected herds, and something of the effect of interfering in the natural course of events, as for instance by prophylactic immunisation against small-pox, diphtheria or typhoid fever. The collection of statistical evidence under field conditions, however, is so beset with difficulties that the assessment of the relative importance of the interrelated factors, determining the course of events in infected communities or herds, has so far proved impossible.

It is to problems of this kind that the method of the experimental epidemic can be applied. Given that continued exposure to risk of infection raises the average resistance of the surviving members of a herd, whether by active immunisation or by selection of the innately more resistant animals, or by both mechanisms together, various questions arise. Is this increase so great that the infection will eventually die out, if none but uninfected immigrants are admitted to the herd? Is it possible by the active immunisation of all entrants to reduce mortality to zero, or eventually to eliminate the infecting organism by depriving it of access to susceptible hosts? If dietetic factors play a significant part in resistance to infection, is their influence so important that on an optimal diet animals will be indifferent, or relatively indifferent, to prolonged exposure to the risk of natural infection? If no single factor is found to exert a decisive effect, will it be possible to attain the desired level by combining several different methods of raising the average resistance of a herd?

To some of these questions the evidence set out in this report offers limited and tentative answers. The work should be regarded

however, as a preliminary survey of the field, rather than as an application of the technique to a well-defined series of problems. Experimental epidemiology has its own technical difficulties and limitations: these are discussed in some detail in Section I. Before one can assess the effect of any method of interference with the natural course of events, it is necessary to obtain an adequate picture of the behaviour of herds that are exposed, over prolonged periods, to the full risks of natural infection. Such a picture is outlined, for mouse typhoid and mouse pasteurellosis, in Sections II and III, and for a virus disease of mice—ectromelia—in Section IV.

The remaining sections are devoted to the influence on herd infection and herd resistance of variations in the virulence and infectivity of the infecting organism, of artificial immunisation of the animal hosts at risk, of changes in diet, of the presence of a bacteriophage, or of dispersal of an infected herd at different phases of the epidemic process. It is of some interest to note that the existence of "epidemic strains" of bacteria, which have been postulated by many epidemiological workers to explain happenings observed in the field, has been confirmed by these experimental studies, and that it has been possible to define certain of their properties.

None of the methods of interference so far attempted has sufficed to eliminate the risks of disease and death under the conditions of severe and continuous exposure to which the herds were subjected in most of the experiments under review. The most promising results were obtained in active immunisation against ectromelia. The negative findings must, of course, be regarded as applying only to the actual infective agents used and to the experimental conditions obtaining: this part of the inquiry is as yet in its infancy.

There can be little doubt that the experimental epidemic affords a more natural, and more severe, method of testing the value of any prophylactic procedure than assays carried out by more artificial tests on individual animals. It can never, of course, replace field observations made under completely natural conditions; but it may well indicate possible solutions to many of the more important practical problems, and so direct the field epidemiologist along the most fruitful lines of inquiry.

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SECTION I

INTRODUCTION

The studies outlined in this report have been in progress for some fifteen years. They form an attempt to place the science of epidemiology on an experimental basis.

Descriptive epidemiology is one of the oldest branches of medical science. The application of statistical methods, though not extending back to the days of Hippocrates and Galen, dates back to the time of Sydenham's contemporary John Graunt; and we would hazard the guess that Graunt's contribution to epidemiology was more significant than Sydenham's. Bacteriology, and its offspring immunology, are of more mushroom growth, little more than half-a-century old. They have already thrown a flood of light on the particulars of many epidemiological problems, but they have as yet attempted little in the way of any adequate synthesis.

In the earliest of our joint communications (Greenwood and Topley, 1925) we attempted a brief review of the epidemiological concepts derived from descriptive and statistical studies, and it is unnecessary to repeat it here; but it may be well to outline very briefly the reasons that seemed to us then—and still seem to us now—to justify the laborious and relatively costly studies of which this report records only the initial steps.

Descriptive epidemiology, as it has emerged from the hands of Hippocrates, Galen, Ballonius, Sydenham, and their followers down to our own time, though it has produced hypotheses that cover some part at least of the observed facts, has left its outlines curiously vague, and has, with a few notable exceptions, failed signally in satisfying the major canon of an empirical science, that a good working hypothesis should lead to action with predictable results, and this in turn to modified hypotheses with further practical applications.

The statistical method, less ambitious but far more precise, has defined the problems of epidemiology in a way that unaided description has been unable to approach. No general hypotheses in this field can ignore the data that the statistician has collected, analysed and presented in a significant and intelligible form. In particular cases the statistical method may permit of the solution of an epidemiological problem, or of a near approach to one; but in those instances in which many unknown variables are concerned, and many assumptions are possible, the statistician is grievously hampered, as many sections of this report will show, by a lack of any adequate indication as to which of the possible assumptions he should select.

The bacteriological and immunological methods of approach have scored their major successes in those parts of the field where the descriptive, and to a less extent the statistical, methods have for the

most part failed. Attacking particular problems, bacteriologists have been able to demonstrate the part played by certain specific parasites, and by the hosts' reactions to them, and by applying the knowledge so gained they have, in some cases at least, been able to interfere successfully in the natural course of events. *Per contra*, the bacteriologist has tended to fail where the statistician, and in a vaguer way the descriptive epidemiologist, have succeeded. He has not been able, indeed he has hardly tried, to put together his pieces of the puzzle in such a way as to achieve a synthesis that bears more than a patchy resemblance to the picture that his colleagues have defined.

With a few possible exceptions, we doubt whether this putting-together process offers any great hope of success. To justify our doubts we may give a few examples.

We know quite well that animal hosts belonging to the same species vary in their innate resistance to any given microbial parasite, though we should be hard put to it if we were asked to describe such variation in terms of its mean and dispersion. We know that there is a general tendency for one attack of an infective disease to leave behind it an increased resistance to further infection by the same organism. We know also that this immunity varies in degree and duration from one infective disease to another, that, for instance, it is very effective following many virus diseases, less effective after many bacterial diseases; but here again we cannot express our knowledge in precise statistical terms. We know that bacteria of the same species vary in virulence, and perhaps in other characters that affect their powers of spread; and many of us suspect that these variations are important from the epidemiological point of view. We know that the results of experimental infection vary with dosage; indeed we know a little, though much less than we should like, as to how they vary. We may be reasonably confident that variations in dosage occur during the contact infection that characterizes epidemics, and that these variations are significant both as they affect the size of dose received at any instant and the rate at which successive doses are received.

Sitting at our desks, or in our armchairs, we can add up these pieces of knowledge in any way we choose. We can describe an epidemic in terms of host resistance. The deaths that occur during the rise and at the crest of an epidemic wave will eliminate the more susceptible members of the herd at risk. With a thus increasing average resistance of the herd the wave will subside, to rise again when births or immigration provide a favourable soil for the parasites that have remained in endemic foci. Alternatively we can vary our picture by giving active immunization pride of place. During the rise of the epidemic not all the infected hosts will contract a fatal illness; many of them will recover and become immune, some at least will develop immunity as the result of an infection that produces no clinical illness. Again the wave will fall because the average resistance of the herd is raised, and again another wave will follow it at a later date when the influx of susceptibles or the loss of immunity

by survivors, or both together, have caused the average herd immunity to fall below some critical level. If we are wise we can adopt these as complementary instead of alternative hypotheses.

But we can, if we please, frame our hypothesis in relation to the parasite rather than the host. We might, as Brownlee (1919) has done, invoke some hypothetical life-cycle of the parasite to account for the periodicity of certain epidemic prevalences. We should not ourselves feel inclined to follow Brownlee's example, because we know of no biological justification for the assumptions that he makes, and they seem to us inherently improbable; but there are other *a priori* possibilities that accord better with our general knowledge of bacteriological behaviour. The parasite, when first it spreads among susceptible hosts, may maintain its initial virulence, and kill a high proportion of those whom it infects. In those whom it fails to kill, either because they possess more than the average resistance *ab initio* or because they have responded to a sub-lethal infection by an immunizing response, it may be unable to maintain its original characters, and mutate into a less virulent form, more suited to a vegetative existence in the situation in which it finds itself. Later, when susceptible hosts accumulate in one of the ways described above, a relatively avirulent mutant may gain access to this more favourable soil, regain its lost virulence, and start the cycle over again.

And so we can go on adding one to one as long as we please; but, though we deal only in perfectly respectable bacteriological units, we shall never know when we have got the right answer. Our hypotheses are working hypotheses, not conclusions, and until we put them to the test they can have no validity, or even usefulness.

The problem at issue is essentially a quantitative one. It is certain that the factors mentioned above, and many others to which no reference has been made, operate in one way or another, and to a greater or lesser degree, in the natural evolution of an epidemic or endemic prevalence. It is the relative importance of these factors that we desire to know, and the exact way in which they interact with each other. In part we desire to know these things to satisfy our intellectual curiosity—the interactions of parasite and host in an infected herd present a fascinating biological problem. In greater part, perhaps—though in an empirical science there can be no sharp cleavage between theory and practice—we desire to gain the power of intelligent and effective interference. Here, again, it may not be amiss to offer a few illustrative examples that have a direct bearing on the control of epidemic or epizootic disease.

Suppose that we can show that, after the rise and fall of an epidemic wave in an infected herd, we are left with a proportion of survivors with a high average resistance, some of whom are infected, and therefore possibly infective; how long will this group of survivors remain a source of danger to susceptible immigrants? Or again, will susceptible immigrants be a source of danger to the old survivors, disturbing the equilibrium on which their immunity, as a herd, depends?

Granting that the natural acquirement of active immunity is an important factor in the later stages of an epidemic, and that we can induce a similar immunity among immigrants by some method of artificial immunization, what will be the effect of such a procedure (*a*) on the immunized hosts themselves, (*b*) on the level of mortality in the herd as a whole, and (*c*) on the continuance of the epidemic prevalence? If the effect on (*a*), (*b*), or (*c*) is significant, what proportion of the total number of hosts at risk must be immunized before a subsidence of the epidemic is induced? If an epidemic can be brought to an end by these means, what is the real condition of the immunized herd? Have we merely eliminated the overt disease, or have we eliminated, or reduced to negligible proportions, the foci of infection? Must we contemplate the immunization of all future immigrants, without an assignable limit in time, or can we, after some stated period, allow non-immunized hosts to mix freely with our immunized herd?

If some non-specific factor, such as diet, is found to have an influence on herd resistance, how important is that influence in relation to other operative factors? Will the provision of an optimal diet increase resistance to such a level that other methods of interference will prove unnecessary, or, if the level attained is less effective than this, will the increased resistance induced in this way, operating in conjunction with the best available method of immunization, produce an effect that neither could produce alone?

Is it possible, as some have claimed, that by the careful selection of an appropriate bacteriophage, we may, by introducing this additional participant into the system of interacting living organisms that constitutes an infected herd, shift the equilibrium in favour of the host and against the parasite?

Here, again, we might multiply our questions *ad infinitum*, but to no good purpose. The point at issue is that we can answer them only by finding out what really happens in an infected herd, not by deducing what *might* happen from our knowledge of what occurs in individual hosts. The herd then must be our universe of study, though we shall need frequent reference to individual hosts, or to smaller groups, for the study and definition of the various interacting factors with which we are concerned.

It is true, of course, that some at least of the information that we require may be attained by the study of human herds with which we have interfered in some particular way; but those who have had experience of the analysis of the field data that have actually been collected will not be over-sanguine in regard to the possibility of rapid advance along these lines; the uncontrollable variables are so many and so complex. It is true, also, that the veterinarian is in the happy position of being able to investigate the diseases in which he is interested in their natural hosts, and that, in studying the behaviour of a herd, he can impose a degree of control that is entirely impossible for the student of human infections. He is, indeed, able to apply the methods of experimental epidemiology directly, avoiding

all arguments from analogy. But cattle, sheep and pigs are less easily controlled than mice ; and they, and their upkeep, are very much more expensive. Finance is, indeed, the limiting factor, and a vitally important one. Experimental epidemiology is essentially the combination of the experimental with the statistical method in an attack on a series of problems that can be solved in no other way ; and its findings must, of necessity, be expressed in statistical terms. It follows that large numbers of experimental animals are essential. The experiments on which this report is based, experiments that have in fact done little more than clear the ground for future studies, have involved the use of somewhere between 100,000 and 200,000 mice.

This, then, seems to us to be the justification for the study of experimental epidemiology, using the small rodents of the laboratory as our test animals and micro-organisms that spread naturally among them, causing fatal diseases, as our test parasites. We are well aware that, in any attempt to apply our findings to similar events in human or animal herds, we are faced with the difficult, and sometimes dangerous, argument from analogy. But the history of bacteriological and immunological research shows clearly how fruitful this indirect method of approach may be ; and we shall at least have narrowed our problem to the question whether what is true of the mouse is true of man, or the cow, or the pig—a limitation that will greatly increase our chance of obtaining a significant answer.

Within a short time after our own studies were commenced, Webster and his colleagues at the Rockefeller Institute initiated an independent investigation along somewhat similar lines. Their angle of approach has differed a little from our own, in that they have occupied themselves more largely with experiments on individual mice, and less with the actual spread of infection among controlled herds. Such studies as they have made on the natural spread of infection have been concerned mainly with epidemics in rabbits and fowls, housed under conditions that left the risk of cross-infection dependent on many variable factors. On the statistical aspects of the problem they have made no serious attack. We have, in each section of this report, endeavoured to correlate their findings with our own.

The General Methods of Experiment

The general methods that have been employed in these studies have been set out in an earlier paper (Topley, 1923), and may be very shortly summarized here, together with certain modifications that have been made within recent years.

The cages employed are cylindrical, 10 in. in diameter and 5 in. in height. Those originally employed were made of zinc, but we have recently substituted cages of the same pattern made of glass. They have lids of perforated zinc, and are provided with cylindrical side-pieces so constructed that any number of cages can be fitted together, end-to-end, thus making a composite cage of the required size. One unit cage is provided for every 25 mice, or for any smaller number in

excess of the nearest multiple of 25; thus a herd of 125 mice would be provided with a cage made of 5 units, a herd of 255 mice with a cage built up with 11 units.

Each day, except Sundays, and in some cases Saturdays, the mice constituting any herd are removed with forceps to a clean cage, provided with food and litter, that has been made ready overnight. The dirty cage, separated into its units with the side-pieces closed, is placed in a sterilizer and steamed for one hour at a pressure of 7 lb. It is then cleaned and prepared for use as a clean cage on the following day. Thus no cage is cleaned before sterilization, and there is no opportunity for excreta to collect. On each day, except Sundays, the mice are transferred to a clean environment, free from the parasite with which they are infected. The assistants who have charge of our infected herds work in sterilized overalls and rubber gloves, the gloves being changed when proceeding from one infected herd to another. They take no part in caring for the normal mice from which our herds are recruited. The houses in which the cages of infected mice are placed are swabbed down daily with a dilute lysol solution, and are fly-proof.

By strict adherence to this technique it has usually proved possible to maintain herds for months or years without the accidental introduction of any extraneous infection; but our success has been by no means uniform. For many years past we have been able to maintain herds infected with different bacterial parasites, such as *Bact. aertrycke* and *Past. muriseptica*, without any cross-infection; but in the case of the virus disease, ectromelia, we have not been so successful, and several experiments have been spoilt by the accidental transference of this virus to a herd infected with some other disease.

Our normal stock has been recruited in part from mice that we have bred ourselves, but in much greater part from mice bred for us by a limited number of small private breeders, who have undertaken to breed for us alone. We have, for many years, ceased purchasing mice from the usual large dealers, since, sooner or later, this procedure has inevitably led to the importation of stock infected with one or other of the diseases that spread naturally among mice. It would clearly be far more satisfactory if we could ourselves breed sufficient mice to meet all our requirements; but the rearing of 15,000 mice or so a year calls for accommodation that is not commonly available in institutes situated in a large city and having to meet an ever-expanding demand for the housing of experimental animals without any open ground for the erection of additional buildings. It may, perhaps, not be altogether amiss to take this opportunity of suggesting that the provision, through some central agency, of an adequate stock of clean and healthy animals of those species which are in common use for experimental purposes would do much to facilitate research work in every field in which such animals are required.

All imported mice are placed in quarantine for 14 days, 12 mice to a cage. The pooled faeces from each cage are tested for the

presence of *Bact. aertrycke* and *Bact. enteritidis*. We may perhaps add that, during the past five years or more, these have not been found. At the end of the quarantine period the mice are moved to the normal stock room, where they are retained until used. All normal mice that die are examined *post mortem*, cultures being taken from the heart and spleen. If a death occurs in any cage, and the post-mortem examination yields no evidence of any known infection, the remaining mice are kept under observation for at least another 14 days before being used. If two deaths occur in any cage the remaining mice are rejected, even though both post-mortems are negative. If any post-mortem yields evidence of any infective disease, even though it is not one of those that spread readily among mice, the remaining mice from that cage are rejected. Mice are not added to our herds, or used for other experiments in this series, until they attain a weight of 18 g., nor are they added after they have attained a weight of 22 g. This usually means that imported mice are under observation for periods of 4–8 weeks before gaining entry to our herds. It may be added that in the quarantine room and normal stock room the cages are treated in the same way as in the rooms in which our infected herds are housed, i.e. they are sterilized each time they are changed, never cleaned before sterilization. With this routine it is almost always possible to limit any infection that may gain access to our normal stock to the cages in which it first appears.

Since it is necessary to identify individual mice in an infected herd we use only a parti-coloured breed. We may add that numerous opportunities have been taken by ourselves and our colleagues to compare the resistance of the mice received from different breeders, and that we have never been able to detect any significant difference.

On entry to an experimental herd, the colour and markings of each mouse are recorded, together with other particulars of weight, origin, date of entry, etc., on the front of a special card. Each mouse that dies is identified by its card, the date of death is noted and on the back of the card are recorded the post-mortem findings, including the results of cultures taken from the heart and spleen, From these cards our statistical records are compiled.

A point of considerable importance is the evidence on which our diagnosis of the cause of death is based, since on the accuracy of this the validity of our statistical records clearly depends. All mice not eaten by their companions are submitted to necropsy and cultures are taken from the heart and spleen. We may, therefore, in any bacterial infection such as mouse typhoid or mouse pasteurellosis have one of the following combinations of findings :—

No characteristic lesions	.. ..	Cultures negative.
No characteristic lesions	.. ..	Cultures positive.
Characteristic lesions	.. ..	Cultures positive.
Characteristic lesions	.. ..	Cultures negative.

The first combination presents no difficulty. The mice are entered in our records as dying from some other cause—as " non-specific " deaths. The last combination is included in the specific deaths but occurs so rarely that it is of no importance. In those very rare instances in which a mouse has died with characteristic lesions without the organism being recovered it has been because our cultures have been overgrown with *Proteus*, or some other spreading organism. The second combination is, however, relatively frequent. The more acute deaths, both in mouse typhoid and mouse pasteurellosis, are usually due to a bacteraemic infection associated with no obvious naked eye lesions, though there is often a little sticky fluid in the pleural or peritoneal cavity, in films from which the causative organism may be detected. In these cases we must clearly rely on the cultural findings; and all mice from which the causative organism is recovered at necropsy, i.e. all mice falling into categories two and three above, are entered in our records as " specific " deaths.

We must, of course, adhere to one method of classification throughout; but in so doing we might clearly be obscuring the true facts of the case. As will be seen in later sections of this report, mice that have survived for a considerable period in our herds, or that have been immunized before entry to them, are, when their deaths are classified on the basis of cultures alone, more resistant than new susceptible entrants. But we know quite well that resistant mice frequently develop latent infections, and they may harbour the causative organism in their tissues, particularly in their spleens, over long periods of time. Such mice, if dying from some other cause, would yield positive cultures at necropsy, and would be entered incorrectly as specific deaths. This might lead to a serious understatement of the real level of resistance of the old residents in our herds, or of the vaccinated mice. That it has in fact led to *some* understatement is, we think, quite certain; but we believe that this understatement has been trivial, for the following reasons. If any large proportion of the mice dying at later cage ages belonged to this " carrier " class, and had in fact succumbed to some unrelated disease, or to some accidental cause of death, the characteristic lesions of the specific disease should show a diminishing frequency with increasing cage age, once the initial period of acute bacteraemic infection had been passed. This is not the case.

As an illustration we may summarize the post-mortem findings in 2,045 necropsies carried out on mice dying during a long-continued epidemic of mouse typhoid. The lesions actually noted in our records are exudate in the serous cavities, any enlargement of the spleen, and necrotic areas in the liver. In the spleen, the degree of enlargement (+, ++ or +++) is noted, and also the presence of small necrotic foci. Of these lesions, gross splenic enlargement and necrotic foci in the liver are the most trustworthy evidence of active salmonella infection of the subacute type. Any mouse

presenting these signs at necropsy may, we believe, safely be regarded as having died from mouse typhoid.

The findings in these 2,045 necropsies are summarized in Table I.

TABLE I

Showing the frequency of various post-mortem findings, omitting minor enlargement of the spleen, in mice dying at different cage ages during a long-continued epidemic of mouse typhoid.

Cage age at death (days)	1–5	6–10	11–15	16–20	21–25	26–30	31–40	40+
Total necropsies ..	34	113	214	276	385	391	259	373
Bact. aertrycke isolated (per cent.).	23·5	73·5	86·9	87·7	94·3	95·1	95·4	83·9
Specific deaths ..	8	83	186	242	363	372	247	313
No lesions (per cent. specific deaths).	—	49·4	29·5	35·1	31·3	29·6	16·6	17·9
Spleen ++ (per cent. specific deaths).	—	1·2	5·9	4·5	3·3	5·4	8·9	8·3
Liver necrosis (per cent. specific deaths).	—	1·4	11·8	12·4	8·8	13·4	10·5	18·8

The significance of these figures seems quite clear. A large proportion of the few deaths that occur during the first five days of herd life are, as we should expect, non-specific. After about the 10th day some 90 per cent. of the deaths are due to the epidemic disease. The proportion of acute bacteraemic deaths, with no obvious lesions, is high during the first ten days of cage life; about a third of the specific deaths that occur between the 10th and 30th days of cage life are of this type, after which there is a considerable decline.

As regards the grosser lesions, these are rare in mice that die within ten days after entry to the herd, but then rise to a higher, though still relatively low, level of frequency, about which they fluctuate throughout all later cage ages. There is no tendency at all for this frequency to fall in mice that die after surviving for 40 days or more. In liver necrosis there is, indeed, a distinct rise. Were the proportion of non-specific deaths—non-specific in the sense that, though *Bact. aertrycke* was isolated, death was in reality due to some other cause—higher in mice dying at cage ages of 40 days or over than in mice dying at earlier periods, one would expect to find a lower proportion of obvious lesions. There is, of course, still the possibility that a proportion of those mice that die at later periods of cage life, and yield *Bact. aertrycke* from their tissues while showing no gross lesions, have in fact died from some other cause. It may be that, among the true specific deaths at advancing cage ages, the frequency of gross lesions rises to a much higher level than that obtaining between the 10th and 40th days of cage life, and that the absence of this rise in our records is due to a dilution of our total specific deaths by deaths that are falsely placed in this category on the basis of the bacteriological findings. It is,

however, quite safe to assert that certainly a quarter, and probably three-quarters or more of the mice dying late in herd life are dying of the specific disease. If, on the basis of our records, we are understating the resistance of such mice, or the resistance of mice vaccinated before entry, we are not understating it grossly.

The problem of post-mortem diagnosis of the cause of death in mouse pasteurellosis differs in no significant way from the problem in mouse typhoid. In the case of the virus disease, ectromelia, it differs substantially, because the method of cultivation of the causative organism is not open to us. It will, however, be more convenient to discuss this question in the section in which the general behaviour of epidemics of ectromelia is described in detail.

There remain to be considered those mice in which no necropsy is possible, the victims of cannibalism. They form an appreciable proportion of the total deaths, and the extent of cannibalism may vary rather widely from one secular interval to another. Table II shows the relevant figures for one epidemic of pasteurellosis and one of mouse typhoid. It will be noted that the degree of cannibalism does not vary in any consistent way from one cage age to another—secular variations are, of course, averaged out. The proportion of specific deaths does not decline with increasing cage age in the pasteurellosis epidemic. In this particular mouse typhoid epidemic the proportion of specific deaths at different cage ages varies rather widely, but there is no consistent fall with increasing cage age.

Taking Tables I and II together, it is clear that the majority of the mice that come to necropsy after the first ten days of herd life show evidence of having died from the prevailing disease; we have therefore, since we must of necessity adopt some arbitrary rule, included the eaten mice among our specific deaths. It is, of course, probable that the proportion of the eaten mice that actually die from the specific disease is low during the first few days of cage life, high between days 10–40, and, perhaps, lower again as cage age advances; but clearly the only method of weighting our records in accord with such assumptions would be to apply to the eaten mice dying at each cage age a weight calculated from the proportion of specific deaths found among the mice dying at that cage age and submitted to necropsy. We are not sufficiently convinced of the reliability of our diagnosis at later cage ages to believe that this method would give us an accurate picture of what was really happening, and the modification that would result would be so slight that there has seemed no point in undertaking the laborious calculations involved.

In regard to the diet on which our mice have been fed, they have, during the whole of the period covered in this report, received a liberal supply of whole oats, and been provided with drinking vessels of the inverted test tube type containing a mixture of equal parts of water and pasteurized milk. These tubes, with their contents, are steamed at 100° C. for ten minutes before being placed in the cages.

TABLE II

Showing the percentage of mice (a) *examined* post mortem *and showing evidence of the specific disease,* (b) *examined* post mortem *and yielding no evidence of the specific disease, and* (c) *not examined* post mortem *because of cannibalism, among mice dying at different cage ages during one epidemic of pasteurellosis and one of mouse typhoid.*

	Pasteurellosis.				*Mouse typhoid.*			
Cage age in days.	*No. of mice.*	*Per cent. specific.*	*Per cent. nil found.*	*Per cent. not examined.*	*No. of mice.*	*Per cent. specific.*	*Per cent. nil found.*	*Per cent. not examined.*
0– 9	141	52·5	19·1	28·4	56	48·2	21·4	30·4
10– 19	299	75·3	7·0	17·7	305	56·7	11·5	31·8
20– 29	264	77·7	4·9	17·4	513	66·7	5·1	28·3
30– 39	102	77·5	4·9	17·6	166	71·1	4·2	24·7
40– 49	64	67·2	10·9	21·9	54	66·7	16·7	16·7
50– 59	27	77·8	7·4	14·8	20	75·0	10·0	15·0
60– 79	39	64·1	12·8	23·1	21	61·9	14·3	23·8
80– 99	31	74·2	6·5	19·4	6	66·7	33·3	0·0
100–149	43	74·4	4·7	20·9	19	52·6	15·8	31·6
150–199	28	75·0	3·6	21·4	11	45·5	27·3	27·3
200–249	9	77·8	0·0	22·2	4	75·0	25·0	0·0

Very recently we have replaced the whole oats in the diet by a mixture of oatmeal, cod liver oil and yeastrel, with a little bran to add bulk. We have made this change because we have found the new diet more satisfactory for our breeding mice and normal stock, and we did not wish entry to our herds to be accompanied by a change in diet. So far as our experience has gone the mice fed on the new diet behave in the same way as those fed on the old.

THE STATISTICAL RECORDS

The nature of the statistical data collected, and the methods employed in analysing them, will be considered in detail in further sections of this report. It may, however, be well to set out briefly the general form of the records that we have most commonly employed, and the purposes for which we have used them.

For each long-continued epidemic in which an infected herd has been recruited over a period of months or years by the daily addition of one or more normal mice, or by the addition of larger batches at regular intervals, we have constructed secular graphs showing for each day of the experiment the total number of animals in the herd and the mortality prevailing in the herd on that day. In measuring the mortality experienced we have used the q_x of the life table, i.e. the probability of dying on that day, or the ratio of deaths during the day to the number alive at the beginning of the day, and not the ordinary death rate of most medical publications, i.e. the ratio of deaths during the day to the average of the populations alive at the beginning and end of the day. There is, in fact, little difference in

the numerical value yielded by the two methods of calculation, but we require q_x values in our life-table constants, and it is simpler to work with the same notation throughout. To reduce large random fluctuations due to the deaths of an unusually large, or unusually small, number of mice within any one 24-hour period, we have used a smoothed q_x. The mortality recorded on any day is the ratio formed by dividing the sum of the deaths for the five days of which that day is central by the sum of the populations at the beginning of each of those five days. Secular graphs of this type will be found facing p. 30. Since their presentation requires the insertion of folding charts, we have not produced in full secular graphs of experiments that have been recorded elsewhere, but they will be found on reference to the original papers.

These graphs tell us what is actually happening in the herd on each day of the whole experimental period. They enable us, for instance, to note the occurrence of successive waves of mortality, the length of such waves, their periodicity, the occurrence of longer or shorter periods of unusually high or unusually low mortality, and so on. If we desire to know what was happening in one herd at any particular time it is to these graphs that we turn. The possible occurrence of seasonal fluctuations in mortality, possible relationships between the rise or fall of an epidemic wave and the composition of the herd in regard to mice of varying cage age at the moment when the rise or fall occurs, indeed, the relationship of any change in mortality to secular events in the herd or its environment, are questions that must be solved by reference to graphs or records of this kind.

There is, however, another kind of problem, in which these graphs do not give us the information that we require. If we wish to know, not what was happening in our herd at any particular time, or in relation to any secular event, but how, on the average, our mice were behaving on any particular day of cage life—on entry to the herd, or on the 10th or 40th day of herd life, and so on—then we need data of quite another kind.

For these purposes the life table gives us exactly the kind of information that we require. It gives a picture from month to month, or year to year, of, say, 10,000 persons, supposed all to be born on the same day, to be observed to the end of their lives, and to suffer at each age the same rates of mortality as those found in the actual population under study. The constants usually given in a life table are (1) the number, l_x, who survive to each given age x; (2) the number, d_x, who die between ages x and $x+1$, where 1 is the arbitrary time interval between successive entries; (3) the probability, q_x, at age x, of dying between ages x and $x+1$; thus $q_x = \frac{d_x}{l_x}$; (4) the expectation of life, $\dot{e}_x$, at age x, which is the average length of life lived after age x of all those who survive to age x.

For our purposes we desire to know, not what happens when a mouse is x days old, but when it has lived x days in the herd. So for

us day 0 is not the day of birth, but the day of entry to our cage. The time interval x is one day. The other constants are calculated on this basis, but we have frequently used two modified constants: ${}_5q_x$, the probability of dying during the five consecutive days beginning with day x, and ${}_{60}E_x$, the expectation of life, or average length of life, following day x limited to 60 days, all mice actually surviving this period being credited with 60 days' life from cage age x.* We have adopted the ${}_{60}E_x$ constant because (*a*) the numbers of mice living to late cage ages are relatively small, and the influence on the unlimited expectation of life at these late ages of a few mice living, say, 200–300 days after day x would be disproportionately large, and (*b*) because the actual unlimited expectation of life, $\mathring{e}_x$, can only be calculated by observing each mouse until it dies in herd, and this means that no mouse alive at the end of the period of observation can yield its proper quota. Even with an e_x value limited to 60 days some proportion of mice must fail us in this respect, for once we have reached the last 60 days of the experiment no mouse, either new entrant or of a higher cage age, has the opportunity of surviving the full 60 days beyond entry or the cage age already attained.

A life table so constructed tends to smooth out all secular variations in the herd or in its environment. It may fail to smooth them out entirely over any relatively short period of observation, for if an unusually high or unusually low mortality was experienced towards the end of that period, it might appreciably affect the total mortality experience of the relatively fewer mice of higher cage ages, while to the total mortality of the more numerous entrants it would contribute proportionately less. When, however, the period of observation lasts over a year or more, and immigrants enter the herd daily, so that the l_x, d_x, q_x and $\mathring{e}_x$ values are the average of well over 300 entries, sampling the herd conditions over the whole experimental period, the smoothing may, in most cases, be regarded as sufficiently complete for our purpose. The fluctuations observable in our life-tables cannot therefore be due to variations in temperature, or in season, or in the risk of infection, or in the exact composition of the herd at any moment. They represent changes in behaviour that depend solely on the length of time a mouse has survived in the herd under the *average* conditions obtaining throughout that particular experiment.

The Mortality of Uninfected Mice under Conditions Similar to those obtaining in the infected Herds

As a standard of reference for many of our experiments it is necessary to have some measure of the effects of mortality that we should expect to observe in mice housed under the same conditions

* The orthodox symbol would be :—$l_{60}\mathring{e}_x$ and ${}_{60}E_x$ is used in actuarial works for the value of an endowment on (x) payable if he survive 60 time units. The orthodox symbol is troublesome to print and, as there is no risk of confusion, we have felt justified in using another letter.

as our infected herds, fed on the same diet, and subject to the same daily immigration. A "blank" experiment of this kind has been carried out (Greenwood, Topley and Wilson, 1931, b).

On 4.x.29, 20 normal mice were placed in one of our experimental cages; from 5.x.29 to 7.x.29 one normal mouse was added daily, and from 8.x.29 to 17.i.30 three normal mice were added daily. This herd was then kept under observation until 27.v.30. In all 329 mice were observed, of which 56 died. In 41 of these mice a necropsy was performed—the remaining 15 were wholly or partially eaten by their companions—and in no instance was any evidence of infective disease discovered. The ${}_{60}E_x$ figures were calculated for each of the first 120 days of cage life, and their values, at ten-day intervals, are given in Table III.

TABLE III

Giving the ${}_{60}E_x$ values for 329 normal uninfected mice living under the same conditions as our infected herds.

Cage age in days.	${}_{60}E_x$
0	56·8
10	57·1
20	56·8
30	56·7
40	58·3
50	58·4
60	58·5
70	58·6
80	58·8
90	58·5
100	59·1
110	59·0
120	59·2

It will be noted that there is a slight rise in the ${}_{60}E_x$ values at about the 40th day of herd life, after which the figures vary so slightly that they may be regarded as constant. This rise may almost certainly be ascribed to an adaptation to the conditions of herd life. Many, perhaps most, of the deaths in a herd of normal mice are due to fighting, and it would seem that an immigrant requires a few weeks to obtain recognition as a member of the herd to whom average toleration must be extended.

There is, it will be noted, no tendency for the limited expectation of life to decrease within the period covered by this experiment, nor would such a decrease be expected. Such data as we possess suggest that mice, on entry to our herds, vary in age from about 70 to 120 days. On the 120th day of cage life few of them will be more than 240 days old. The 60 days allowed in our limited expectation of life will take such mice to their 300th day of life. Few figures are available with regard to longevity in mice; but from such data as we have been able to find (*see* Greenwood, 1928) it would appear that a normal mouse will live for two years or more, so that our ${}_{60}E_x$ limit will not carry any of our mice into the range of senility.

A word may perhaps be inserted here with regard to our herds as social units, since they diverge widely from any herds observed or maintained under natural conditions. In experiments that last eighteen months or more some mice probably die of old age, but the numbers of such mice must be relatively very few. There are births in the community, but they contribute nothing to its numbers. The mice born are invariably eaten at or soon after birth, and litters are rarely seen. Some few females probably die from the risks associated with pregnancy and parturition. An appreciable, but not a large, number of deaths is due to fighting. The herd relies for its recruitment entirely on immigrants, males and females, all in early adult life, entering the herd at regular, usually at daily, intervals.

Of the mortality within such a herd, in the absence of any contagious disease, the figures set out in Table III give, we believe, a reasonably accurate picture. We should accept a ${}_{60}E_x$ value of about 58 days as representing the limited expectation of life of a mouse exposed to no risk of infection, or so resistant as to be indifferent to the risk to which it was exposed; and we shall take this figure as a standard for comparison when considering the behaviour of the mice in our infected herds.

The Method of the "Closed Epidemic".

When studying the effect of some particular factor on the mortality within an infected herd, it is often convenient to employ a method that differs from that which we have described above.

An appropriate number of mice is taken and injected intraperitoneally with a constant dose (usually 1,000 bacteria) of a suspension of the organism under study. These mice are divided into batches of 25, and to each batch are added 100 normal mice, making a herd of 125 mice, of which 25 are infected and potentially infective and 100 are exposed to risk. One, or sometimes more, of these herds are kept as controls. The method of interference under study—a special diet, or the artificial immunization of the mice at risk, or the administration of a bacteriophage, and so on—is applied to one or more of the remaining herds. All herds are then observed for 60 days, and the deaths among the 100 mice exposed to risk are recorded. We can compare the behaviour of the exposed mice in the test and control herds by noting the difference in the number of survivors alive on the 60th day, or by a comparison of the ${}_{60}E_x$ value calculated from day 0, the day on which the normal mice were brought into contact with their infected companions; in the majority of our experiments we have used the latter method.

In experiments of this type the 100 mice at risk in each group are exposed, at the same moment, to contact with the same number of mice, that have each been injected with the same number of bacteria, and it would be reasonable to assume that, *provided the mice themselves are random samples and all other factors influencing the spread of infection are distributed between the groups in a purely*

random fashion, the mice in each group will behave in the same way, within the limits covered by random sampling errors. But the condition of randomness may not be fulfilled, and it can certainly not be assumed.

In determining the most suitable ratio of infecting to exposed mice (Topley, 1926, b) this aspect of the problem was submitted to experimental trial. A series of five herds was set up, in each of which 100 mice were exposed to risk, and in each of which the same suspension of *Bact. aertrycke* was employed for infection, but in which the number of infected mice varied from 100 to 5. The ${}_{60}E_x$ values and their standard errors were as follows :—

No. infecting mice.	*No. exposed mice.*	${}_{60}E_x$ *exposed mice.*	*S.E.**
100	100	34·1	1·96
50	100	34·3	1·96
20	100	35·4	2·09
10	100	38·1	1·84
5	100	45·7	1·73

There is no appreciable difference between the ${}_{60}E_x$ values in the groups with 100 and 50 infecting mice respectively, and no significant difference between either of these groups and the group with 20 infecting mice. With a decrease in the number of infecting mice to 10 and 5 there is, however, a rise in the ${}_{60}E_x$ value, so that the average duration of life was distinctly longer in the cage with five infected animals and somewhat longer in the cage with 10 mice. These differences raised the question as to whether the variation between sets

* It should be noted that " standard errors " attached to such measures as ${}_{60}E_x$ ought to be treated with circumspection. It is a matter of statistical routine to compute the mean and standard deviation of a " population " of values and to use these " constants " in determining the standard error attaching to a sample. But obviously the " population " frequency of, say, ${}_{60}E_x$ is very skew ; its upper limit is by definition 60 days. Therefore one of the fundamental assumptions of the routine treatment is not fulfilled. It is of course well-known that the means of large samples drawn from a skew population usually approximate to a normal distribution. But if and when a critical conclusion turned upon some individual difference of sample means, a more refined test than a mere comparison of standard errors would be necessary. We do not think that in a general analysis such as this it is necessary to interpolate particular discussions.

In the actual calculation of these standard errors, and of those applicable to the values obtained from long continued epidemics, the following method was adopted. The mean and standard deviation of length of life limited to 60 days were calculated for all mice in the cage, whatever secular point of time they entered the cage. The standard error of ${}_{60}E_x$ for any cage age x was taken to be this standard deviation divided by the square root of the number of mice alive at cage age x. Where the death-rates from specific causes only were used in the construction of the life tables, the mean length of life limited to 60 days for all mice dying of specific causes was similarly found. The standard deviation of these values was not calculated directly but indirectly on the assumption that the coefficient of variation was the same for all causes and specific causes. To reach the standard error this standard deviation was divided by the square root of the number of mice alive at cage age x.

of identical trials would be greater when the ratio of infecting to susceptible mice was decreased to a point at which the reduction began to affect the average survival time of the exposed mice than when it was maintained at a level at which further increases had no appreciable effect.

To test this point two further series, each of five identical groups, were set up. In each group of the first series 5 infected mice were added to 100 normal mice. In each group of the second series 25 infected mice were added to 100 normal mice. The same bacterial suspension was used to infect the 5 mice in each group of the first series. Another strain of *Bact. aertrycke* was used to infect the 25 mice in each group of the second series. Neither was the same strain as that used in the series referred to above. It is not, however, any possible difference between the series that concerns us here. Our interest lies in the consistency or inconsistency of the groups within any one series.

The $_{60}E_x$ figures for these two series of tests were as follows :—

Series.	*Group.*	*No. infecting mice.*	*No. exposed. mice.*	$_{\infty}E_x$ *(exposed).*	*S.E.*
A	1	5	100	29·67	1·85
	2	5	100	32·24	1·74
	3	5	100	20·04	1·32
	4	5	100	30·62	1·78
	5	5	100	37·18	1·84
B	1	25	100	40·74	1·94
	2	25	100	44·62	1·94
	3	25	100	46·41	1·78
	4	25	100	41·63	1·82
	5	25	100	38·64	1·94

It is clear at a glance that the $_{60}E_x$ figures for the five groups in series A differ more widely than those for the five groups in series B. In each series 10 pairs of values are available for comparison. If the differences between these pairs of $_{60}E_x$ values are compared with their standard errors in each of the two series, we get the following results.

Ratio Difference/S.E. of Diff.	*Number of pairs*		
	Series A (5 infecting mice)	*Series B (25 infecting mice)*	*On basis of normal curve*
0–1	2	3	6·83
1–2	1	4	2·72
2–3	3	3	0·43
3–4	0	0	0·03
Over 4	4	0	0·00

Clearly our groups are not random samples in either series, the distribution of ratios bears no resemblance to that expected on the basis of the normal curve of error ; but the results with 25 infecting mice were more consistent than those with 5 infecting mice ; in the former no observed difference was three times its

standard error and the average difference between the ten pairs of values was 3·88 days; in the latter four of ten differences were more than four times the standard error and the average difference between the ten pairs was 7·37 days. We must not, however, lose sight of the fact that the groups are small and that when differences are compared the influence of the very divergent A.3 is considerable; we have, however, no biological ground for excluding it.

In connection with a series of experiments recorded on pp. 148–52 an opportunity was taken to test again the consistency of results reached by this method of procedure. Four series, each consisting of five identical tests were set up, with 25 infected and 100 exposed mice in each test. The strain of *Bact. aertrycke* used differed from one series to another, but here again we are concerned, not with differences between the series but with consistency or inconsistency of the behaviour of the five groups of which each series is composed. The results are set out in Table IV.

TABLE IV

Showing the $_{\infty}E_x$ *values obtained in five different groups of* 100 *mice exposed to the same risk of infection.*

Four series of tests

Series.	*Group.*	$_{\infty}E_x$	*S.E.*
A	1	30·07	1·72
	2	34·83	1·81
	3	29·63	1·65
	4	28·39	1·73
	5	33·42	1·99
	Mean 1–5	31·27	1·08
B	1	36·67	1·68
	2	40·01	1·66
	3	39·52	1·62
	4	31·75	1·57
	5	38·20	1·59
	Mean 1–5	37·23	1·33
C	1	53·52	1·57
	2	52·85	1·54
	3	53·09	1·48
	4	51·50	1·57
	5	52·12	1·57
	Mean 1–5	52·62	0·33
D	1	45·72	2·11
	2	53·87	1·53
	3	55·01	1·65
	4	52·33	1·68
	5	54·14	1·44
	Mean 1–5	52·21	1·50

These four series, each consisting of five identical tests, yield between them 40 duplicate pairs of $_{\infty}E_x$ values in which a difference can be compared with its standard error. If we do this we get the following results.

Ratio Difference/S.E.	*No of pairs*	
	Series A—D	*On basis of normal curve*
0–1	23	27·30
1–2	7	10·88
2–3	5	1·71
3–4	5	0·11

Here, again, it is clear that we are not dealing with random samples. If we compare these figures with those of series B above, in which the same number of infecting and exposed mice were employed, we see that approximately the same proportion of duplicate pairs gives differences that are less than twice their standard errors. But, whereas in the small series of tests in series B the remaining pairs gave difference/S.E. ratios of 2–3, in the larger series there is an appreciable number of ratios of 3–4.

Taking this experience as a whole, we should incline to the view that, when using this method, any difference between a single test and a single control should be regarded with extreme suspicion unless the difference is five to six times its standard error, which means that we cannot, in such tests, detect with any certainty a difference that, if significant in the statistical sense, might well be important in its epidemiological implications. The best method of avoiding this difficulty would appear to be by setting up all such tests in duplicate or triplicate, using as our measure of significance the standard error calculated from the separate mean ${}_{60}E_x$ values whenever this exceeds the standard error calculated from the grouped 200–300 values given by the individual mice. This, for instance, is the method we have followed in the experiments recorded in Section VI and Section VIII of this report.

Such a procedure, unfortunately, demands a large expenditure of mice—500–750 mice to compare one bacterial strain with another, or the influence of one diet with that of another—but anything, in our view, is to be preferred to basing conclusions on data that will not bear their weight, and so adding further confusion to problems that are already obscured by a mass of ill-founded hypotheses.

In some of the experiments recorded in later sections of this report it will be noted that a method has been followed very similar to, but not identical with, that of the closed epidemic as described above. In such instances any modification that has, for one reason or another, been introduced has probably had a tendency to reduce the sampling errors discussed in this section, as, for example, when exposure to infection has been allowed to occur in one large herd, and the exposed mice have subsequently been divided into separate groups that have been treated differently. In such cases we have no empirical measure of the actual sampling errors involved, but we have tried, when comparing the proportions of deaths or survivals, or the ${}_{60}E_x$ values, to be conservative in our estimate of the significance that attaches to our findings.

With these preliminary remarks on the general methods we have employed, and some of the difficulties we have encountered, we may pass to a consideration of the results that we have so far obtained.

Many of the experiments dealt with in this report have already been recorded in detail elsewhere, and these we have merely summarized. The observations on ectromelia (Section IV), on prophylactic immunization against this infection (Section V), on the infectivity of immunized and infected animals (Section VII), and on the varying epidemicity of different strains of *Past. muriseptica* (Section VI) have not hitherto been published.

SECTION II

THE COURSE OF EVENTS IN AN INFECTED HERD RECRUITED BY CONTINUOUS IMMIGRATION

It would clearly be useless to attempt to determine the effect of any particular method of interference in the course of events in an infected herd subject to continuous immigration, without an adequate knowledge of how such a herd behaves when left to its own devices. We have, therefore, spent much time and labour in studying the course of events, over periods varying from many months to several years, in herds infected with mouse typhoid, mouse pasteurellosis or infectious ectromelia, and subject to the immigration, at a rate that has been constant for any one experiment but has varied from one experiment to another, of normal, uninfected mice.

The ectromelia experiments, which have not been recorded elsewhere, are described in some detail in Section IV of this report. The mouse typhoid and mouse pasteurellosis experiments have all been described in previous papers (Greenwood and Topley, 1925; Greenwood, Newbold, Topley and Wilson, 1926, 1928, 1930; Newbold, 1927; Greenwood, Topley and Wilson, 1931, a, c; Hill, 1933) and it will be convenient in this section to summarize these experiments and the conclusions that we should draw from them, discussing in Section IV any modifications suggested by our experience with the virus disease.

The Secular Course of Events in an Infected Herd

We may, perhaps, most conveniently start our description of the secular course of events in an infected herd by eliminating one particular working hypothesis. Assuming, as we are certainly justified in doing, that mice vary innately in their resistance to any given bacterial infection, and that some at least of the immigrants to our cages will become actively immunized as the result of sub-lethal or latent infections, it is clearly possible that the continuous elimination by death of the more susceptible of the immigrants might result in the selection of a residue so resistant as to be indifferent to all further attacks of the parasite. With continuous immigration, even of small numbers, there will always be some susceptibles at risk; but the ratio of susceptibles to resistants might well fall to a low level, and such an event might be marked by a progressive fall in the specific mortality, by a longer or shorter intermission in the epidemic prevalence, or even by a complete cessation of the epidemic as the susceptibles became more and more diluted with resistants.

One of our infected herds was under continuous observation for a period of seven years and eight months, several for two years or more. In different herds we have varied the rate of immigration from six mice a day to one mouse every third day. In some cases

we have replaced the daily immigrants with larger batches added to a herd at intervals of 4–6 weeks. In no instance has an epidemic prevalence come to an end, and in no instance has the mortality from the specific disease fallen to negligible proportions, or, in the long run, undergone any great diminution. Under the conditions that obtain in our experimental herds, the immigration of normal uninfected mice, even in small numbers or at considerable intervals of time, will suffice to maintain indefinitely any infective disease that has once gained access to the herd.

To this statement we would add one proviso: a particular epidemic disease may, under these conditions, be completely replaced by another with equal or greater powers of spread. This has happened once only in our experience—in the long-continued epidemic, lasting in all over seven years and eight months. This, at its inception, was an epidemic of mouse pasteurellosis. Within a year sporadic deaths from mouse typhoid began to occur. These grew more frequent and then died away. Later, towards the end of the third year of observation they increased again, probably as the result of the introduction of a few infected immigrants—our quarantine was not then as efficient as it has since become. Coincidently with this increase the frequency of deaths from pasteurellosis declined, and within a few months ceased to occur. For the last four years of observation this herd suffered only from mouse typhoid. The lesions of pasteurellosis were never observed at necropsy, nor was *Past. muriseptica* ever isolated.

Although this is the only example that we have observed of the complete elimination of one infection through replacement by another, we have no doubt that it would be of frequent occurrence if events were allowed to take their course after the purposeful or accidental introduction of an alien infection to a herd already suffering from another disease of lower epidemic potency. Webster (1930, c, d) records an experience very similar to that which we have outlined above; and in a few instances in which ectromelia has gained access to one of our herds that has been suffering from a bacterial infection, the happenings during the few succeeding weeks have left little doubt that the bacterial disease would, sooner or later, have been wiped out by the virus infection. There is, of course, nothing surprising in this. The persistence of a particular disease in a herd depends, as will be seen, on the survival through long periods of mice suffering from latent infections. There is no valid reason to suppose that these infected survivors are more resistant to a new type of infection than are recent immigrants to the herd. A high mortality resulting from the new disease will eliminate these old inhabitants within a certain period, longer or shorter as the case may be, and with them the only existing foci of the old infection, since, as has been noted, there is, in our experiments, no opportunity for a persistent environmental infection —the mice are transferred each day to cages that have been sterilized and cleaned.

This aspect of epidemiology, the replacement of one infection by another, or the possible stimulation of one by another, clearly invites more detailed study by these or similar methods. But, for the solution of the preliminary problems with which we have been concerned, mixed infections of this type are quite unsuitable, and, with the exception of the long-continued epidemic mentioned above and a few of our earlier epidemics that were continued for a few months after the entry of an alien infection, it has been our practice to sacrifice an infected herd on the first sign of the entry of any adventitious disease. The records that follow, unless the reverse is specificially stated, may be taken as referring to herds infected with a single epidemic disease.

Secular Fluctuations in Mortality

We may consider first the events in herds that are receiving a steady influx of immigrants in numbers that ensure that recent entrants shall form an appreciable proportion of the total mice at risk. We may take as an example an epidemic of mouse pasteurellosis that was initiated on 14.ii.24. To the original herd of 20 infected mice, 6 normal mice were added daily. The population rose more or less steadily until the end of June, when it fluctuated within the limits of 212 and 226. At this period, therefore, between one-fifth and one-sixth of the total population at risk were being renewed each week by new immigrants. Even under these conditions the rate of mortality was not steady, the smoothed q_x curve showed a succession of waves. But the fluctuations were small, and became progressively smaller, and it appeared that we were approaching the condition of a population receiving immigrants at a constant rate, suffering from a constant rate of mortality, and hence stabilizing itself at a constant total number. Fig. 1 shows in graphic form the events in this herd from 14.ii.24–14.vii.24. Unfortunately, *Bact. aertrycke* gained entry to our herd soon after this date and the experiment had to be abandoned.

As a contrast to this we may take the two herds in which the rate of addition has been lowest. Both were infected with mouse pasteurellosis, both were started on the same date (14.ii.24) as the experiment described above, and both with 20 infected mice.

To one of these herds one mouse was added every third day. The total population of this herd never rose above its original 20 mice and seldom sank much below it. Susceptible immigrants were accumulating very slowly. The history of this herd (Fig. 2) began with three moderate waves of mortality as measured by the smoothed q_x curve—the actual range of the fluctuations, depending in this case on a very small number of deaths, had of course little significance—there followed a quiet period of 70–80 days, then a sharp wave of specific mortality in July, then quiescence again, followed by the occurrence of a death from mouse typhoid.

To the second herd (Fig. 3) one mouse was added every other day. At its maximum the population reached 40, but for the rest it usually

fluctuated between 20 and 30. There were at first four small waves of specific mortality; then a quiet interval of 59 days; then two well-defined waves; then irregular fluctuations till the end of September.

This last herd suffered from no overt mouse typhoid infection, but it had shared in the latter period of observation in immigrants from a suspect stock, and the experiment was therefore discontinued.

With this low level of immigration there were, it will be noted, quite definite intermissions, lasting over periods of 8–12 weeks; but in each case the specific infection broke out again after a quiescent period. The course of events in these two herds over the period 14.ii.24–1.viii.24 is shown in Figs. 2 and 3.

With intermediate rates of immigration (one mouse a day, two mice a day, or three mice a day) the fluctuations of the q_x curve were of a type intermediate between that displayed by the six-mice-a-day herd on the one hand and the one-mouse-every-third-day herd on the other. There were no real intermissions, but there was a succession of well marked waves of mortality, sometimes well separated, sometimes tending to fuse for a period into a fluctuating mortality at a high or a low level. An illustrative graph is given in Fig. 4.

This irregular alternation of periods of higher or lower mortality, each marked by a succession of waves or fluctuations, has convinced us that, in judging the effect of any intentional interference with the course of such an epidemic, any effect that appears to have been produced must, before significance is attached to it, be shown to persist over a considerable period of time—several months at least—and be reproduced in several trials of an identical kind. Any temporary change in mortality following a single intentional change in the condition of a herd may well be due to chance, even though the change, in terms of the mortality rates prevailing before and after the intentional interference, is quite unmistakable.

Before passing to a discussion of our life-table data, which raise many problems concerning the mechanisms by which the secular behaviour of an infected herd is determined, we may note three other points that arise directly from a consideration of the secular graphs.

If we compare the daily rate of immigration, the average daily specific death rate, and the average daily total population for a number of infected herds, each observed over a period of many months or several years, we obtain the figures in Table V.

It will be seen, so far as can be judged from this small series of experiments, that the average rate of mortality does not rise or fall with a change in the number of immigrants. The figures for Experiment P 0·3, and to a less extent those for P 0·5, are based on a relatively small number of mice observed over a relatively short period of intermittent mortality, and are therefore more subject to sampling errors than those for the remaining experiments. The figures for mouse typhoid afford a more reliable guide

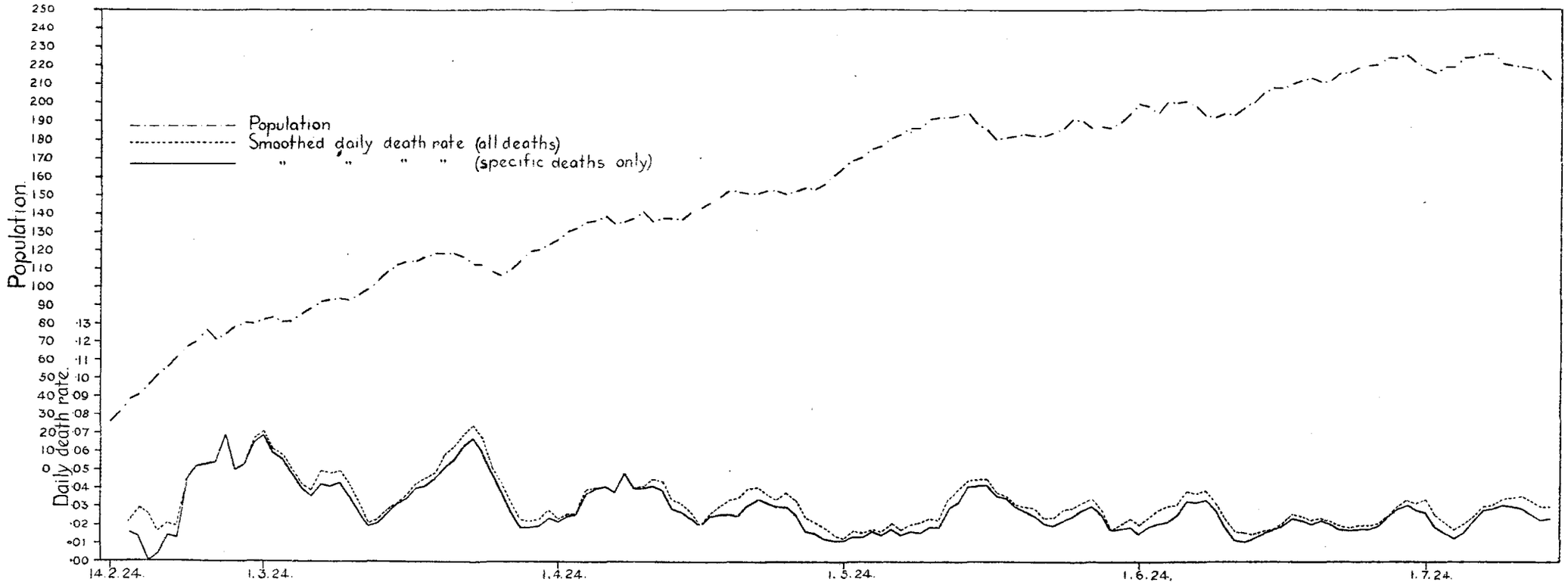


FIG. 1.—Secular death-rate and population in the first five months of an epidemic of mouse pasteurellosis. Six mice added daily. (Exp. 3, Greenwood & Topley, 1925.)

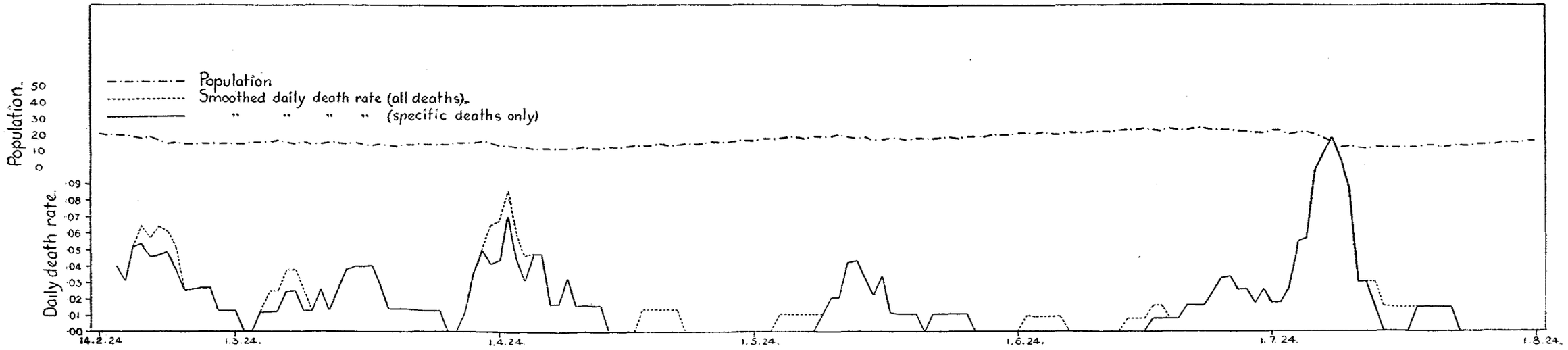


FIG. 2.—Secular death-rate and population in the first five months of an epidemic of mouse pasteurellosis. One mouse added every third day. (Exp. 6, Greenwood & Topley, 1925.)

[*To face p. 30.*]

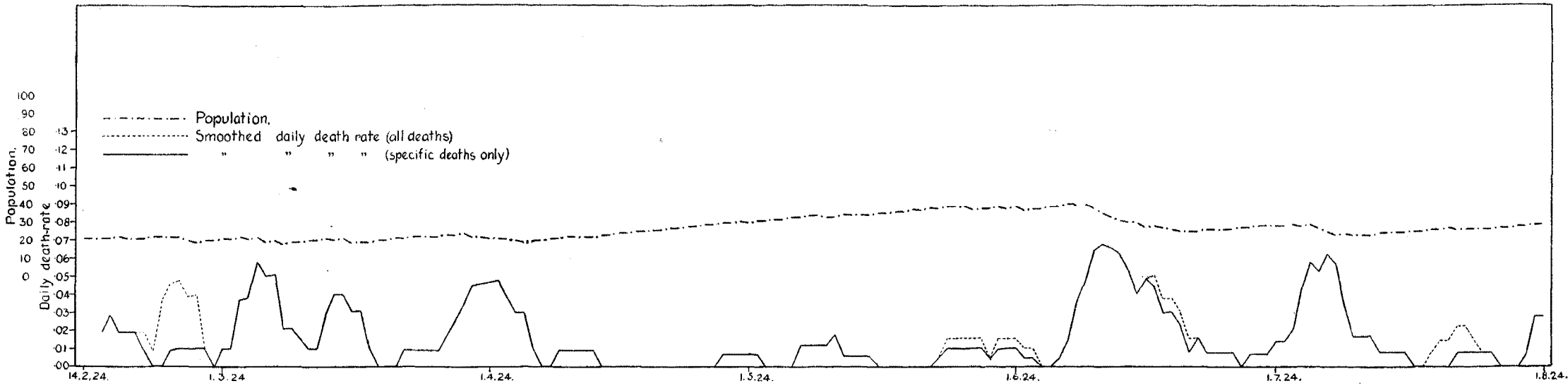


Fig. 3.—Secular death-rate and population in the first five months of an epidemic of mouse pasteurellosis. One mouse added every other day. (Exp. 5, Greenwood & Topley, 1925.)

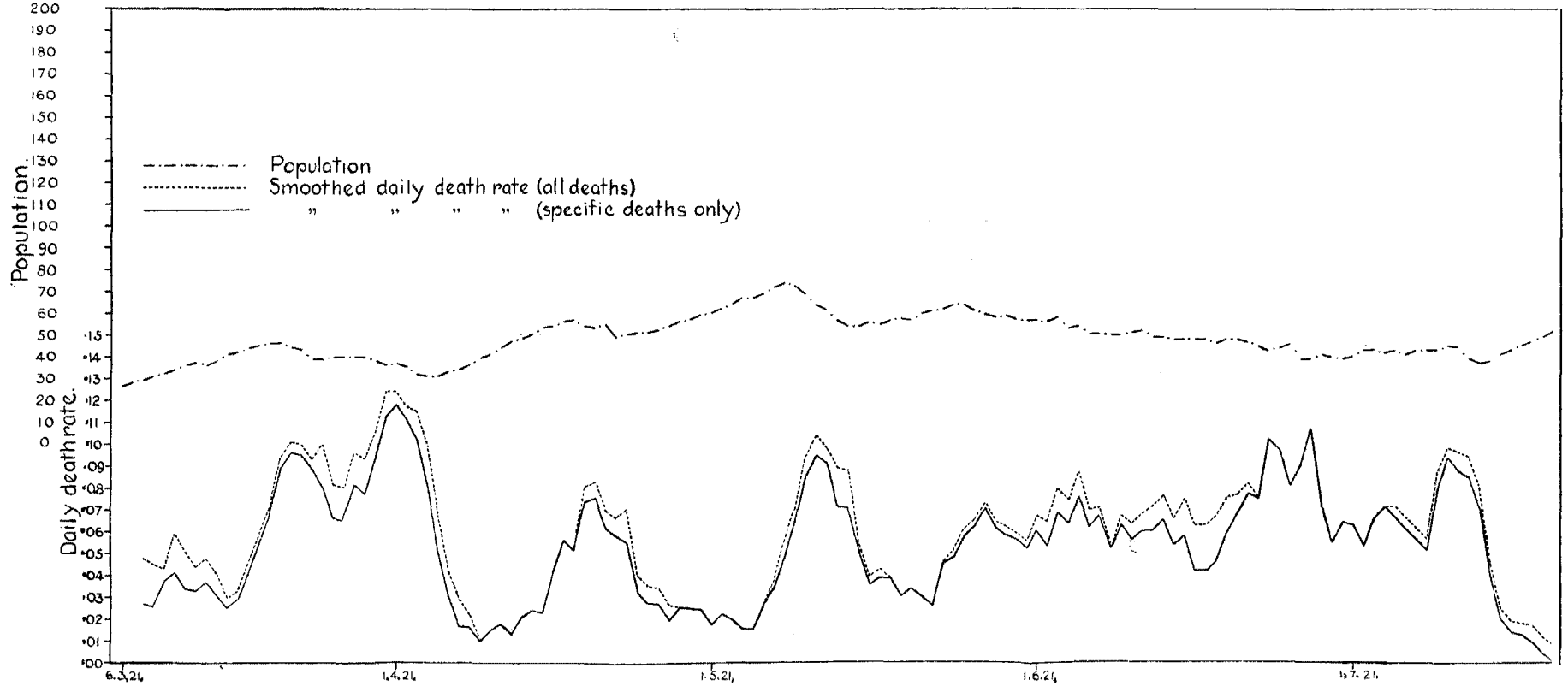


FIG. 4.—Secular death-rate and population in the first four months of an epidemic of mouse pasteurellosis. Three mice added daily. (Exp. 2A, Greenwood & Topley, 1925.)

than those for pasteurellosis. In two of the latter experiments (P 3 and P 1) a small proportion of typhoid infections was occurring, which may have exerted a significant effect. In two others (P 6 and P 0·3), although the epidemic was a pure pasteurellosis during the greater part of its course, typhoid infection was introduced towards its close. The remaining *Pasteurella* epidemics (P 3 N and P 0·5), and the three mouse-typhoid epidemics (A 6, A 3 and A 1), were not subject to the occurrence of any adventitious infection.

TABLE V

Experiment.	*Nature of infection.*	*No. of mice added daily.*	*Average daily q_x (specific).*	*Average total population per day.*
P 6	Pasteurellosis	6	0·0256	155·7
P 3 N	,, ...	3	0·0244	108·9
P 3	,, ...	3	0·0352	63·5
P 2	,, ...	2	0·0253	60·6
P 1	,, ...	1	0·0180	40·8
P 0·5	,, ...	0·5 (*a*)	0·0194	21·9
P 0·3	,, ...	0·33 (*b*)	0·0147	17·8
A 6	Mouse typhoid ..	6	0·0190	237·2
A 3	,, ..	3	0·0261	103·1
A 1	,, ..	1	0·0230	32·7

(*a*) One mouse added every other day. (*b*) One mouse added every third day.

It will also be seen that the average population increases, though not uniformly, with increasing rates of immigration. This must clearly be the case if, as these experiments suggest, the death-rate remains relatively steady over a wide range of different immigration rates. Experiments P 1, P 0·5 and P 0·3 suggest that when the immigration rate is considerably lowered there will be a point at which the average death-rate is effected, due to intermittencies in mortality. But to attain that level of mortality, under the conditions of our experiments, the number of immigrants in unit time must be so greatly reduced that the average total population is very small. It would seem, therefore, that the average size attained by an infected herd, living under conditions in which the death-rate is allowed to find its own level has been determined, in the main, by the rate at which the herd was increased by the addition of fresh susceptibles.

The second point is the periodicity of the fluctuations in mortality displayed by the q_x curves in the secular graphs. We have noted in our introductory remarks that Brownlee, to account for a supposed rigid periodicity in successive outbreaks of measles, postulated a life cycle of the parasite with a correspondingly rigid period. We also stated why we should not, even though a fixed periodicity in Brownlee's sense were demonstrated beyond cavil, assent to that particular hypothesis in regard to its cause. In our own experiments the question arises, is there any fixed periodicity the cause of which requires discussion? The answer is that there is not. There is a

periodicity of a kind in the succession of waves that occur in many of our herds. In the *Pasteurella* experiments referred to above the average wave interval when one mouse a day was added was 22 days; when three mice were added the average interval over different periods was 19–22 days; when six mice were added, and the fluctuations were at their slightest but most regular, it was 18–19 days. But the individual waves varied fairly widely, and, as has already been noted, sometimes they were clearly separated, sometimes fused together, while, with the lower rates of immigration, there were considerable intermissions. Moreover, periodogram analysis, the method used by Brownlee, has not, in our experiments, revealed any significant period (Greenwood and Topley, 1925).

We should, therefore, regard these waves or fluctuations as displaying an irregular and variable, not a fixed and steady, periodicity; though, under certain conditions, the fluctuations may become more regular at the same time as they diminish in range. In framing any hypothesis as to the mechanisms that determine the occurrence of these waves of mortality we should picture an interacting system that would be capable, in response to changes in the various factors concerned, of giving rise to short waves, or long waves, or definite intermissions, or minor fluctuations, or even, under exceptional conditions, to a perfectly steady level of mortality. It will be more convenient to discuss the probable nature of this system when we have considered the data afforded by our life-table figures.

We may, however, note here certain experiments that have a direct bearing on this particular problem. It has been shown (Topley, 1921; Amoss, 1922) that if an epidemic of mouse typhoid is allowed to subside, no additions of susceptible mice being made over a long interval, the addition at any later date of a number of such susceptibles is followed, after a lag-phase of varying length, by a fresh epidemic wave. In this wave the newcomers suffer first, and most severely; but the survivors from the previous epidemic prevalence share in the mortality, and eventually they may all succumb. In this instance the time-relation between intermittent immigration and intermittent waves of mortality is unmistakable.

The third point that calls for attention is the possible occurrence of "seasonal" fluctuations in the mortality in our infected herds. We use this term because it has achieved currency in the relevant literature (*see*, for instance, Pritchett, 1925, a, 1926, a; Glenny and Waddington, 1928; Blake and Okell, 1929; Mayer and Sulzberger, 1931; Sulzberger and Mayer, 1931; Orr, MacLeod and Mackie, 1931). All these observers note differences in the mortality rates following the injection of bacteria or toxic substances into groups of laboratory animals when similar tests are carried out at different times in the year, and ascribe their results to seasonal fluctuations in the resistance of their experimental animals. Pritchett's experiments may be considered in rather more detail, since they were carried out as part of a systematic investigation into the factors that determine epidemic spread.

The two main series of experiments on which Pritchett bases her conclusions were carried out on mice of several different breeds, since she was concerned with differences in resistance between the breeds as well as with seasonal differences within each breed. During the first year (1923–24) five breeds were under study; during the second year, four breeds. Each month a group of mice of each breed, about 50 mice per group, received an injection of about 5,000,000 *Bact. aertrycke* into the stomach through a fine catheter. All groups were observed for 56 days and the deaths were noted. We are here concerned only with seasonal fluctuations in mortality, so that the breeds may be grouped. The total number of animals thus becomes large—3,120 mice were tested during the first year, 2,100 during the second. In a subsequent paper Pritchett (1927) gives records of similar monthly tests carried out on mice on different diets that include a group belonging to one of the breeds tested during the years 1923–25 and fed on the same diet. This affords a further comparison between the mortalities observed in different months of the year. The monthly figures are given in Table VI. Those for the 1923–25 period have had to be read from small-scale charts so that they may not be entirely accurate. Those for 1926 were given in tabular form. The latter are, it should be noted, based on much smaller numbers of animals belonging to a single breed.

TABLE VI

Showing monthly mortalities among groups of mice per cent.

Month (a).	*1923–1924.*	*1924–1925.*	*Unweighted mean, 1923–25.*	*1926.*
January ..	74 (+ *1*)*	58 (− *21*)	66·0	87 (+ *17*)
February ..	85 (+ *12*)	52 (− *27*)	68·5	78 (+ *8*)
March	78 (+ *5*)	79 (*0*)	78·5	52 (− *18*)
April	75 (+ *2*)	94 (+ *15*)	84·5	63 (− *7*)
May	89 (+ *16*)	86 (+ *7*)	87·5	63 (− *7*)
June	62 (− *11*)	93 (+ *14*)	77·5	57 (− *13*)
July	57 (− *16*)	80 (+ *1*)	68·5	74 (+ *4*)
August	73 (*0*)	84 (+ *5*)	78·5	73 (+ *3*)
September ..	86 (+ *13*)	95 (+ *16*)	90·5	88 (+ *18*)
October ..	70 (− *3*)	65 (− *14*)	67·5	71 (+ *1*)
November ..	55 (− *18*)	70 (− *9*)	62·5	82 (+ *12*)
December ..	70 (− *3*)	92 (+ *13*)	81·0	52 (− *18*)
Yearly average (unweighted).	73	79	76	70

(*a*) In 1923–24 and 1924–25 the experiment actually began in October of the first-mentioned year and ended in September of the latter.

* The values in brackets are the deviations from the mean for the year.

On the basis of the 1923–24 results Pritchett concluded that the mice of all breeds showed a seasonal fluctuation in resistance resulting in a high peak of mortality during the spring, a lower death rate during summer, and a subsequent autumn rise. She considers that the second year's experience was in agreement with the first.

This view seems a little difficult to justify. February, 1923–24 gave the highest monthly mortality but two, February, 1924–25 the lowest monthly mortality for the respective years. June, 1923–24 gave the lowest monthly mortality but two, June, 1924–25 the highest but two. Taking the mean of the monthly mortalities for these two years, most of the significant fluctuations disappear. There is a rather higher mortality in April and May, and a definite peak in September; but a glance at the differences between the mortalities recorded in the same month of two successive years makes the significance of the " seasonal " variations very doubtful. In fact the correlation coefficient between the monthly values of the two years is $+0{\cdot}002$. This doubt is intensified by the 1926 experience. We cannot include these figures in any mean valuation for the reasons stated above, but their monthly trend would be expected to follow that of the 1923–25 period. Actually it does not. The mortality in March, April and May was well below the average, while that for July and August was above. The single feature that could be regarded as consistent with the earlier experience is the high mortality in September. A further comparison may be made by comparing the sign of the deviations from the mean in each year and thus seeing whether a month in which the mortality is below the yearly average in 1923–24 is also below the average in 1924–25 and 1926. This comparison gives 36 values of which 13 are of the same sign, 19 are of opposite sign and the remaining four are 0 in conjunction with a $\pm$ sign. It does not seem to us, therefore, that Pritchett has succeeded in demonstrating a seasonal fluctuation in resistance, or in mortality. Whether the fluctuations in mortality that are apparent when certain monthly tests are compared with other monthly tests in the same or another year are due to a fluctuating resistance in the mice is, of course, a different problem. Here we need only note that fluctuations in resistance, to induce such results as these, would have to be determined by some factor external to the mice themselves, since they would have to be synchronous. Independent fluctuations in the resistance of the mice constituting different groups of adequate size would not alter the *average* resistance of one group as compared with that of another, or the average resistance of a single group from one time to another.

It may be well, at this point, to consider a little more closely exactly what the term " seasonal " implies. Mice kept in a modern animal house, with reasonably adequate temperature control, and fed on such an artificial diet as our mice have received, are clearly not subject to seasonal influences in any way comparable to those encountered by animals living a natural life in the world outside our laboratories.

It is, however, unlikely that all seasonal influences have been eliminated. It is quite possible that a diet of whole oats, milk and water may be subject to seasonal variations in certain important constituents, though perhaps a little unlikely, in view of the observations outlined in Section VIII that such variations would greatly

affect the resistance of the mice. It is, however, very probable that the mice themselves, even when housed under such uniform conditions as ours have encountered, may be subject to seasonal physiological rhythms. There are, for instance, observations that suggest seasonal variations in fertility. But, from our present point of view, we are not so much concerned to know whether synchronous fluctuations in resistance are seasonal, as to know whether they in fact occur. Pritchett's findings suggest that they may, and Wilson (1930) has noted synchronous fluctuations in the mortalities following the injection of three different strains of *Bact. aertrycke* into mice during a sixteen months period, all three strains being tested synchronously on fifteen occasions. These fluctuations, however, showed no evidence of a seasonal distribution.

Our own data are as follows. Over a period of several months we had six different epidemics of pasteurellosis under simultaneous observation. Over a period of 76 days we correlated the daily death rates in every possible pair of herds. In only five of the possible fifteen pairs was the correlation coefficient significant, and in each of these pairs the experiment had been started on the same day, so that the initial waves of mortality would inevitably tend to occur at about the same period. One of six herds was in a much later stage of epidemic spread. With the fluctuations of mortality in this herd, none of the others showed any significant correlation. Moreover, in general, our long-continued epidemics have failed to show any tendency for periods of high or low mortality to recur at definite seasons of the year. We have certainly not shown that synchronous fluctuations in resistance do not occur—reference to the section on ectromelia will afford a strong suggestion that, under exceptional conditions, they do. The most that we can say is that, under the environmental conditions to which our mice are normally subjected, we have at present no evidence that would lead us to suppose that they play a significant part in determining the secular fluctuations in mortality that have been observed.

It is almost superfluous to emphasize that here, at least, there can be no argument from the mice in our cages to the natural world beyond them. In that world we have no doubt at all that seasonal influences, and factors that, though not seasonal in any meteorological sense, exert their effects at particular seasons of the year, play an exceedingly important part in the genesis and evolution of epidemic disease.

SECTION III

THE COURSE OF EVENTS IN A LONG-CONTINUED EPIDEMIC AS REVEALED BY A CAGE-AGE LIFE TABLE

Complete cage-age life tables, and a detailed discussion of the conclusions that can be based upon them, will be found in the section dealing with ectromelia. Similar tables giving the constants for various epidemics of pasteurellosis or mouse typhoid will be found in the papers in which these epidemics have been described. For the purposes of the present section it will suffice to quote the values observed for a few of these constants in certain of these epidemics, confining ourselves to examples that serve to illustrate the particular points under discussion. To avoid any complication that might be introduced by the existence in a herd of an infection other than that under study, we have selected only those experiments that have been entirely free from any adventitious infective disease.

In Table VII and Figs. 5 and 6 will be found tabular and graphic records of the l_x and ${}_5q_x$ values for various days of cage age between day 0, the day of entry to an infected herd, and day 100–200. These records refer to two epidemics of mouse typhoid (A 6 and A 3) and one epidemic of pasteurellosis (P 3 N). In epidemic A 6, six mice were added to the herd each day, in epidemics A 3 and P 3 N three mice were added.

TABLE VII

Showing the l values of one herd infected with mouse typhoid (A 6) and the ${}_5q_x$ values (probability of dying within the next five days) for two herds infected with mouse typhoid (A 6 and A 3) and one herd infected with pasteurellosis (P 3 N).

Cage age in days.	A 6			A 3	P 3 N
	l_x (all deaths).	${}_5q_x$ (all deaths).	${}_5q_x$ (specific deaths).	${}_5q_x$ (specific deaths).	
0	10,000	·0230	·0139	·0077	·0324
5	9,770	·0575	·0477	·0302	·0774
10	9,281	·1079	·0994	·0934	·0964
15	8,215	·1564	·1466	·1642	·2311
20	6,930	·2496	·2408	·3237	·2650
25	5,201	·3185	·3106	·4061	·1960
30	3,544	·2638	·2556	·3469	·1508
40	2,225	·1031	·1455	·1836	·1244
50	1,805	·0527	·0499	·1280	·0644
60	1,618	·0518	·0416	·0973	·0757
70	1,461	·0467	·0428	·0965	·0308
80	1,301	·0132	·0132	·0357	·0671
90	1,226	·0619	·0619	·0000	·0859
100	1,115	·0334	·0334	·0644	·0805
200	535	·0500	—	—	—

Starting with the trend of l_x values shown in the first column of Table VII and graphically in Fig. 5, it will be remembered that these show the way in which a population of mice dies out when exposed at each cage age to the average mortality rate at that age obtaining during the whole of the epidemic period on which the figures are based. In this case we have taken 10,000 as the arbitrary starting figure of our life-table population. The *actual* number of mice concerned in Experiment A 6 was 2,226, and the mortality rates observed among these mice at each cage age have been applied to the hypothetical 10,000. It will be seen from Fig. 5 that there is very little mortality during the first ten days of herd life. This is natural enough. If a mouse is infected on the day of entry it will not, on the basis of experimental infections with measured doses given *per os*, be likely to die until 5–10 days later. If it escapes infection for a few days, its death, supposing it to be fatally infected, will be

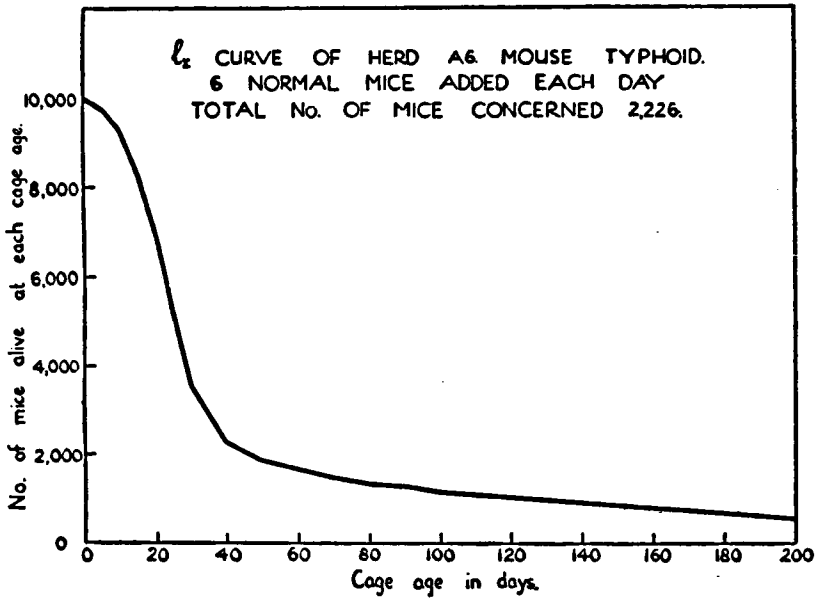


FIG. 5

correspondingly delayed. The deaths that occur during these first ten days are, in fact, largely non-specific, though a few mice contract, soon after entry to the herd, a rapidly fatal infection of the septicaemic type. After the tenth day the rate of mortality from mouse typhoid rapidly increases, and usually reaches a maximum about the 25th day of cage life. At this point the l_x curve is dropping steeply. About the 30th day, by which time some two-thirds of the entrants are dead, the fall of the curve becomes less steep. After about the 40th day, by which time little more than a fifth of the entrants remain alive, the fall becomes slower still. Our 10,000 are reduced to about a tenth of their original number by the hundredth

day, and to about a twentieth by the two-hundredth. These figures, of course, apply only to one particular epidemic of a particular infective disease, but, though pasteurellosis and ectromelia, or even other epidemics of mouse typhoid, show minor differences in their l_x curves, these are not so great as to alter the general picture presented. Fig. 5 may, indeed, be taken as illustrating in a general way the average fate of mice entering a herd that is infected with a highly contagious disease associated with a high mortality.

The ${}_5q_x$ columns of Table VII and the curves depicted in Fig. 6 tell the same story in a slightly different way. The mortality rate

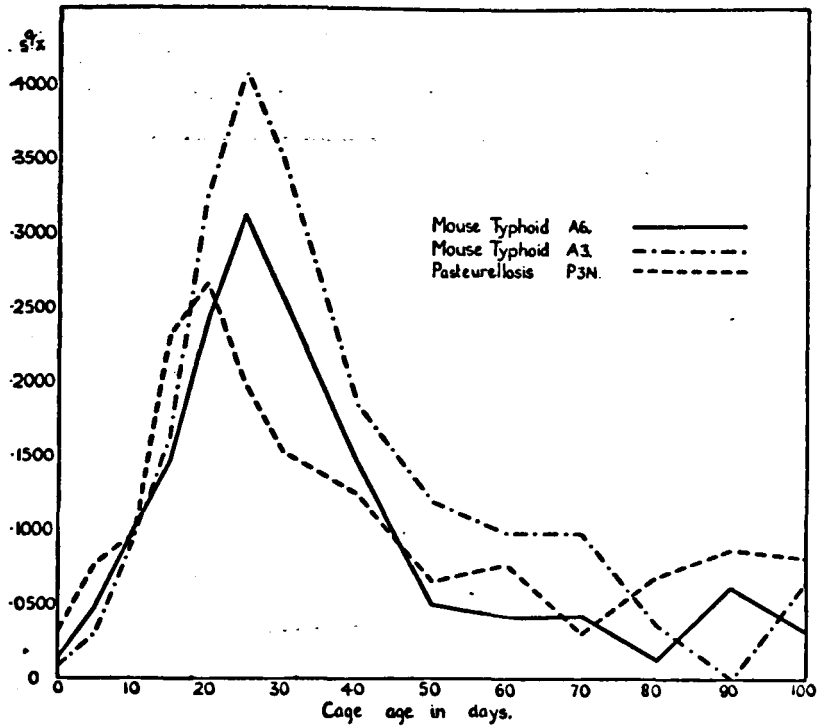


FIG. 6.—The probability of dying within the next 5 days (${}_5q_x$) in two herds infected with mouse typhoid (A 6 and A 3) and one herd infected with pasteurellosis (P 3 N).

rises rapidly during the first three weeks following entry to an infected herd, reaching a maximum at about the 25th day of cage age in the mouse typhoid epidemics, at about the 20th day in the herd infected with pasteurellosis. There is then a rapid fall, which becomes more gradual after the 30th–50th day. From this point onwards the curves tend to fluctuate; but it must be remembered that the mortality rates at later cage ages are calculated on a relatively small number of mice and that, with the low mortality rates then prevailing, small differences in the actual number of mice dying during the arbitrary

five-day period will make an appreciable difference in the ${}_5q_x$ value. In the light of our experience with many epidemics of this kind we are inclined to believe that these fluctuations result merely from sampling errors, and that the mortality rate tends to a steady value after about the 60th day, the population thereafter dying out logarithmically; it would, indeed, be difficult to conceive any biologically probable hypothesis to account for fluctuations in the ${}_5q_x$ curve at this part of its course. It will be noted that these ${}_5q_x$ curves are based on specific deaths—specific in the sense defined on pp. 13–14*. It will be seen, therefore, that our tables and curves demonstrate that the specific death rate never reaches zero. However long a mouse survives it is still liable to die of mouse typhoid, or of pasteurellosis; indeed, its risk of dying seems to alter little once the critical period of 60 days or so has been passed.

TABLE VIII

Showing expectation of life limited to 60 days, ${}_{60}E_x$, on different days of cage age for normal uninfected mice and for herds infected with mouse typhoid (A 6, A 3, Av D, Av 3C and A 1 D) and with pasteurellosis (P 3 N). Normals, all deaths. Others, specific deaths.

Cage age in days.	Normal.	A 6	A 3	Av D	Av 3C	A 1 D	P 3 N
0	56·8	30·6	27·5	30·8	34·9	27·1	29·5
5	57·4	26·9	23·0	26·8	30·8	22·6	26·2
10	57·1	23·9	19·0	23·1	26·5	18·4	24·1
15	57·1	22·2	16·0	20·8	23·3	15·2	22·2
20	56·8	21·7	14·1	19·4	21·3	13·0	24·3
25	56·7	24·0	15·2	20·8	19·9	13·3	28·7
30	56·7	30·6	20·0	25·7	20·3	16·9	31·8
40	58·3	41·5	31·4	38·9	22·1	26·4	36·5
50	58·4	46·1	39·6	45·1	30·3	36·9	40·6
60	58·5	47·1	44·3	48·4	38·0	40·8	40·9
70	58·6	47·3	48·2	50·9	47·0	46·4	43·0
80	58·8	48·7	50·8	51·2	46·8	46·4	40·7
90	58·5	48·3	49·1	50·6	49·0	47·2	40·0
100	59·1	49·2	47·1	53·4	47·5	47·3	41·2

* It must be remembered that life table constants, whether rates of mortality or expectations of life, based upon rates of mortality in computing which certain deaths have been excluded, are at two removes from reality. A life table constructed on the basis of all deaths observed among the exposed to risk through a particular period is subject to the assumption that there is no secular change (*vide* pp. 18–19). If one constructs a life table on the basis not of all deaths but on that of some fraction of them it has a meaning only if we assume that had the cause which produced the excluded deaths *really* been eliminated, then the rates of mortality from all other causes would not have been affected. Farr, who constructed several life tables in this way, has discussed the assumptions in detail (*see* Supplement to the 35th Annual Report of the Registrar-General, pp. xxxviii *et seq.*). We certainly do not feel justified in claiming that a table based only on what we call specific mortalities is a probable picture of the course of events under any circumstances. But, for the particular purposes for which we use such tables, viz., of comparison, we think they may be helpful.

We may view the same facts from still another angle by considering the ${}_{60}E_x$ values of our life tables—the expectation of life limited to 60 days. In Table VIII are set out the ${}_{60}E_x$ figures for normal uninfected mice living under our herd conditions, for five epidemics of mouse typhoid (A 6, A 3, Av D, Av 3 C and A 1 D) and for one epidemic of pasteurellosis (P 3 N). In Fig. 7 are shown the ${}_{60}E_x$ curves for the normal uninfected mice, and for three of the epidemics (A 6, A 3 and P 3 N). Taking either the tables or the charts, it will be noted that, in accord with our expectation, the ${}_{60}E_x$ values fall to a minimum at the cage age period when the ${}_5q_x$ values reach their maximum, and rise as the latter fall. The main interest of these figures, however, lies in the relations that they bear to the ${}_{60}E_x$ values for normal

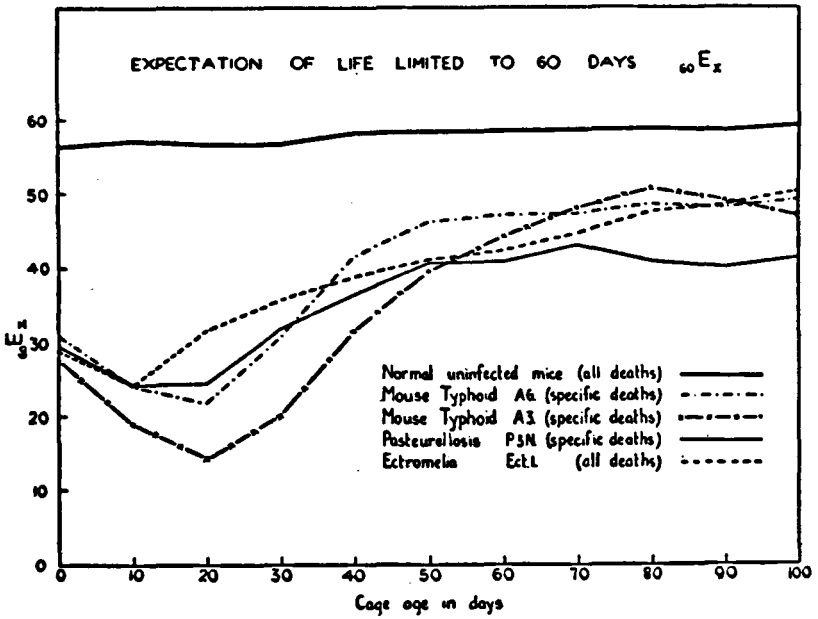


FIG. 7

uninfected mice. During the period of highest death rate, the 20th–25th days of cage age, the mice in the infected herds have approximately one-quarter to one-half the limited expectation of life of those in the uninfected control herd. But from this point onwards the difference diminishes; on the 50th day of cage age the ${}_{60}E_x$ of the mice in the infected herds varies from 30·3 to 46·1 days as compared with a normal figure of 58·4 days, and on the 100th day of cage age it varies from 41·2 to 53·4 days against a normal figure of 59·1 days. The limited expectation of life of the mice in the infected herds never reaches the normal level, but it tends towards it, and in a few instances the residual difference is not very great.

It will, however, be noted that the proportion of entrants that survives the risks of the first 50–100 days of cage life, and reaches

the period of low specific mortality, is by no means large. For example, in experiment A 6 only 18 per cent. of the entering mice are alive on the 50th day, only 11 per cent. on the 100th (compared with 91 and 87 per cent. in our normal uninfected herd). This is the order of dying out we have repeatedly observed when exposure to risk of infection has been free and continuous. What would happen if contact were more restricted we do not yet know.

What are the mechanisms that underlie the consistent story told by these different life tables? At the risk of tiresome repetition we may note again that they must be sought in the mice themselves—environmental factors are averaged out. The mice behave as they do because their average quality has become altered as the result of 10, or 20, or 50, or 100 days' survival in an infected herd. Clearly this is only another way of saying that the average resistance of the mice increases with cage age; and there are two obvious ways in which this average resistance may be altered. Assuming, as we may safely do, that the innate resistance of the entering mice varies over a significant range, the susceptible mice will be the first to die, and the average resistance of the survivors at any day of cage age after the mortality has reached a significant figure will rise as the result of a simple selection of the more resistant individuals. But mortuary selection will not be the only factor at work. Not all the infected mice will die, and those that live will have gained some degree at least of specific immunity. Natural immunization will certainly be proceeding in our herds synchronously with the march of disease and death. Our problem is to determine, if we can, the relative importance of these two factors—selection and immunization. Here, as elsewhere in this report, discussion of the relevant data must be preceded by a careful definition of our terms, since "resistance" or "immunity," used without qualification, would mask differences in mechanism that are of the first importance from our present point of view.

The Qualitative Demonstration of Resistance, or Immunity, and its Quantitative Measurement

Before attempting to differentiate one kind of resistance from another it is necessary to consider, very briefly, the methods at our disposal for detecting, or measuring, this character in any animal or group of animals. Suppose that we take a group of 50 mice, of the same breed, and of about the same age and weight, and inject them by the same route with the same dose of the same bacterial suspension. Suppose that we observe them for a period well beyond that which we know from experience covers the normal range between infection and death, and suppose that, at the end of this period, half have died and half are still alive and in apparent health. Are we justified in concluding that the 25 survivors had a natural resistance greater than that of the 25 mice that died? This view is quite clearly adopted by Webster (1923, a), who attaches primary importance to differences in innate resistance. He would

indeed accept its validity when considering differences in behaviour much less clear-cut than that between survival and death. Thus, in discussing the significance of a series of tests in which groups of mice were injected *per os* with a suspension of *Bact. aertrycke*, he says," Mice bred at the Rockefeller Institute vary in their susceptibility to mouse typhoid infection. . . . . This graded variation may be roughly analysed as follows; 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."

As a statement of what happens this needs no comment; but the assumption that the happenings are determined solely, or even mainly, by innate differences in the mice is clearly open to question.

To take a simple analogy; suppose that we were faced with 50 discs lying on a table, half black and half white, and were told only that they had been drawn out of a bag containing many hundred such discs, tossed one by one, and fallen in their present positions. Two assumptions, at least, would be compatible with the observed distribution of blacks and whites. The black discs might be black on both sides, and the white discs white. The difference in colour would be due to a difference in the discs. The element of chance in the observed distribution would have arisen during the withdrawal of the discs from the bag; the tossing would have had nothing to do with the matter, and we might assume that the half-and-half frequency of blackness and whiteness indicated, within the errors of random sampling, that the bag contained an equal number of black and white discs. But a different hypothesis would fit the facts equally well. All the discs might be exactly alike, black on one side and white on the other. In this case the draw from the bag would have had no effect on the result. The distribution would be determined by the tossing, and the fact that we saw 25 white and 25 black discs would depend on the equality of the chances of falling black or white face uppermost. We could not, on this single observation, tell which of these hypotheses was true; but we could easily settle the matter by turning the discs over, or, if this was not allowed, by tossing them all again several times and seeing whether the blacks continued to show black, and the whites white. But we cannot do this with our mice. Some are dead, and we can never know how they would respond to a subsequent inoculation. Those that are alive we can inoculate again, but if they proved resistant we cannot tell whether this is because they were resistant *ab initio*, or because we have immunized them during our first test. Actually, of course, some will die in the second test and some will survive, and the proportion of survivors will almost certainly be higher than that observed among a control group not previously inoculated; but our difficulty remains, there is no simple

crucial test that will enable us to decide whether our original mice behaved as they did because of differences in innate resistance, or whether their resistance was approximately equal and their behaviour was determined by chance influences of the kind that determine whether a penny falls heads or tails.

We should not, in reality, stop to consider either alternative to the exclusion of the other. We can be quite sure that, even if all mice were possessed of exactly the same degree of innate resistance, using that term in any recognized sense, *some* dose of bacteria could be found that would, on the average, kill 50 per cent., and leave the other 50 per cent. alive. Chance certainly plays some part, and the observed distribution of deaths and recoveries is certainly not an exact indication of the distribution of innate resistance. But we can be equally sure that mice do differ in resistance, and that the average innate resistance of those mice that survive will be higher than that of those mice that die. What we cannot tell by direct experiment, and what we chiefly want to know, is the relative weight that we should attach to each of these two factors, chance and innate resistance.

It will help in the definition of our terms if we consider the hypothesis of "chance" a little more fully from the biological side. The result of injecting a fixed dose of bacteria into the stomach of a mouse may well vary according to the reaction of the gastric juice at the moment of injection, the interval since the last meal, the rate of emptying of the stomach, and so on. It may vary according to the temporary condition of the blood plasma, or leucocytes, according to the rate of lymph flow, according to the number and state of activity of the reticulo-endothelial cells in the sinuses through which the bacteria happen to pass, and so on *ad infinitum*. It would be true enough, in a sense, to say that the mouse in which all these factors were, at the moment the injection was given, in a state unfavourable to the parasite, was temporarily more resistant than another mouse in which they presented fewer obstacles to bacterial invasion; but this is not what we usually mean by resistance, certainly not by "innate resistance." We should, at least in the discussions contained in this report, define "innate resistance" as a genetic character that endows its possessor with a lasting resistance to the infection under consideration, lasting, that is, over the period between adolescence and mature adult life, which is the only period with which we are concerned. We should make no assumptions with regard to the mechanisms on which this immunity depends, nor as to its specificity or non-specificity. It might be an immunity that was operative against the parenteral injection of bacteria by any route; or it might depend on some defensive mechanism operating only at the body surfaces, including of course the intestinal and respiratory mucosae; it might, or might not, involve the intervention of specific or non-specific humoral factors. Though making no assumptions of this kind *in our definition*, we should, on general grounds, expect many

of these heritable mechanisms to be less narrowly specific than those brought into play in active immunization, and we should hence accept a demonstration of sharp specificity, in the resistance of an animal that had previously been exposed to infection, as a suggestion, though certainly not as proof, that this particular example of resistance was more likely to belong to the "acquired" than to the "innate" category.

We should, for convenience of discussion in the ensuing pages, limit the meaning of "acquired resistance," unqualified by any phrase or adjective, to that type of immunity that is known to follow an attack of an infective disease, or a latent infection that gives rise to no overt signs or symptoms, or artificial immunization by any of the usual methods. Such immunity is, in almost all known instances, specific in the immunological sense. We should, therefore, regard a demonstration of non-specificity as a clear suggestion, though again not as a proof, that the particular example of resistance concerned was more likely to belong to the "innate" than to the "acquired" category. As to the duration of the acquired type of immunity we can make few assumptions. We should guess, from all analogy, that it would last for many weeks at least, probably for months, and possibly throughout a mouse's short life; but it would be only a guess.

These are the two kinds of resistance with which we are here mainly concerned; but there are other conditions that fall naturally into the same generic class, and that we cannot entirely ignore.

There is, for instance, a type of non-specific immunity, largely local but to some extent more general in its effects, that follows experimental infections in animals, or the injection of various bacterial or non-bacterial agents into their tissues. In the former case it is often associated with an acquired immunity of the specific kind. It appears to depend in part on the persistence of a local inflammatory reaction, in part on a relatively transient increase in the bactericidal powers of the blood. Examples will be found in the section on vaccination. It is the proved existence of this kind of immunity that prevents the acceptance of a demonstration of non-specificity as a proof that a given example of resistance is innate, not acquired.

There are, again, reasons for believing that immunizability as well as immunity may be subject to genetic variations; and it is at least possible that, in respect of any particular infection, these characters may be more or less highly correlated. An animal that responds briskly and effectively to the stimulus of the infection concerned may well be at once less likely to die from a large dose of the causative organism, and more likely to acquire immunity from a small dose. The existence of this class of genetic differences will not, however, seriously complicate our problem. In so far as the character of innate immunizability renders an animal resistant to any ordinary dose of the causative organism, that animal will be regarded as possessing innate resistance. In so far as it is

ineffective until the stimulus of a latent or sub-lethal infection has been applied, the animal, after response to the stimulus, will be regarded as having acquired resistance.

There is one further point that we may conveniently mention here, though it will be discussed in more detail in a later section—the occurrence of fluctuations in innate or acquired resistance. We are not including as fluctuations, in this present sense, those transient changes that we referred to in discussing the factor of chance, nor those impressed fluctuations, following alterations in the environmental conditions that may affect simultaneously all the mice in a herd. The fluctuations we have in mind are different. In innate resistance, although we have assumed the permanence, or semi-permanence, of a level of resistance above the average of the race or species, it is clear that various physiological crises, such, for instance, as pregnancy or various pathological states not related to the prevailing epidemic infection, might for a time lower, or perhaps raise, the level of innate resistance. The same is true of acquired immunity, but here we have the added factor of a specific increase or decrease, due to repeated doses of the parasite.

Finally, we must emphasize that resistance is a relative not an absolute term, and that the resistance we encounter is almost always partial, not complete. Resistant mice may all die eventually from the specific disease; if they live longer than the mice of a control group they fall, by definition, within the resistant class. We are, indeed, almost always concerned with differences in the average resistance of different groups; and this is fortunate because, though it is impossible to obtain any satisfactory measure of the resistance of any individual mouse, and very difficult to assess in any absolute terms the average resistance of a group, it is much easier to compare the average resistance of one group with that of another.

The Existence and Degree of Innate Immunity

We have given above the reasons for our inability to accept the mere demonstration that some mice live and some mice die after receiving a constant dose of bacteria as satisfactory evidence that those that live owe their survival entirely, or in the main, to an innate resistance; and we have pointed out that the character of such an experiment precludes us from the direct confirmation or refutation of this view by testing the survivors over again. There are, however, several indirect methods of attacking this problem; and Webster and his colleagues, although they attach more significance than we should ourselves to the results of such tests as those referred to above, have applied these indirect methods in seeking for additional evidence on this particular problem.

If genetic differences in resistance exist they should be inherited; one breed or strain of any animal species will probably be found to be more or less resistant than another; and, within any one breed, it should be possible, by selective breeding, to build up a strain of mice, or of any other host species, that has an average resistance

significantly higher or lower than that of unselected individuals. Moreover, such studies should afford some measure of the degree of immunity that these genetic factors are capable of affording. This method has been followed not only by Webster and his colleagues (Webster, 1924, b, 1925, a, 1933, a, b; Pritchett, 1925, b, 1926, b), but by many other workers who have approached the problem from the genetic rather than the epidemiological side. The copious, but somewhat confusing, literature of this subject has recently been reviewed by one of us in some detail (Hill, 1934) and it is unnecessary here to do more than quote the conclusions reached in that report. The available experimental data appear to have established quite clearly the existence of significant differences in resistance between different strains within the same species, e.g. between different strains of mice or rats. They strongly support the view that genetic differences in resistance exist within a strain, and that these differences can be segregated by selective breeding in such a way as to allow the production of derived strains of relatively high or relatively low resistance. That this evidence is less decisive than that adduced in support of the existence of differences between different breeds of mice or rats is due to experimental difficulties that are discussed in the report referred to. As regards the degree of average resistance that can be induced by selective breeding throughout a series of generations, it may be noted that the recorded examples vary over a range of differences extending from a slight delay in time to death, as compared with unselected controls, to the survival of a relatively high proportion of the selected group. Taking the most favourable records, however, there is no instance in which animals of the selected strain were uniformly resistant to bacterial infection, even when the test of resistance was the injection of the bacteria concerned in a dose that failed to kill 100 per cent. of the unselected controls. We cannot tell how selected resistants would behave under the conditions to which our infected herds are subjected. Moreover, in those herds there is no opportunity for selective breeding; so far as innate differences in resistance are concerned they must fall within the range covered by unselected mice.

We have noted above that, without attaching undue weight to this particular piece of indirect evidence, a demonstration that the resistance of surviving mice was specific would favour the hypothesis that it had been acquired during the original test, or during exposure to infection, while a demonstration that it was non-specific would suggest that it was due, at least in considerable part, to genetic factors.

Webster (1924, a) records a series of experiments from which he concludes that non-specific factors are largely concerned. Six groups of mice were injected *per os* with different strains of salmonella bacilli; three of these were strains of *Bact. aertrycke*, of somewhat different virulence, two were strains of *Bact. enteritidis*, and one was a strain of *Bact. paratyphosum B*. The survivors of these groups,

except those of one of the groups infected with *Bact. enteritidis* in which almost all the mice died in the original test, were reinfected with one of the strains of *Bact. aertrycke*, together with a group of control mice that had not been previously infected. All the surviving groups showed a lower mortality (27–52 per cent.) after the second inoculation than that shown by the previously uninfected controls (80 per cent.). The relatively high resistance of the groups previously injected with the three strains of *Bact. aertrycke* would be expected as the result of a specific acquired immunity, as also would that of the group previously injected with *Bact. paratyphosum B.*, since that organism possesses the same somatic antigens as *Bact. aertrycke*. The evidence of the existence of a non-specific factor, in this experiment, rests on the increased resistance to *Bact. aertrycke* of the survivors of the group infected with one of the strains of *Bact. enteritidis*, since this organism does not share either of the characteristic somatic antigens of *Bact. aertrycke*. Moreover, these survivors were more resistant to the second injection than those from two of the other four surviving groups. It may be noted, however, that the number of mice in this particular group was very small (15); and there is a statement in the text, the exact significance of which is not easy to assess. It is stated that whenever the original bacterial strain administered for immunizing purposes was recovered at necropsy the mice yielding it were considered to be chronic carriers, not true survivors, and were hence excluded from the final computations. There is no obvious method by which the *Bact. aertrycke* administered at the first injection could be differentiated from that administered at the second; so in these three groups all deaths would be included. It is quite possible to distinguish the closely related *Bact. paratyphosum B.* and *Bact. aertrycke*, but the method of differentiation employed is not stated. *Bact. enteritidis*, on the other hand, differs sharply from *Bact. aertrycke* in its antigenic structure. The application of the rule given above to this group would certainly lead to the elimination from the records of some of the mice that died, but of none of the mice that lived. We should not ourselves regard this experiment as a satisfactory proof that the resistance of the surviving mice was non-specific.

In the same paper Webster records an experiment in which 13 mice that had survived one dose of mouse-typhoid bacilli, 48 mice that had survived two doses of mouse-typhoid bacilli and 20 controls, not previously inoculated, were injected *per os* with 0·0016 g. of bichloride of mercury. All the controls died, while 2 of the 13 once-infected mice and 12 of the 48 twice-infected mice survived. Moreover the deaths occurred more rapidly in the controls than in the previously infected animals. These differences would, by the usual statistical tests be regarded as doubtfully significant in regard to the final mortality, clearly significant in regard to the deaths during the first ten days of observation. This resistance, such as it is, is clearly non-specific. That it is entirely referable to innate as opposed to acquired factors is less certain. We have noted that

some degree of non-specific immunity may be acquired against a bacterial infection, and it is at least possible that the single or double infection with mouse typhoid bacilli may have altered the reactions of the tissues to the metallic poison injected into the stomach. It would, at all events, be unsafe to assume that acquired non-specific factors played no part.

In other papers Webster and his colleagues have recorded experiments indicating the influence of non-specific factors in the resistance of strains of mice selected by breeding, and in the resistance of mice fed on a special diet; but in neither of these cases could there be any question of an acquired immunity, so that these findings do not concern us here.

We may refer here to a small experiment that we have carried out on lines somewhat analogous to those followed by Webster in the experiment referred to above (Topley, Wilson and Lewis, 1925, a). A group of 185 mice was fed, on several occasions during a period of four weeks, with a broth culture of *Bact. aertrycke*. At each feeding 0·02 c.c. of a 1/10 dilution of an 18 hr. culture was administered into the mouth with a dropping pipette. The faeces of all mice were examined daily. One month after the last feeding the position was as follows. Of the 185 mice, 92 had died, in most cases from typical mouse typhoid; of the remaining 93, 61 had excreted *Bact. aertrycke* in the faeces on one or more occasions. The remaining 32 mice had never yielded cultures of this organism. These 32 mice, together with 30 controls, were injected intraperitoneally with 2×10^5 *Bact. aertrycke*. During the following 21 days, 23 of the 30 controls died and 7 of the 32 survivors. The 25 mice that survived both feeding and intraperitoneal injection were then injected intraperitoneally, together with 25 controls, with 2×10^7 *Bact. aertrycke*. All the controls were dead by the 11th day, by which time 3 of the 25 doubly-surviving mice had died. These were observed for 68 days, when 11 in all had died. The 14 survivors from this test, together with 20 normal controls were then injected intraperitoneally with 500 *Past. muriseptica*. All the controls died within 4 days, 13 of the 14 trebly-surviving mice died in 2 days. The single surviving mouse was given the same dose of *Pasteurella* 10 days later. It died in 24 hours, raising the suspicion that it had not received the correct dose at the first inoculation.

These mortalities may be summarized in percentage form as follows:—

	Test mice. per cent.	*Controls.* per cent.
(A) Survivors from feeding tested with 2×10^5 *Bact. aertrycke.*	21·9	76·7
(B) Survivors from (A) tested with 2×10^7 *Bact. aertrycke.*	44·0	100·0
(C) Survivors from (B) tested with 500 *Past. muriseptica.*	92·9	100·0

The mice tested in (A) were of the kind that might be expected to display innate resistance. They had survived repeated feeding with *Bact. aertrycke*, and had never excreted it in the faeces, i.e. so far as this particular test was concerned they had shown no evidence of infection. They were found to be significantly more resistant than normal controls; but the possibility of immunization by latent infection could not, of course, be excluded. The survivors from this test, retested with a larger dose of the same organism, were again significantly more resistant than normal controls, though, allowing for the larger dose and the longer period of observation, there is no evidence of any measurable increase of resistance. The fact that, in this test, all the controls died in eleven days, while 56 per cent. of the doubly-surviving mice were alive and well 68 days later, suggests that selection could hardly be the only factor at work, since no normal mouse of the stock employed was able to resist this dose. In test (C) the trebly-surviving mice showed no significant advantage over normal mice when injected with the unrelated organism *Past. muriseptica*. The immunity at this stage was clearly specific; and in this respect the results of this small series of tests differ from those recorded by Webster.

The Relative Importance of Innate and Acquired Resistance in Long-Continued Epidemics

We may now turn to the indirect evidence bearing on this problem that we have obtained in the study of our infected herds.

We may refer first to an experiment (Greenwood, Newbold, Topley and Wilson, 1926) which was carried out to confirm the general conclusions based on our life-table figures.

For the purposes of this experiment we started two infected herds, A and B, using a single strain of *Past. muriseptica* as our infecting agent. Each herd received three daily normal immigrants. In addition herd A received at regular intervals larger batches of normal mice. From the survivors of these larger batches, mice were removed after 10, 20, 30, 40, 45, 50, 55, and so on, days in herd A and transferred to herd B. With each of these transferred batches, herd B received also a batch of normal mice, which may be referred to as C mice. Judging from our life-table figures we should expect that those transferred A mice that had lived 30 days or more in A, would live longer in herd B than the C mice entering with them, and that their advantage would increase up to some limit with length of exposure in A. This is what happened, as may be seen from Table IX.

The figures of our life table were, in fact, very closely reduplicated in regard to their general trend. The mice with 10 days' residence in A were very slightly shorter lived in B than the controls added with them; many of the former had already contracted in A an infection from which they died in B. From the 20th day onward the ${}_{60}E_x$ value for the life of the transferred mice in B rises steadily up to and including the group that had lived 50–60 days in A. The last group, composed of those mice that had lived 65–165 days in A,

had a somewhat shorter life in B. But the numbers here are becoming small, and the standard errors large, so that the fall in the ${}_{60}E_x$ value between the oldest-but-one and oldest groups is not more than might have arisen by chance. We have, however, confirmed by this transference experiment, the fact that survival in an infected herd increases the resistance of the survivors up to a limit that, while considerably greater than the average resistance of previously unexposed mice, is quite insufficient to ensure to such survivors the normal expectation of life when they are exposed to a continuous risk of re-infection.

TABLE IX

Mice.	*Length of exposure in A (days).*	*No. of mice in group.*	*Length of life in B limited to 60 days (${}_{60}E_x$).*	*S.E.*
C ..	0	887	22·4	0·53
A ..	10	173	21·3	1·63
A ..	20	163	25·5	1·68
A ..	30	128	32·6	1·90
A ..	40 and 45	90	33·1	2·25
A ..	50–60	73	37·4	2·51
A ..	65–165	58	30·3	2·82

We have employed the data provided by this experiment in an attempt to throw some light on the selection-*versus*-immunization problem. The secular death rate in herd A, as in all our herds, showed fairly wide fluctuations. We were thus able to select groups of A mice that had never experienced a high mortality in A and compare their behaviour in B with that of all A mice, as tabulated above. The arbitrary level of mortality that we selected was 7.5 per thousand per day, less than one third of the average mortality in A. There were 212 A mice that had never experienced a mortality higher than 7·5 per thousand before transference to B; and there were 493 C mice (normal controls) that were admitted to herd B at the same time as these particular A mice.

The ${}_{60}E_x$ figures at entry for these groups were: A mice, 25·1 days; C mice, 20·9 days; difference, 4·2 days, with a standard error of 1·44. The corresponding figures for all A's and C's taken from Table IX were: A mice, 28·4 days; C mice, 22·4 days; difference, 6·0 days, with a standard error of 0·98. It will be seen that the advantage of all A mice was greater than that of the A mice that had never experienced a high mortality in A; but the difference is not large, whereas the difference in the severity of mortuary selection was very considerable. These figures seem to us to suggest that active immunization must have played a large, perhaps a preponderating, part in increasing the average resistance of the migrants from A to B.

We have also analysed the data of this experiment by the method of correlation; but, before considering the results, we should wish to emphasize that their correct interpretation is not easy. One is

seldom, in an investigation of this kind, in the biometrician's paradise, where the characters are not very variable—have coefficients of variation of not more than 15–20 per cent.—regressions are linear, and the dependent variable is highly correlated with a number of independent variables, which among themselves are not highly correlated. It is much more usual, as in this case, to have coefficients of variation ranging up to or beyond 100 per cent., regressions of more than dubious linearity, and "independent" variables displaying obstinately high correlations one with another.

With this proviso we may note that the total and partial correlations between various measures of exposure in A and the length of after-life in B entirely confirm the conclusion that the average resistance of the migrants from A to B had been raised by their experience in A. When, by the method of partial correlation, we attempt to differentiate the effect of selection from the effect of natural immunization, the values obtained, if taken at their face value, would suggest that immunization was the more important process.

Thus we may note the following partial correlations :—

(*a*) Length of life in B and length of previous exposure in A (keeping constant average death-rate during previous exposure in A and average death-rate in B) ·194, S.E. ·024.

(*b*) Length of life in B and death-rate in A for 10 days previous to transfer (keeping constant length of previous exposure in A and average death-rate in B) ·021, S.E. ·025.

(*c*) Proportion of mice surviving 28 days in B and length of previous exposure in A (keeping constant average specific death-rate in B) ·554, S.E. ·065.

(*d*) Proportion of mice surviving 28 days in B and average specific death-rate in A during previous exposure (keeping constant average specific death-rate in B) ·195, S.E. ·090.

These figures suggest that the resistance of the migrants to B is far more highly correlated with the length of time that they lived in A than with the mortality rate, i.e. the stringency of mortuary selection, that they experienced in that herd. But since the regression in (*b*) and (*d*) departs much more widely from linearity than in (*a*) and (*c*), a comparison of these partial correlations is dangerous. Moreover, it must be remembered that the exposure of a mouse to a high death-rate in A shortly before its transference to B may shorten its subsequent life in B, not because it lacks resistance to the risks experienced in the latter herd, but because it has already received in A an infection of which it dies in B.

Although we first used the method of partial correlation in connection with this particular experiment, in which mice were transferred from one infected herd to another, it is clearly possible to employ it in analysing the data of any long-continued epidemic, since we can correlate the behaviour of any mouse subsequent to any arbitrary day of cage age with its length of previous exposure, or with the death-rate that prevailed during that period, or during

any part of it. When this was done for the long-continued epidemic of pasteurellosis mentioned in an earlier section of this report, in which a certain number of deaths occurred from mouse typhoid, the results were in close general accord with those obtained in the migration experiment, as may be seen from the following partial correlations (Newbold, 1927) :—

(*a*) Length of after-life and length of previous exposure (keeping constant death-rate during previous exposure and death-rate during after-life) ·150, S.E. ·022.

(*b*) Length of after-life and death-rate for the last 10 days of previous exposure (keeping constant length of previous exposure and death-rate during after-life) —·052, S.E. ·024.

(*c*) Length of after-life and death-rate during previous exposure (keeping constant length of previous exposure and death-rate during after-life) —·018, S.E. ·024.

The fact that the two latter partial correlations are in this case negative, even though insignificant, can, we think, only be explained by supposing that the increased average resistance of the surviving mice due to the elimination by death of their more susceptible companions is more than counterbalanced by the chance of carrying over, from the period of previous exposure to the period of after-life, a fatal infection contracted during the earlier period. This view is strengthened by the fact that the negative correlation is higher when the death-rate during the last ten days of previous exposure is taken as one of the variables than when we select the average death-rate during the whole of the previous exposure.

Later (Greenwood, Newbold, Topley and Wilson, 1928) the same method was applied to a subsequent phase of the same epidemic prevalence, when mouse typhoid had completely replaced pasteurellosis, no deaths from the latter disease were occurring, and six normal mice were being added daily to the herd. In this case the relevant partial correlations were as follows :—

(*a*) Length of after-life and length of previous exposure (keeping constant death-rate during previous exposure and death-rate during after-life) 0·307, S.E. ·021.

(*b*) Length of after-life and death-rate during the last ten days of previous exposure (keeping constant length of previous exposure and death-rate during after-life) —0·278, S.E. ·021.

(*c*) Length of after-life and death-rate during previous exposure (keeping constant length of previous exposure and death-rate during after-life) —0·282, S.E. ·021.

These figures are much more striking than those previously quoted. The relatively high negative correlations under (*b*) and (*c*) must again probably be attributed to the carrying over of an ultimately fatal infection from one period to the other, though it will be

noted that, in this case, the negative correlation is as high when the mortality during the whole period of previous exposure is considered as when we confine our attention to the last ten days of previous exposure.

For the reasons set out above we do not think that complete reliance can be placed on the values given by partial correlation in attempting to differentiate between natural immunization and selection; but they do, we think, suggest quite definitely that, of the two, immunization is the more important factor.

We may refer here to the results of another experiment, described in more detail in Section VI of this report. The relevant comparison was between the behaviour of four groups of mice, when submitted to contact infection with mouse typhoid by the method of the closed epidemic. Of these groups only three concern us here, the first (*a*) consisted of mice that had survived closed epidemics of mouse typhoid of low average mortality, the second (*b*) of mice that survived epidemics of high average mortality, and had hence been submitted to a far more rigorous selection than the mice in (*a*), the third (*c*) of normal mice that had not been submitted to any risk of infection. The ${}_{60}E_x$ figures for these groups of mice were as follows :—

(*a*) 48·8, S.E. 1·86 days.
(*b*) 48·3, „ 1·84 „
(*c*) 37·5, „ 2·61 „

It will be seen that the greater rigour of mortuary selection that had been exerted on the (*b*) mice gave them no advantage over the less-rigorously selected but at-least-as-well-immunised (*a*)'s.

In concluding this section we may return for a moment to the question of specificity of resistance, and note a few observations made during an epidemic of pasteurellosis in which normal immigrants were admitted, not daily, but in large groups. Towards the close of this epidemic *Bact. aertrycke* gained entrance to the herd. The subsequent course of events could not be taken as a basis for an analysis of the usual kind, but it provided an opportunity to gain additional information on this particular problem. Taking the periods marked off by the addition of large groups of normal mice, we were able to compare the behaviour, during any period, of new entrants with that of mice that survived one, two or more periods of previous exposure to risk. The earlier periods of unmixed pasteurellosis showed the usual advantage of survivors over new entrants. Thus the happenings during the fourth period were as follows, neglecting the small group of longest-surviving mice.

Periods passed through.	*No. of mice.*	*Deaths during fourth period.*	*Mortality per cent.*
2	44	9	20·6
1	29	7	24·1
0	50	22	44·0

At the beginning of the fifth period mouse typhoid made its appearance in the herd. The events during this period were

Periods passed through.	*No. of mice.*	*Deaths during fifth period.*	*Mortality per cent.*
3	35	34	97·1
2	22	20	90·9
1	28	22	78·6
0	50	45	90·0

The advantage of the surviving mice had largely disappeared, and the results obtained at necropsy left little doubt as to the cause. A proportion of the mice were eaten, and could not be examined *post mortem*. Of 34 necropsies on new entrants 23·5 per cent. showed evidence of pasteurellosis (the mice showing a double infection are excluded), while in 76 necropsies on the surviving mice the corresponding figure was 11·8 per cent. Clearly the survivors were, as before, more resistant to pasteurellosis. For *Bact. aertrycke* infections the corresponding figures were, new entrants 41·2 per cent., old survivors 38·9 per cent., an insignificant difference.

A somewhat similar analysis was made in another of these early experiments, in which a herd suffering from pasteurellosis became infected with mouse typhoid. In this herd additions were being made daily and the method employed was to select two groups of mice, (A) a group that had immigrated well before the first case of mouse typhoid occurred, and (B) a group that immigrated during the three weeks following the appearance of this disease. The fate of these two groups was then followed through two later periods, i.e. subsequent to the three weeks during which group B was immigrating. During the first period the mortality from pure pasteurellosis among the B mice was 24·7 per cent., among the surviving A's it was 13·7 per cent. The pure mouse-typhoid mortality was 5·2 per cent. among the B's, 6·0 per cent. among the A's. Here again, mixed infections are excluded. The survivors were more resistant to the old-established disease, but not to mouse typhoid, the newcomer. During the succeeding 25-day period the position changed. The pasteurellosis mortality was 18·2 per cent. among the B's, 23·2 per cent. among the A's, an insignificant difference. The mouse-typhoid mortality was 25·8 per cent. among the B's, 10·1 per cent. among the A's. It appeared, therefore, that both groups were about equally resistant to pasteurellosis, but the A's more resistant to mouse typhoid. The latter difference is readily explicable. All the A mice were resident in the herd during the whole of the earlier period and hence exposed to *Bact. aertrycke* infection. The B mice, on the other hand, entered *during* the period, so that many of them were exposed for only a short time, and so had less opportunity of being actively immunized. The lack of any difference in the resistance of the A and B mice, during this period, to pasteurellosis is less easily explicable.

These observations, though we should certainly not regard them as decisive, are in entire accord with our earlier experiments in regard to the specificity of the resistance that exposure and survival confer in these two diseases; and, while not disputing that an innate resistance might show this differentiation, we should regard these findings as giving some measure of indirect support to the view that active immunization is an important factor.

Summarizing our views on this problem, we wish to make it clear that we have no doubt at all that innate differences in immunity, and immunizability, exist and are important. We do not, on the other hand, believe them to be decisive. One point is abundantly clear, innate immunity is relative, not absolute. If the 20–30 per cent. of mice that Webster regards as resistant on the basis of their response to a single injection *per os* were solidly immune, there would, in any long-continued epidemic, be a piling-up of those resistant mice that would give secular and life-table mortalities utterly different from those that we have consistently observed. Given this relativity of innate as of acquired immunity, we should ourselves be inclined to attach greater importance to natural immunization than to mortuary selection as a determining factor in the epidemic process, in part for the reasons that have been set out in the preceding pages, but no less from our general knowledge of the behaviour of epidemic disease in man and animals under natural conditions, in which the decisive importance of natural immunization is beyond dispute.

The Spread of Infection in relation to Mortality

For the reasons we have just discussed we have concluded on the basis of our life-table figures and certain other experimental data that active immunization plays a very important part in the epidemic process. If this is so, it follows that infection must be far more frequent than the deaths themselves indicate, since many non-lethal immunizing infections must be occurring. That infection occurs very rapidly under the conditions to which our herds are subjected there can be no doubt. Reference to the l_x column of Table VII, p. 36, shows that, in the mouse-typhoid epidemic on which those figures are based, 48 per cent. of the mice at risk were dead by the 25th day of herd life. Unless we suppose that all mice that receive a dose of the infecting organism develop a rapidly fatal infection—a supposition that would be absurd in view of all our knowledge of the way in which infected animals behave—it is clear that a very large proportion of all the animals at risk must have received a dose of the infecting organism within this time. How they will respond to the reception of such a dose is, however, another matter. It is at least conceivable that some of them, possessed of an innate immunity depending on an unusually high efficiency of those mechanisms that hinder the access of bacteria to the tissues, might suffer no change in their initial state. The possible occurrence of this type of genetic immunity has arisen in connection with our

studies on ectromelia, and will be discussed in more detail in the relevant section. We can, however, be quite sure that a proportion of the mice will develop sub-lethal immunizing infections, and it is the proportion of such infections that concerns us here. We cannot obtain the information we need from experiments of the kind hitherto described, which yield data only in relation to the progress of mortality. In order to obtain this information an experiment was carried out on a more elaborate plan (Topley, Ayrton and Lewis, 1924).

Broth cultures of *Bact. aertrycke* were fed to a number of mice on three successive days. The faeces of these mice were examined daily and, on the fifth day after the last feeding, five mice were selected that were excreting *Bact. aertrycke*. These mice were placed in one of the usual experimental cages, and to them were added 20 normal mice. On the following day, and on every day thereafter until the 115th day, one normal mouse was admitted to the herd. On the 118th day all survivors were killed and examined *post mortem*. All mice dying during the experimental period were examined in the usual way. In addition, on each day except Sundays, the faeces of every mouse living in the herd were collected separately and examined for the presence of *Bact. aertrycke*; we had, therefore, in addition to our records of deaths and post-mortem data, a complete record of how each mouse had behaved, as regards excretion of *Bact. aertrycke*, during the whole of its life in the herd.

The more important data are set out in Tables X and XI. Both tables are constructed on the "life-table" principle, that is, they refer not to secular events, but to the average behaviour of the mice during the whole experimental period. In both cases the 5 infected mice and the 20 mice added with them have been ignored. Table X gives figures for mice up to the 25th day of cage age; the mice that were exposed for a shorter time than this have therefore been omitted, leaving 90 of the added-daily mice for consideration. The second column of the table gives the percentage of these mice that first showed evidence of infection during the time interval shown in the first column. In the great majority of cases "first infection" means the first isolation of *Bact. aertrycke* from the faeces. Occasionally, however, a mouse died from mouse typhoid without having previously excreted this organism. In such a case death has been taken as the first evidence of infection. The third column gives the percentage dying in each time interval. The fourth, the total percentage infected by the end of each time interval, i.e. the sum of the appropriate entries in column 2. The fifth, the total percentage dead at the end of each time interval, i.e. the sum of the appropriate entries in column 3. The sixth, the percentage alive. The seventh, the percentage of those still alive at the beginning of each interval that are known to have been infected in previous intervals or to have become infected in the current interval, i.e. in calculating the figures for this column the number

of mice alive at the beginning of each time interval has been divided into the number of mice alive at any time during that interval that have, at one time or another up to the end of that interval, excreted *Bact. aertrycke* in the faeces. In other words, it shows the maximum infective state at each cage age. In the construction of Table XI, showing the proportion of mice that live for different times in herd after having excreted *Bact. aertrycke* in the faeces, we have had to base our figures on 43 mice, since only this number had 60 days available after their first excretion and before the last day of the period of observation. An experiment of this type is extremely laborious, and it was impossible to continue it over a protracted period; but, although the numbers involved are small, we do not think that there can be any doubt as to the general significance of the results.

TABLE X

Showing the proportions of infections and deaths among 90 mice, exposed to mouse typhoid, for different cage ages.

	Per cent. of total population.					*Per cent. of mice alive in each interval infected.*
Cage age in days.	*First becoming infected in each age interval.*	*Dying in each age interval.*	*Infected by end of age interval.*	*Dead by end of age interval.*	*Alive at end of age interval.*	
1– 5	30·0	0·0	30·0	0·0	100·0	30·0
6–10	13·2	3·3	43·3	3·3	96·7	43·3
11–15	17·8	8·9	61·1	12·2	87·8	59·8
16–20	14·4	22·2	75·5	34·4	65·6	73·4
21–25	7·8	7·8	83·3	42·2	57·8	76·3

Turning to Table X it will be noted that no less than 30 per cent. of the mice at risk are infected during the first five days of herd life; another 13 per cent. received their first infection—or rather show the first sign of having received it—during the second five days interval, and a further 18, 14 and 8 per cent. become infected during the succeeding intervals, so that (*see* column 4) by the 25th day 83·3 per cent. of entrants have become infected. Deaths, naturally enough, do not occur so soon. Their frequency in successive time intervals is set out in column 3, and it will be noted that the greatest mortality occurs during the 16–20 day period of herd life. By the 25th day (column 5) 42·2 per cent. of the mice are dead, a figure that does not differ significantly from that observed in most of our mouse-typhoid epidemics. The last column of the table shows the condition of the mice still living in each cage-age period, and it will be noted that the percentage infection figure steadily increases. At the end of the first 5-day period all entrants are alive and only 30 per cent. of them are infected. At the end of the fifth 5-day period more than half of them are still alive (57·8 per cent.), but 76·3 per cent. of these survivors have become infected. Only 14 of

the original 90 mice survive 25 days without showing signs of infection. A glance at Table XI gives a clear indication of the different ways in which the mice react; 62·9 per cent. of them die within 30 days of showing the first sign of infection. It is a reasonable assumption that the majority of these mice have contracted a fatal infection on first contact with the parasite. A small proportion die between the 30th and 60th days, perhaps of a more chronic type of infection, perhaps of a relapse due to the lighting-up of a latent infection, or perhaps as the result of a reinfection from other mice. But the majority of those mice that become infected without contracting a rapidly fatal illness survive to beyond the 60th day, and this quota represents 30·2 per cent. of the total entrants.

TABLE XI

Showing length of life in herd after first excretion of Bact. aertrycke *for 43 mice exposed to mouse typhoid.*

Length of after-life in days.	*No. of mice.*	*Per cent. of mice.*
1–10	7	16·3
11–20	10	23·3
21–30	10	23·3
31–40	2	4·7
41–50	1	2·3
51–60	0	0·0
Over 60 ..	13	30·2

It may be added that the full records of this experiment, which are set out in the original paper, show that the majority of these infected but surviving mice excreted *Bact. aertrycke* on many occasions during the period of observation, i.e. their infection was not transient, but was either persistent over a relatively long period or was renewed by fresh contact infection.

At the end of this experiment there were 46 survivors that had lived in the herd for 14 days or more. In the necropsies on these mice the usual cultures were taken from the spleen and, in addition, the serum of each mouse was tested for the presence of agglutinins acting on *Bact. aertrycke.* The findings were as follows:—

Mice with positive spleen cultures 27
Mice with positive agglutinins 7
Mice with negative spleen cultures and negative agglutinins 17

Of the 17 mice that gave entirely negative findings, 11 had excreted *Bact. aertrycke* during life.

We may add that the post-mortem findings at the conclusion of this experiment are in entire accord with those we have noted among surviving mice that have been killed at the end of a considerable number of closed epidemics.

We think, therefore, it has been clearly demonstrated that, under the conditions of our experiments, (*a*) infection occurs very rapidly, (*b*) only a small minority of mice remain uninfected after the first three to four weeks of herd life, and (*c*) a considerable proportion of these infections are sub-lethal. It follows that conditions are extremely favourable for the occurrence of the natural immunization that, on the basis of other experiments, we have been led to regard as the most important factor in the increased resistance of surviving mice.

The Condition and Ultimate Fate of the Resistant Survivors

Before summarizing our general conception of the course of events in an infected herd, there is one further point that needs discussion. We have emphasized that our longest-lived survivors are never solidly resistant; however long they have lived in a herd they are liable at length to die of the reigning disease. Why is this so?

Many descriptions of the effect of repeated sub-lethal doses of a bacterial parasite, or its products, would suggest that, with each repetition of the stimulus, the partially immunized animal should become more and more resistant, at least until a high level of immunity had been attained. But our observations seem hard to reconcile with such a view, unless the ultimate level of immunity is of a relatively ineffective order; for our mice are exposed to such frequent reinfections that there should be no opportunity for an acquired immunity to decline for lack of repeated stimuli.

There is, however, no real evidence that the immunity gained as the result of sub-lethal infection is maintained at a steady level, even over a relatively short period of time. In many cases, at least, surviving animals are infected as well as resistant to re-infection, and there is no reason to doubt that these persistent infections may at times be activated by various non-specific factors; so that a resistant animal may eventually die of the infection that it has carried for months or years. The occurrence of relapse-deaths of this kind would, however, solve only part of our problem; for our surviving mice are not indifferent to the mortality prevailing in the herd of which they are a part. In some of our earlier experiments there seemed indications that, with advancing cage age, mice did become less and less responsive to fluctuations in the general death rate; but a recent re-examination of this question in the light of a far larger collection of data has not confirmed this hypothesis (Hill, 1933). When the expectation of life limited to 60 days from day x is compared for different death-rates for the five days preceding day x, this comparison being made for mice of various cage ages, it is found that the ${}_{60}E_x$ figure decreases with increasing general death-rates, and that this remains true even at cage ages of 80–100 days. Thus, for a combined mouse-typhoid experience, the ${}_{60}E_x$ value at entrance to the herd was 28 days when the death-rate for the five days preceding entrance was 0·01–0·02, and 27 days when

it was 0·04–0·05. At 20 days' cage age—the period of minimal expectation of life—the ${}_{60}E_x$ was 18 days when the prevailing death-rate for days 15–19 had the lower value, 11 days when it had the higher. At cage age 60 days the corresponding figures were 48 and 38 days respectively. Thus, although resistance increased with cage age, whatever the prevailing death-rate, increasing experience did not render the survivors indifferent to fluctuations from a lower to a higher general death-rate in the herd. The figures for epidemics of pasteurellosis gave analogous results; and analysis by correlation confirmed the indications given by simple inspection of the grouped figures. We must then conclude that many of our surviving mice die as the result of reinfection by their companions.

There is, indeed, nothing improbable in the view that reinfection may lower resistance instead of raising it, and may thus either activate a pre-existing infection, or implant a new one in the temporarily susceptible host, or perhaps exert both effects simultaneously.

An experiment carried out to obtain light on this point (Topley, 1933) has given very suggestive results. A large number of mice was injected at weekly intervals with a constant dose (500×10^6 bacilli) of a suspension of *Bact. aertrycke* killed by heating at 55° C. for one hour with the addition of 0·25 per cent. formalin. After the 1st, 2nd, 3rd, 4th, 6th, 8th, 12th and 13th injections 30 mice were set apart and, together with 30 normal controls, were injected with 1,000 living *Bact. aertrycke* seven days after the last immunizing injection. All injections were given intraperitoneally, and all mice were observed for 28 days after the injection of living bacilli. The 13th test was performed in duplicate. The results, as regards survivals in the various groups, were as follows:—

No. of immunizing doses.	*Percentage of mice surviving 28 days.*			
	Immunized.	*Controls.*	*Difference.*	*S.E.*
1	43·3	0·0	43·3	10·6
2	70·0	3·3	66·7	12·4
3	76·7	6·7	70·0	12·7
4	83·3	13·3	70·0	12·9
6	83·3	3·3	80·0	12·8
8	66·7	6·7	60·0	12·4
12	53·3	10·0	43·3	12·0
13(*a*)	46·7	3·3	43·4	11·2
13(*b*)	36·7	3·3	33·4	10·3

Taking the difference between the immunized and control groups as the best measure of the effect of repeated doses of killed bacterial suspension, it will be seen that the effect of repetition up to the sixth dose was to increase the immunity up to a relatively high level. Beyond this point, however, repetition of the doses produced a progressive decrease in immunity, so that after 12 injections the resistance of the mice had fallen back to the level attained after a single dose.

It may be noted that this effect appeared to be specific, and not due to any non-specific lowering of the general resistance of the mice. Another batch of mice that had received 13 immunizing injections were tested by the injection of 1,000 *Pasteurella muriseptica*, together with 30 controls. There was no significant difference in the death-rate in the two groups, indicating that the many-times-vaccinated mice were no more susceptible to this organism than normal unvaccinated animals.

Observations that would seem to have a similar significance have been recorded by Neufeld and Etinger-Tulczynska (1933). They injected mice with repeated doses, given at varying intervals, of pneumococci and of other organisms, and found that animals that were initially resistant might succumb to later injections of the same bacterium. So far as could be judged from the small number of animals employed, it appeared that many of these repeatedly-injected mice had become at least as susceptible as normal untreated controls.

It would serve no useful purpose to discuss in any detail the possible mechanisms involved in this process of de-immunization, but we may note that there is a host of immunological analogies on which we might draw. From the days when the " negative phase " was first noted, a wide variety of observations have shown that the effect of any injection of antigen into the tissues depends on the immunological condition of the animal at the time the injection is made, the size of the dose injected, and the time-relation of the injection to any others that may have preceded it. It is by no means uncommon, in the presence of particular combinations of these factors, for an injection of antigen to lower " immunity," so far as immunity can be measured by antibody-titrations. It is true that this lowering, after a single dose, is usually followed by a rise up to, or beyond, the previous level; but with repetitions of antigenic stimuli during the depression phase the low level may be maintained over long periods. In part, of course, the lowering is the direct result of the neutralization of circulating or fixed antibody by the injected antigen, but this is probably not the whole story.

Clearly we should, in any adequate discussion, have to consider here the possible relation of allergy, or hypersensitiveness, to the phenomena we have observed. Sensitization may be playing a part ancillary to, or perhaps more important than, de-immunization. But such a discussion would take us altogether too far afield.

It would seem natural to consider here the possible relation to this problem of Dudley's (*see* Dudley, 1923, 1926) hypothesis of the importance of the velocity of infection; but this will fall more conveniently for discussion in a subsequent section, and we may conclude with a brief summary of our present conceptions of the immunological state of the older survivors.

We should, then, regard these survivors—say the mice of 60 days cage age or over—as forming an extremely heterogeneous class, and the ${}_{60}E_x$ value calculated for this group as affording a measure

not of the mean value of some one character "resistance" shared in varying degree among these mice, but simply of their average behaviour under a particular set of conditions, the behaviour itself depending on the interaction of a large number of variable factors, some interdependent, some relatively independent.

Thus, a group of survivors whose average behaviour is measured by some particular ${}_{60}E_x$ value, say 48 days, will include at least the following categories: (*a*) a large proportion of mice, say 30–60 per cent., that are harbouring the bacterial parasite in their tissues, many of which will have been first infected during the first 14–21 days of residence in the cage; (*b*) another large class that have been infected during some previous period of exposure, but have subsequently freed themselves of the infecting parasite; and (*c*) a very small class, perhaps negligible in numbers, which, though they must have received many doses of the parasite, have never contracted any infection, and have hence undergone no change in state. In the particular case of mouse typhoid, it will be noted that this is an observed distribution, not an assumed one.

Of the class (*a*) mice it is probable that many would eventually die of the specific infection altogether apart from happenings in the herd, e.g. if they were removed from all contact and isolated in single cages (*see* pp. 101–104). They are in a condition of unstable equilibrium; relatively, but not absolutely, indifferent to reinfection from without, they are liable to activation of their own infection by various non-specific causes. If left in the herd they may be affected by reinfection from their fellows, and their susceptibility to such reinfection probably varies widely from time to time according to the temporary balance of the equilibrium existing in their own tissues. Mice of class (*b*) will be more effectively "resistant." If removed from the herd they will run no further risk of dying from the reigning disease. Left in the herd they are liable to reinfection; and if this is massive, or too frequently repeated, they may either develop the disease in an acutely fatal form, or pass again into class (*a*), from which at some earlier period they had emerged. Analogy with the results of the experimental infection of partially immunized mice would suggest that the latter is a very frequent event. The small minority of (*c*) mice will, unless in the meantime they die of some other cause, eventually succumb to infection, or pass into the (*a*) class, and perhaps thence into the (*b*)'s. That they will not remain permanently uninfected is attested by the fact that, in epidemics running continuously over several years, there is no effective piling-up of completely resistant mice.

We should, then, regard the "resistance" of long-surviving mice not as a character remaining over long periods at a steady high level, but as subject to wide fluctuations. At any one moment in secular time some survivors will be almost solidly immune, others may be no more resistant than new entrants; at a later instant of time any given mouse may have shifted from the

" resistant " to the " susceptible " class, or *vice versa*. Still other mice will, from time to time, move from either of these classes to the class of the fatally infected, either because of a change of equilibrium within their own tissues, or as the result of reinfection from without. Sooner or later every mouse will receive a reinfection at a moment when its resistance is at a low ebb, or will succumb to an activation of its own latent infection. Eventually all will die of the specific infection, unless death from some other cause intervenes.

The General Course of Events within an Infected Herd

Before passing to the consideration of the particular problems that fall for discussion in later sections of this report we may give a brief outline of the conceptions that we have been led to form of the course of events in any herd, infected with an epidemic associated with a high mortality, and subject to the immigration of susceptible animals. Our picture is, in general terms, as follows.

The great majority of mice are infected shortly after entrance, so that the reacting system at any moment contains a relatively small proportion of animals presenting a virgin soil. The subsequent course of events in the individual mice has been considered in the previous section, and we may here confine our attention to the behaviour of the herd as such. After the first wave of disease and death that always follows the aggregation of an infected herd, the epidemic settles into a state of unstable equilibrium. With few daily immigrants, the mortality curve tends to show relatively wide and relatively regular fluctuations. We do not think that these fluctuations are caused by any " life cycle " of the parasite, or by any " seasonal " variation, or, indeed by any single determining factor. We should liken them rather to the swings of a balance that has been thrown out of equilibrium. With a steady immigration of susceptibles in relatively large numbers these fluctuations in mortality tend to die down, and the herd shows an almost steady death-rate. If we could work with infinite numbers of mice under unvarying environmental conditions, we believe that it would remain entirely steady throughout an indefinite period of time. There would be so-many entrants and so-many deaths in each time interval, and the total population of the herd would be unvarying. But, even with a high immigration rate, the equilibrium will remain unstable, and a sudden disturbance, due to any one of a large number of possible environmental causes, will produce a swing in the system, and not a single swing. The system will be set swinging again, and it may be many months before it regains its equilibrium, perhaps upon a new level.

We have not, in this section, given any consideration to the part played in this reacting system by changes in the characters of the parasite as opposed to changes in the characters of the hosts, beyond expressing our disbelief in any fixed and recurrent life-cycle. This particular problem is discussed in Section VI.

SECTION IV

A STUDY OF THE EXPERIMENTAL EPIDEMIOLOGY OF *ECTROMELIA* INFECTION

In the previous sections we have given a brief review of the general characters of epidemics among mice initiated by such bacterial parasites as *Bact. aertrycke* and *Past. muriseptica*, and of the mechanisms on which we believe these characters to depend.

During the last few years we have been interested in the study of a biologically different type of infection. The description by Marchal (1930) of a natural virus disease in mice—ectromelia—afforded an opportunity of comparing the epidemic behaviour of a virus disease with that of the bacterial infections already studied. We wish to express to Miss Marchal our thanks for supplying us with the strain of virus with which the epidemics described in this report were induced.

Experimental Procedure

The general procedure followed in these experiments has not differed from that employed in initiating and maintaining earlier experimental epidemics. The relevant figures in regard to additions and deaths will be set out in later sections. The nature of the disease has, however, necessitated a change in the diagnostic criteria employed in determining the cause of death, and the significance of these criteria must be briefly discussed.

As in all our experiments, all dead mice left uneaten by their companions have been examined *post mortem*. The average rate of cannibalism has not been high. Of 4,955 mice dying during these experiments 4,410 have been submitted to necropsy. From each of these mice a culture has been taken from the spleen to exclude the presence of bacterial infection. There has been no evidence at any time of the infection of our herds with *Bact. aertrycke, Past. muriseptica* or any other bacterial parasite that is known to cause epidemic disease in mice.

In determining the cause of death in epidemics of bacterial infection, our final criterion has always been the isolation of the bacterial parasite from the tissues. A majority of the dead mice have shown the characteristic lesions of mouse typhoid or of mouse pasteurellosis, but the remainder have shown no recognisable lesions, or lesions so doubtful that a diagnosis would have been impossible apart from the bacteriological findings.

In ectromelia the cultivation of the virus is not available as a diagnostic method, and we have had to rely on other evidence. It is unnecessary to repeat here the excellent description of the lesions of ectromelia given by Marchal. It will suffice to note that the lesions in the liver and the spleen, and particularly the characteristic discoloration of the former, enable a diagnosis to be made with a high

degree of probability in a majority of cases. The frequent enlargement of the lymphoid follicles of the intestine affords another serviceable criterion; but these follicles are unequally developed in normal mice, so that an apparent enlargement cannot be accepted as certainly diagnostic of the disease.

Of 4,116 mice submitted to necropsy—those dying during the period 20th August to 30th September, 1932, are omitted from these figures for reasons that will become apparent later—2,600, or 63·2 per cent. were diagnosed on post-mortem appearances as having died from ectromelia.

To assess the significance of these figures it is necessary to consider them in relation to cage age at death. In ectromelia, as in mouse typhoid and pasteurellosis, there is good evidence that long residence in an infected herd is associated with great increase in resistance, and it would obviously be desirable to determine, if possible, the ratio of specific deaths to total deaths in the different cage-age groups, in order that the full effect of this acquired immunity, and of the concomitant elimination by death of the more susceptible mice, might be assessed as accurately as possible.

In Table XII the relevant figures are set out for 1,565 mice that died in the herd labelled " Ectromelia 1 " between 18th November, 1930, and 19th August, 1932, and for 1,368 mice that died in the herd labelled " Ectromelia 2 " between 16th January, 1931, and 19th August, 1932—periods extending in each case from the commencement of the epidemic to a heat wave that exerted a dramatic effect on the epidemic prevalence.

TABLE XII

Showing the percentage of mice dying at different cage ages with the characteristic post-mortem appearances of ectromelia

Cage age at death in days.	*Ectromelia 1.*		*Ectromelia 2.*	
	No. examined.	*Per cent. positive.*	*No. examined.*	*Per cent. positive.*
0– 10 ..	154	60·4	145	60·0
11– 30 ..	819	67·8	754	65·4
31– 50 ..	239	69·0	157	59·9
51– 75 ..	137	72·3	91	63·7
76–100 ..	49	46·9	37	67·6
101–200 ..	71	33·8	71	35·2
201 and over ..	96	13·5	113	13·3
Total ..	1,565	62·1	1,368	58·3

There is a slight difference in the run of the figures during the first hundred days of cage life. In Ectromelia 1 the frequency of positive post-mortem findings increases from 60·4 per cent. in the cage-age group 0–10 to 72·3 per cent. in the group 51–75, and then drops sharply to 46·9 per cent. in the group 76–100. In Ectromelia 2

the frequencies fluctuate irregularly between 59·9 per cent. and 67·6 per cent. during this period of cage life. Beyond the 100th day each experiment tells the same story. The frequency of positive post-mortem findings falls sharply, and among the mice dying after more than 200 days' residence in the herd it reaches a very low figure (13·3–13·5 per cent.). So far, then, as can be judged from post-mortem appearances alone, the total death rate of mice dying at late cage ages overstates the deaths due to ectromelia to a considerably greater extent than does the same rate applied to mice dying at earlier periods.

But it is quite certain that the figures based on post-mortem appearances alone understate the specific ectromelia death rate at all cage ages, just as a similar criterion will understate the death rate from a bacterial infection. To obtain accurate figures it is clearly necessary to supplement the naked-eye diagnosis by some other test. Two such methods are available, though they are too laborious to be employed in the routine examination of very large numbers of mice. Sections of the tissues, especially of the intestines, may be stained and examined for the presence of the characteristic inclusion bodies, and a filtrate may be prepared from the liver of a dead mouse and tested for the presence of the virus by injection into a small sample of normal mice. Both these methods have been employed in the examination of reasonably large groups of mice dying in our infected herds, in order to test the reliability of our naked-eye diagnosis.

In searching for inclusion bodies, the method employed has been to fix the entire small intestine and prepare paraffin blocks containing a series of parallel segments which are cut longitudinally, since sections cut from such blocks display the maximum number of lymphoid follicles, and it is in the epithelial cells in the near neighbourhood of such follicles that inclusion bodies are, in our experience, most commonly found. Table XIII shows the correlation between the naked-eye post-mortem appearances and the detection of inclusion bodies in sections from 262 mice dying during the earlier phases of these epidemics.

TABLE XIII

	Inclusion bodies found.	*Inclusion bodies not found.*
Post-mortem appearances positive ..	103 (39·3%)	59 (22·5%)
Post-mortem appearances negative ..	11 (4·2%)	89 (34·0%)

It is clear that reliance on the detection of inclusion bodies alone would decrease rather than increase the proportion of deaths diagnosed as ectromelia, and since most of the mice in which a positive naked-eye diagnosis was accompanied by a failure to detect inclusion bodies presented unmistakable lesions of the disease, we

have no doubt that the microscopical diagnosis was at fault. We do not, of course, doubt that a more thorough search of the tissues would have enabled us to demonstrate inclusion bodies in a far higher proportion of cases ; but such extensive histological examinations are scarcely possible in dealing with large numbers of mice.

In testing the infectivity of the tissues, suspensions of emulsified liver tissue were filtered through sand and paper pulp, 0·5 per cent. phenol was added to the filtrate to destroy any contaminating bacteria and the phenolized filtrate was then stored in the ice chest for three days before being injected in 0·1 c.c. doses into normal mice. Such filtrates, when prepared from mice dying of typical ectromelia, have almost uniformly given rise to typical infection in normal mice. In most cases five mice have been injected with the filtrate under test ; later in the investigation three mice were injected instead of five, since the earlier results indicated that this would prove an adequate number. In a few cases the liver suspension was allowed to settle instead of being filtered. The results of all such tests may, we think, be justifiably considered together.

Since our experience with mice dying of the typical disease at early cage ages, or after experimental infection, led us to the view that virus would certainly be detected in the livers of such mice, and since the expense involved in testing the liver of each dead mouse by the injection of three or five normal animals necessarily limited the scope of this particular part of our enquiry, we confined our attention, so far as mice dying during the first 75 days of cage life were concerned, to those in which the naked-eye diagnosis was negative. We have records of 71 such mice. Of these, 18 (25·4 per cent.) yielded infective liver filtrates. On this basis we should have to transfer about one quarter of our negative group to our positives, as diagnosed by naked-eye appearances, in order to arrive at a correct specific death rate. This would give us, for the cage ages in question, 70–79 per cent. of the total deaths.

We were, however, particularly interested in the deaths occurring at later periods of cage life, since naked-eye post-mortem diagnosis had suggested that the divergence between the specific and total death rates was more considerable at these cage ages. We therefore selected at random 100 mice dying after a residence of 75 days or more in Ectromelia 1 and 100 dying after a similar period in Ectromelia 2, and tested liver filtrates by the same method. The results are set out in Table XIV.

TABLE XIV

Mice dying at cage ages 75 days or over

	Liver filtrates positive.	*Liver filtrates negative.*
Post-mortem appearances positive ..	49 (24·5%)	24 (12·0%)
Post-mortem appearances negative ..	12 (6·0%)	115 (57·5%)

It will be noted that the post-mortem findings agree with the test of the liver filtrate in 82 per cent. of these mice. Twelve mice of 127 that gave negative post-mortem appearances yielded infective liver filtrates, suggesting that about one-tenth of these mice should be transferred to the specific deaths, as compared with one quarter at earlier cage ages. If this is a true indication, the real specific death rate at cage ages over 75 days, and particularly over 100 days must have formed a relatively small proportion of the total death rate. The probability that this was so is increased by confining our attention to mice dying after prolonged residence in an infected herd. Of the 200 mice referred to above, 106 died at cage ages of 200 days or over; 19·8 per cent. of these showed the naked-eye appearances of ectromelia, as against 36·5 per cent. of the total 200 mice.

Turning again to Table XIV it will be seen that 24 of 73 mice diagnosed at *post mortem* as having died of ectromelia gave non-infective liver filtrates. This is not surprising. Such filtrates contain products of the host's tissues as well as the virus, if the latter is present, and all those who have worked with such material have noted the irregularities that may result from the presence of specific antibodies. This factor will come into play more frequently in mice dying after long residence in an infected herd than in those dying shortly after entrance, since many of the former will have acquired a partial immunity, even if of too low a grade to enable them completely to resist infection. It is at least possible that some of the lesions noted in these mice represented healed or healing foci of infection. It may be noted in this connection that a correlation has been observed between the extent and distribution of the lesions found at necropsy and the infectivity of the liver filtrates. Many of the mice yielding inactive filtrates have shown minimal lesions, confined chiefly to the spleen.

In summary, we do not think that we can adduce pathological criteria sufficient to justify the use of a specific death rate. We have therefore employed total death rates throughout. We believe, for the reasons given, that these rates under-estimate the relative resistance of mice of advanced cage age; but since the advantage of such mice is sufficiently obvious when total death rates are used it seems better to understate the case than to apply an estimated correction that would, of necessity, rest on an insecure foundation.

Another way in which ectromelia differs from mouse typhoid and mouse pasteurellosis is that it gives rise, in a proportion of cases, to a characteristic swelling of the feet that is recognizable during life. In such cases it is possible, as it has not been possible in the bacterial infections studied, to follow the subsequent history of mice that have passed through a clinically obvious attack of the disease, and have apparently recovered from it. The proportion of mice showing these lesions of the feet is not large. Of 4,661 mice dying in Ectromelia 1 or Ectromelia 2 only 332 (7·1 per cent.) developed this condition during life. The after history of 311 of these mice, which include those dying in all phases of the epidemic

except that of the 1932 heat wave and the few subsequent weeks (19th August to 30th September, 1932) is of some interest. Table XV gives the post-mortem findings in these mice grouped according to the number of days they survived after first developing swollen feet. Those that died within five days showed lesions in the spleen or liver in 51·9 per cent. of cases. The foot lesions in these mice were clearly a part of the acute infection to which they succumbed. As the number of days lived after the appearance of the foot lesions increases, the proportion of mice showing characteristic lesions in the internal organs decreases, at first rapidly then more slowly. Of 149 mice surviving for more than 30 days only 30 (20·1 per cent.) would have been diagnosed on the post-mortem findings as having died from ectromelia. The persistence of the foot condition varies very widely in mice that survive for several weeks after its first appearance, but in the majority of those that live for relatively long periods it disappears before death. In 107 of the mice included in Table XV recovery from this local lesion had occurred. These figures clearly suggest that a mouse that has passed through an attack of ectromelia and recovered from it is relatively unlikely to develop a fatal attack of the disease at a later date; but, equally clearly, this increased resistance is not absolute, for 15·9 per cent. of mice that lived for 200 days or over after developing foot lesions eventually died with the post-mortem appearances of the disease. There is evidence of the same fact in the occurrence in some mice of two or more attacks of swollen feet, with a considerable interval between them; the second or third attack sometimes terminated fatally. Thus, a particular mouse developed a characteristic swelling of the foot on 3rd June, 1931, which had disappeared by 8th June. A second foot lesion developed on 31st August and lasted until 14th September. Another attack commenced on 14th March, 1932, terminated in the death of the mouse, with typical post-mortem lesions, on 23rd March.

TABLE XV

Showing the frequency of positive post-mortem findings in mice dying at various intervals after the appearance of foot lesions.

Days of subsequent life.	*Number in group.*	*Per cent. in group.*	*P.M. +*	*Per cent. P.M. +*
0– 5 ..	81	26·0	42	51·9
6– 10 ..	38	12·2	14	36·8
11– 30 ..	43	13·8	12	27·9
31– 50 ..	20	6·4	5	25·0
51–100 ..	25	8·0	6	24·0
101–200 ..	41	13·2	9	22·0
200 + ..	63	20·3	10	15·9
Total ..	311	99·9	98	31·5

It is, of course, entirely possible that these recurrent foot lesions, or a foot lesion followed after a long interval by an acutely fatal

illness in which the feet are not affected, may be an expression not of second or third attacks, in the sense of fresh infections, but of exacerbations of a single infection that persists over months or years. The very variable duration of the foot lesions, sometimes lasting for a few days, sometimes for many weeks, with recurrences sometimes after a short, sometimes after a long interval, is perhaps in favour of this view, although many of these mice remain in apparent health for months after one lesion has subsided and before another occurs. In either case, it is clear that clinical recovery is not always associated with permanent immunity to relapse or reinfection.

Epidemiological History of the Herds

Enough has now been said to make the pathological foundation of the epidemiological study clear and we pass to a description of the epidemiological history of the herds.

These have been two, which we distinguish as Ectromelia 1 and 2.

Ectromelia 1 herd was inaugurated with 20 inoculated and 25 normal mice on 18th November, 1930, and maintained to 19th March, 1934, i.e. for more than three years. Ectromelia 2 herd was inaugurated with 10 inoculated and 25 normal mice on 17th January, 1931, and maintained until 20th October, 1932, i.e. for 1 year, 9 months. In both experiments three normals were added daily.

Over the period through which the two herds existed side by side their experiences were similar. On the whole, Ectromelia 2 had a lower rate of mortality than Ectromelia 1 and its maximum population reached a higher figure than that of Ectromelia 1, viz., 322 in July, 1932, as compared with 262 attained at about the same time in Ectromelia 1. The similarity of the events is, however, evident. After the peaks of mortality invariably found in the early days of a colony of infected mice, both herds settled down to a relatively steady rate of mortality which was low enough to permit a *slow* increase in population. From the beginning of May, 1931, the population increased from 150 to 235 a year later in Ectromelia 1. In Ectromelia 2 the population increased from 190 to 300 in the year ending 1st August, 1932. In both herds this phase ended with the occurrence of a number of deaths from heat stroke during the night of 19th–20th August, 1932, which marked the maximum of a short but intense heat wave. On Tuesday, 16th August, 1932, the maximum air temperature at Greenwich was 77·1°; on Wednesday it reached 88·3°; on Thursday 91·7° and on Friday, 19th August, 98·9° was recorded. The maxima of the three following days were 93·0°, 79·0° and 67·6°. The record of 19th August, 1932, was within 1·1° of the highest temperature ever recorded at Greenwich (100° on 9th August, 1911). As the Ectromelia 2 herd was broken up shortly after this—although not before its mortality had become affected—it will be necessary to rely on Ectromelia 1 for a further account, but, before taking this up, it will be of interest to consider in a little detail what was happening during the stage of virtual equilibrium or rather slow increase.

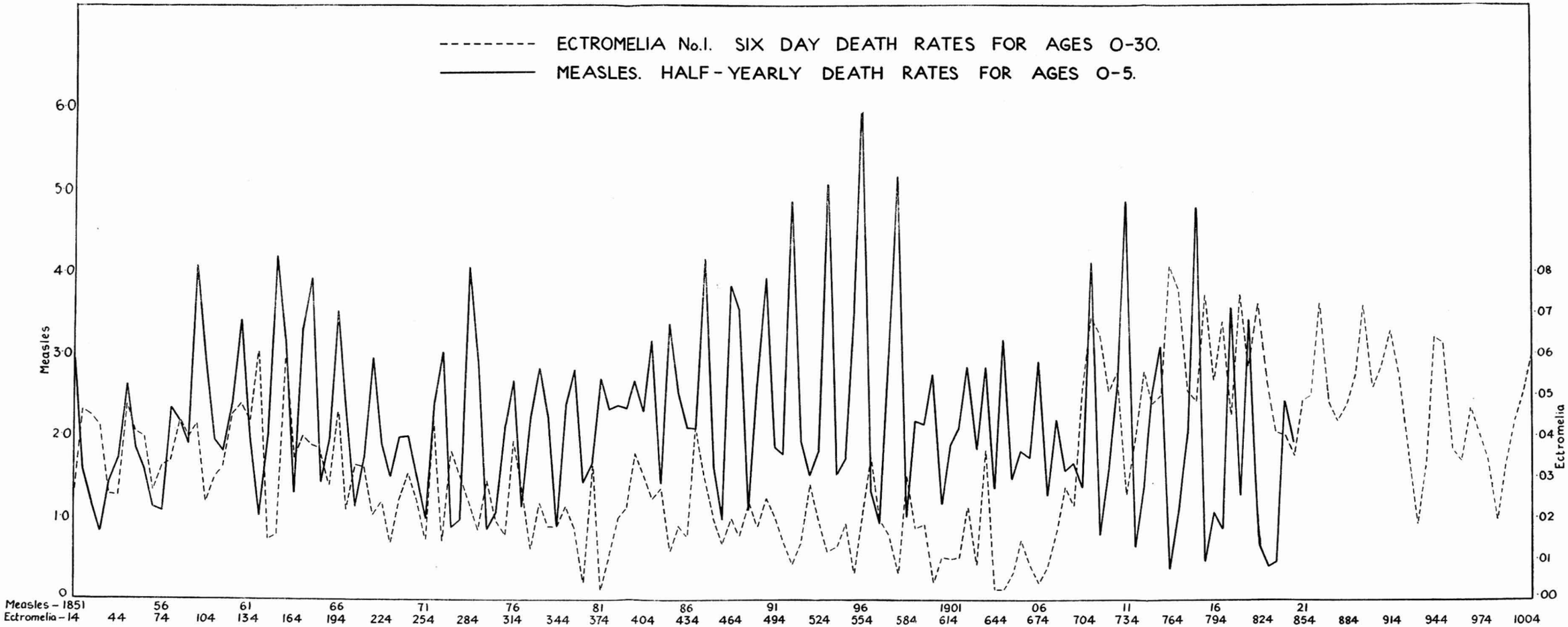


FIG. 8—The secular trend of the death-rate from measles per 1,000 at ages 0–5 years in London from 1851 to 1921 and of mortality in the cage from ectromelia at cage ages 0–30 days over approximately two-and-three-quarter years.

[*To face p. 70.*]

It has been said that through this period rates of mortality were fairly steady. This remark applies to a smoothed average, viz. the daily death rate obtained by taking the average of five days, the central point moving one day forward for each value graphed. Such a moving average is much smoother than a daily death rate—based upon a single day's figures and was indeed adopted with the object of obtaining a less jagged and irregular trend. Further it is based upon an all-age mortality and so, in favourable circumstances, tends to be weighted by the increasing population of mice of considerable seniority in herd, which mice always have a lower rate of mortality than their juniors, especially so in the infection now under consideration. If attention be directed to younger mice the smooth appearance of mortality disappears as the following result demonstrates. In Fig. 8 we have plotted the rate of mortality of mice of age less than 30 days in herd using a time interval of six days and also the mortality from measles in children aged 0–5 years (London, 1851–1921) taking a six months' interval. The time scales were chosen because (as we have often pointed out) on the scales of respective longevities, a month of human life corresponds to a day of mouse life. It will be seen that the ectromelia rates show a succession of peaks which have a certain similarity to those characterizing measles. Human measles is the disease *par excellence* which displays a regular periodicity to account for which various hypotheses have been made, such as, (*a*) that the wave-like movement is determined by the accumulation of susceptibles; when the rate of accession of susceptibles is greater than the rate of their depletion through the occurrence of new cases, a point in time will be reached when the case rate rises and will continue to rise until the balance is inclined the other way and then the case rate declines, a harmonic rise and fall of cases being thus produced; (*b*) that what, in our nomenclature, would be called the infectivity of the virus or organism undergoes secular changes of a periodic character; (*c*) that seasonal factors determine the movement; (*d*) that most of the previous factors and, in addition, variations of resistance of the assumed susceptibles are involved.

Roughly speaking, hypothesis (*a*) is that originally enunciated in quantitative form by Hamer; (*b*) is the hypothesis of Brownlee, who first investigated the form of the periodicity on a large scale; (*c*) combined with (*a*) has found favour with many field epidemiologists and (*d*) has the support of Stocks' recent work, although this author is sceptical of (*b*), at least in the sense in which Brownlee enunciated it.

As we are not concerned in this report with the very complex problem of human measles we have not to adjudicate upon the evidence, but since, in our experiments, the rate of accession of susceptibles is accurately known, we think it will be of interest to describe carefully how hypothesis (*a*) can be formulated and tested in arithmetical terms and shall give an account of the late H. E. Soper's investigation (1929). It is supposed that the disease

with which we are concerned conforms to the following herd conditions.

(1) All who enter the herd are susceptible to it and all equally susceptible.

(2) All new cases are generated from pre-existing cases.

(3) The period of time during which an infected individual is infective is infinitesimally short and an infection produced by him is effective after an interval (the incubation period) which is the same in all cases.

(4) The number of new cases generated in a unit of time does not differ greatly from the number of accessions to the herd in a unit of time.

The imposition of these conditions which (except (2)) are biologically improbable, even impossible, renders the arithmetical formulation simple. To abandon (1) would introduce considerable mathematical difficulties. To abandon either of the clauses of (3) would not fundamentally affect the situation, the result would merely be to blur the sharp outlines of the waves which, as we shall see, are generated by the assumed mechanism.

One first thinks of such a herd as in a state of equilibrium. In each unit of secular time, which we will suppose to be the incubation period of the disease, a new cases of the disease occur and a new susceptibles are added to the herd; the total number of susceptibles present in the herd remains constant. This constant number we may call the steady state quota of susceptibles for the particular herd in equilibrium. As, by hypothesis, all who enter are susceptible and such susceptibles enter in constant numbers in each time unit, we may think of this steady-state quota, m, say, as some multiple of the constant accessions, equal to $s.a$. The s will be a constant characteristic of the herd, if it is very large the conditions of the herd are such that it can support a large population of susceptibles in disease equilibrium, and conversely.

Now one sees that, arithmetically, the postulated equilibrium can be destroyed in one of two ways. At the beginning of any interval, in the state of equilibrium, we have a infecting cases, and the new cases are supposed to be generated by multiplying this a by $\frac{\text{Susceptibles present}}{s.a}$ which, as the numerator is supposed to be $s.a$ produces again a cases; then as a fresh susceptibles are added, the numerator in the next interval will again be $s.a$. But suppose we alter the a existing cases to $a + b$; then our unit multiplier will give us $a + b$ fresh cases and the next multiplier will not be unity, for the numerator instead of being $s.a$ will be $s.a - b$ and at the next stage we shall multiply $a + b$ by a factor less or greater than unity in accordance with the sign of b.

We should equally destroy the equilibrium if, keeping a untouched, we had altered the multiplier by increasing or decreasing the number of susceptibles present in the herd. Whichever course is adopted,

the result will be to change a dead level into a simple harmonic oscillation, such that (assuming that the disturbance is not very great) its period will be precisely equal to $2\pi\sqrt{s}$. In actual practice, the theoretical regularity of oscillation so engendered will not be arithmetically reproduced but, as the following illustrations show, simple arithmetic does give us essentially what the theory requires.

Take for instance a herd for which the equilibrium quota is 100, supporting, with an accession rate of 10, and an $s = 10$, 10 new cases. Let us destroy equilibrium by increasing the number of susceptibles present from 100 to 120 (we might think of this being done by the deliberate introduction to the herd of 20 non-immunes over and above the ordinary additions on one occasion). Then the multiplying factor is changed from 100/100 to 120/100 and the new cases are 10 times $1{\cdot}2 = 12$. The next multiplier is $(120 - 12 + 10)/100 = 1{\cdot}18$, and the new quota of cases $12 \times 1{\cdot}18 = 14{\cdot}16$. The next multiplier therefore is $(118 - 14{\cdot}16 + 10)/100$ and so on. We obtain the third column of Table XVI, with a repetition after 20 terms (the theoretical number is $2\pi\sqrt{10} = 19{\cdot}87$). Had the constant s been 5 and the addition rate 20, then starting as before with a steady state of 20 cases we should reach the figures of the second column with a period of approximately 14 terms. At first sight there may appear to the biologist to be an absurdity involved in this way of looking at the matter.

TABLE XVI

Periodic series.

Unit of time.	$s = 10$, $a = 10$.	$s = 5$, $a = 20$.
1	12	24
2	14	28
3	16	30
4	17	30
5	17	26
6	16	22
7	14	17
8	12	15
9	9	13
10	8	12
11	6	13
12	6	14
13	5	17
14	5	20
15	5	24
16	5	
17	6	
18	7	
19	8	
20	10	
21	12	

Suppose, for instance, that we disturbed the equilibrium not by altering the number of cases in the steady state, nor by increasing or decreasing the numerator, but by changing the *denominator*,

e.g. by doubling the addition rate. Then, in our illustration of a steady state of 100 with cases 10 and additions 10, the result of doubling the accession rate would be instantly to halve the number of new cases. This both looks absurd and *is* absurd. The point is that, under the postulated conditions, s cannot be maintained constant, for to do so would involve the absurdity that by merely increasing the rate of addition we could raise the equilibrium state of the herd, which would be just as absurd as holding that if we increased a population its equilibrium store of provisions to avert scarcity might be left constant. Clearly if we double the rate of addition we must, other things equal, suppose that we halve s and retain the equilibrium quota. What will be changed will be the period of oscillation and of course the equilibrium will be *ipso facto* destroyed, for starting with 10 cases, and a multiplier 100/100, the next multiplier will become $(100-10+20)/100=1\cdot10$ and the case rate will at once begin to rise and a cycle of approximately 14 terms be introduced.

This method then is a quite satisfactory method of describing periodicity on the stated conditions. Since the likelihood of a disturbance from the steady state arising in a small community is great, it would be expected that in any such community harmonic oscillations would certainly be generated. But it would be expected that they would be blurred by quite fortuitous events. If we are concerned—as indeed we are—with populations of the order of magnitude of our arithmetical example, it will be seen that the change of a unit at many stages of the operation would swing the course from one harmonic cycle to a quite different form. Therefore we cannot arithmetically *prove* that this method of description is inapplicable. But we think we can make it probable that it is, in its simple form, unhelpful, that there is not too much but too little oscillation in our small communities. Take, for instance, the information contained in Table XVII. From day 220 to day 340 the population varied but little, we might reasonably call its steady state *total* 169. Now if we identified this total with the steady state number of susceptibles of the previous theory, the position would be that between day 340 and 360 mortality decreased; instead of 60 deaths (three mice enter this herd daily) there were 48, allowing the population to increase from 169 (we assumed this value, for it is the average of the seven values from day 220 to 340 inclusive, but the argument would not be affected if the observed value of 172 were used) to 181. The multiplier of 48 will be $181/169 = 1\cdot0710$, giving for the new quota of deaths $51\cdot408$, and the new population $181-51$ (working to the nearest unit, as we are only illustrating an argument) $+60 = 190$. The next multiplier is accordingly $190/169 = 1\cdot1243$, giving $57\cdot80$ deaths and a new population of 192. Now 190 agrees reasonably well with the observed value of 194 at day 380 and 192 computed for day 400 is not bad agreement with the observed 199, but our multiplier has now reached a value which will make the deaths exceed the accessions, so that the total will begin to decline

steadily. Actually the total increased almost steadily over the next 280 days, and attained a maximum of 262.

It will, however, at once be objected that the total population cannot possibly be identified with the susceptible population of Soper's schema, because it will include an undetermined number of animals which have already passed successfully through an attack, other animals infected and destined to die, others naturally resistant. The suggestion therefore offers itself that we might do better by considering the population aged less than 40 days. These are augmented by the steady accessions and depleted by death or by transfer to a class of seniority which will certainly consist, as to a large majority, of animals which have received an infective dose. Counting from day 100 the populations are :—

100	62.	620	100.
120	70.	640	97.
140	58.	660	102.
160	74.	680	103.
180	60.	700	90.
200	64.	720	50.
220	73.	740	60.
240	84.	760	54.
260	84.	780	47.
280	75.	800	48.
300	81.	820	44.
320	80.	840	49.
340	82.	860	58.
360	89.	880	52.
380	95.	900	49.
400	84.	920	45.
420	79.	940	68.
440	91.	960	53.
460	84.	980	58.
480	91.	1,000	67.
500	87.	1,020	60.
520	92.	1,040	56.
540	92.	1,060	46.
560	92.	1,080	52.
580	87.	1,100	56.
600	87.	1,120	53.
		1,140	50.

The disturbance which, in our view, affected the whole course of the experiment took place after day 640. Down to that point there is no indication of regular periodic movement and if we were to suppose that the steady state population of susceptibles numbered 100, then, as 60 entrants arrive in the unit of time, s must be equal to 1·6667.

The incubation period of ectromelia must be short, probably not more than four days on the average. This is 0·20 of the unit here taken, so that if there were a periodicity its length would be $(1{\cdot}6667 \times 0{\cdot}20)^{\frac{1}{2}} \times 6{\cdot}283 = 3{\cdot}63$. The cycle would be gone

through from maximum to maximum in about four time intervals. Obviously this has not happened. It appears that by no arrangement can a schema with Soper's *s* held constant describe the facts of our experience.

In our view, a more hopeful scheme of generating multipliers is the following. Let us suppose that at the beginning of a unit interval the susceptibles of the herd number N. We shall generate new cases by multiplying N by a fraction which in Dudley's terminology may be called the infection pressure. Its denominator we may still regard as a constant, for its numerator we shall take some function of the number of potentially infective animals present in the herd, viz. animals actually ill of the disease and destined to die, together with carriers. The new quota of cases will be the product of N and this infection pressure. At the next stage N will be depleted by the realised infections and augmented by the constant accessions, while the multiplier will also be modified; but, although the assumptions involved seem to us reasonable, we do not, at present, attach sufficient importance to the results to feel justified in discussing them at length. The present section is, perhaps, a little out of scale, but, as the mathematical analysis of periodic or quasi-periodic phenomena is difficult to follow, we believe that a purely arithmetical survey of an important method may add to the interest of our study.

We shall now return to the description of changes in the ectromelia herds and will begin with a closer scrutiny of the age constitutions set out in Table XVII. In a herd of normal mice shielded from infection, few die and those mostly from violence at cage ages less than 100 days. Such a herd receiving fixed numbers of daily accessions would by the end of 100 days have a fairly stable age composition, viz. about three animals at each cage age under 100. In Ectromelia 1 the age constitution is stable at ages under 10. If none died in the first ten days of cage life the population aged less than 5 (i.e. of recorded ages 0, 1, 2, 3 and 4 days) and at 5–10 (5, 6, 7, 8, 9) should be respectively 15 and 15. In fact over a secular period of 1,140 days, the first total has differed from 15 only four times and only by one unit on each occasion. The second total was precisely 15 thirty-two times, 14 twenty-one times, and less than 14 only four times. During this period the total population of the herd has varied between 50 and 262. So, practically speaking, the absolute population at ages under 10 is invariant. Passing now to the next age group, 10–20, its invariant population would be 30. In the 57 censuses of the table the actual population in this age group has been less than 10 five times, less than 20 twenty-five times, less than 25 forty times, and 25 or more seventeen times. This is very far from invariance; still, between day 300 of the experiment and day 640 (the epoch of the heat wave) the population at these ages did not fall below 25 in thirteen out of seventeen censuses. In the next age group, 20–40, the theoretical number of 60 is never approached closely, although on four occasions the population exceeded 40. The absolute fluctuations are greater than in the younger groups.

TABLE XVII

Age constitution of Entromelia 1 *herd.*

		Age group (days).						
Day.	*Total.*	*0–4.*	*5–9.*	*10–19.*	*20–39.*	*40–79.*	*80–149.*	*150 and over.*
0								
20	50	15	15	20				
40	61	15	14	12	20			
60	84	15	15	13	21	20		
80	104	15	14	21	16	38		
100	111	15	14	15	18	29	20	
120	130	15	15	21	19	25	35	
140	131	15	14	21	8	30	43	
160	148	15	15	21	23	20	43	11
180	146	15	14	19	12	20	41	25
200	157	14	15	17	18	23	38	32
220	170	14	15	21	23	21	34	42
240	170	14	15	24	31	15	28	43
260	169	15	15	26	28	24	17	44
280	161	15	15	22	23	26	15	45
300	169	15	14	28	24	21	16	51
320	171	15	15	19	31	22	18	51
340	172	15	14	26	27	20	18	52
360	181	15	15	28	31	24	16	52
380	194	15	14	23	43	33	11	55
400	199	15	15	24	30	44	16	55
420	194	15	14	25	25	40	23	52
440	216	15	15	27	34	34	41	50
460	218	15	15	25	29	40	49	45
480	225	15	14	27	35	34	52	48
500	223	15	15	25	32	33	50	53
520	232	15	15	25	37	34	40	66
540	252	15	14	29	34	48	37	75
560	262	15	15	25	37	49	39	82
580	260	15	14	26	32	39	52	82
600	230	15	15	22	35	16	47	80
620	250	15	15	27	43	32	37	81
640	254	15	15	28	39	53	22	82
660	253	15	15	27	45	50	27	74
680	256	15	14	29	45	55	24	74
700	231	15	15	22	38	54	27	60
720	137	15	14	13	8	14	20	53
740	142	15	15	20	10	9	17	56
760	131	15	14	16	9	10	19	48
780	112	15	13	14	5	11	15	39
800	90	15	12	15	6	7	10	25
820	67	15	15	9	5	8	6	9
840	66	15	14	13	7	6	5	6
860	80	15	15	15	13	9	7	6
880	81	15	14	12	11	17	7	5
900	86	15	15	9	10	19	11	7
920	78	15	13	11	6	10	15	8
940	101	15	15	22	16	7	17	9
960	92	15	15	7	16	13	18	8
980	108	15	15	17	11	24	14	12
1000	121	15	15	19	18	21	13	20
1020	117	15	14	21	10	15	22	20
1040	110	15	15	9	17	13	24	17
1060	111	15	13	7	11	21	22	22
1080	105	15	15	14	10	14	14	23
1100	108	15	14	16	11	14	15	23
1120	109	15	14	15	9	18	16	22
1140	106	14	14	16	6	15	17	23

We may perhaps regard the period bounded by the 480th and 680th days as one of epidemiological equilibrium; at the beginning the total population was 225, at the end 256, and the maximum was 262; the total population had ceased to increase before the heat wave. The death rate was not absolutely constant; there are indications of waves near the 560th and 590th days. But the waves are rather ripples than waves; the amplitude of deviations from a low average line of mortality is small. The proportions of very young mice fell slightly, that of mice aged 20–79 increased fairly substantially; mice aged 80–149 decreased in proportional frequency, while the oldest mice at first increased, but after day 600 had become almost constant.

One may summarize these results by saying that over the pre-heat wave period (1) mice of age under 10 are almost invariant in numbers. (2) The numbers of mice aged 10–20 fluctuate, but do not vary greatly from the mean, which is 22·9 with standard deviation 4·3. (3) The numbers of mice aged 20–40 and 40–80 fluctuated widely, especially those in the age group 40–80. (4) The oldest mice increased slowly between day 220 and day 500 from 42 to 53, then rapidly from 53 to 82, around which total they fluctuated for 120 days. So during the pre-heat wave phase one has a constant population of very young mice, a fairly stable population of mice aged 10–20, widely fluctuating populations at older ages, with a clear tendency for the proportion of oldest mice to increase, but to increase in spurts rather than uniformly.

We now turn to the events succeeding the heat wave. The mortality rates at ages are set out in Table XVIII. Let us take the results seriatim, beginning with mice at ages 0–10. They suffered severely in the heat wave, and 24 days later there is another peak of mortality. Next over a period of 30 days deaths cease, then there is a resumption of high mortality—a peak at day 800 considerably exceeds the heat wave effect—and thenceforward mortality remains high. It must, however, be remembered that the exposed to risk number at the most 30, so that these secular changes are conditioned by one or two deaths only. Still it is permissible to conclude that, even for the previously invariant group, conditions are somewhat less favourable than before.

TABLE XVIII

Ectromelia No. 1.—Average daily death rates, five days smoothed, in age groups, after heat wave.

Date.	*Day.*	*Cage age in days.*					
		0–9.	*10–19.*	*20–39.*	*40–79.*	*80–149.*	*150 and over.*
20.8.32	642	·054	·040	·030	·046	·054	·031
	3	·049	·048	·026	·052	·056	·029
	4	·009	·008	·020	·018	·011	·003
	5	—	·008	·005	·014	—	·003
	6	—	·008	—	·014	—	—

TABLE XVIII—*continued*

Date.	Day.	Cage age in days. 0–9.	10–19.	20–39.	40–79.	80–149.	150 and over
20.8.32	647	—	·008	—	·014	—	—
	8	—	—	—	·010	—	—
	9	—	·008	·005	·010	—	·003
	650	—	·008	·005	·009	—	·005
	1	—	·009	·005	·009	—	·005
	2	—	·009	·005	·005	—	·005
	3	—	·009	·005	·004	—	·005
1.9.32	4	—	—	—	·004	·009	·003
	5	—	·009	·004	—	·009	·003
	6	—	·009	·009	—	·017	·003
	7	—	·009	·009	—	·016	·003
	8	—	·008	·022	·004	·016	·003
	9	—	·008	·027	·008	·016	·005
	660	—	·007	·028	·016	·023	·003
	1	—	·014	·034	·020	·023	·005
	2	·007	·014	·035	·035	·023	·005
	3	·020	·014	·026	·031	·031	·008
	4	·027	·014	·021	·027	·024	·005
	5	·027	·007	·015	·020	·016	·005
	6	·035	—	·020	·024	·008	·003
	7	·028	—	·020	·012	·008	·003
	8	·014	—	·015	·024	·008	·003
	9	·007	—	·015	·029	·008	·003
	670	·007	—	·015	·029	·024	·003
	1	·007	—	—	·025	·032	·003
	2	·007	—	—	·021	·032	·005
	3	·007	—	·005	·013	·024	·003
	4	·007	—	·005	·008	·024	·003
	5	·007	—	·005	·008	·008	·003
	6	—	—	·013	·012	—	·003
	7	—	—	·018	·012	·008	—
	8	—	—	·022	·008	·008	—
	9	—	—	·022	·011	·016	·005
	680	—	—	·022	·015	·016	·011
	1	—	—	·018	·007	·016	·014
	2	—	·007	·013	·007	·016	·014
	3	—	·021	·004	·007	·016	·014
1.10.32	4	—	·021	·017	·018	·016	·012
	5	—	·036	·030	·014	·023	·006
	6	—	·037	·026	·014	·031	·003
	7	—	·030	·034	·018	·023	·009
	8	—	·015	·035	·017	·031	·009
	9	—	·015	·023	·007	·031	·006
	690	—	·015	·018	·014	·023	·009
	1	—	·029	·023	·014	·023	·012
	2	—	·044	·019	·017	·023	·009
	3	—	·051	·020	·020	·022	·018
	4	—	·059	·026	·024	·015	·028
	5	—	·061	·021	·024	·029	·025
	6	—	·071	·021	·032	·029	·022
	7	—	·057	·021	·025	·029	·020
	8	—	·067	·037	·026	·029	·013
	9	—	·080	·037	·030	·029	·003
	700	—	·065	·043	·023	·029	·010

TABLE XVIII—*continued*

Date.	*Day.*	*Cage age in days.*					
		0–9.	*10–19.*	*20–39.*	*40–79.*	*80–149.*	*150 and over.*
18.10.32	701	—	·039	·044	·027	·036	·010
	2	—	·039	·058	·039	·043	·010
	3	—	·029	·048	·048	·036	·010
	4	—	·046	·068	·050	·037	·011
	5	—	·054	·066	·065	·037	·004
	6	—	·087	·077	·064	·030	·007
	7	·007	·097	·090	·073	·031	·007
	8	·007	·107	·108	·071	·047	·004
	9	·014	·090	·084	·076	·065	·004
	710	·021	·099	·113	·068	·051	·007
	1	·035	·083	·115	·071	·063	·004
	2	·028	·106	·114	·076	·066	·007
	3	·028	·102	·127	·081	·050	·015
	4	·028	·089	·152	·082	·031	·019
1.11.32	5	·034	·108	·131	·082	·031	·015
	6	·020	·128	·125	·091	·020	·015
	7	·027	·123	·132	·071	·010	·011
	8	·027	·143	·098	·058	·010	·004
	9	·020	·182	·100	·049	·010	—
	720	·014	·145	·102	·054	·010	—
	1	·014	·164	·104	·044	·010	·004
	2	·020	·177	·044	·032	·010	·004
	3	·021	·177	·064	·033	·010	·004
	4	·021	·156	·065	·035	·010	·004
	5	·014	·152	·046	·036	·011	·004
	6	·028	·103	·047	·038	—	—
	7	·014	·097	·048	·039	—	—
	8	·014	·092	·024	·043	—	·004
	9	·028	·090	—	·023	—	·004
	730	·035	·088	—	·025	—	·004
	1	·028	·099	·021	—	—	·004
	2	·028	·101	·020	—	—	·004
	3	·028	·080	·019	—	—	—
	4	·014	·081	·018	—	·012	·007
	5	·007	·107	·037	—	·012	·007
	6	—	·080	·020	—	·012	·007
	7	·007	·038	·021	—	·012	·007
	8	·007	·035	·022	—	·024	·011
	9	·007	·052	·023	—	·012	·004
	740	·007	·059	·023	—	·012	·007
	1	·007	·086	·022	—	·012	·007
	2	·013	·096	·020	·021	·012	·011
	3	·013	·098	·058	·021	—	·011
	4	·020	·083	·053	·021	—	·011
1.12.32	5	·021	·116	·065	·021	—	·008
	6	·021	·120	·109	·022	—	·011
	7	·007	·126	·109	—	—	·008
	8	·007	·131	·111	—	—	·008
	9	—	·131	·121	—	—	·008
	750	—	·072	·130	—	—	·008
	1	—	·035	·078	—	·010	·004
	2	—	·033	·080	—	·010	·004
	3	—	·040	·042	—	·010	—
	4	·007	·065	·043	—	·010	—

TABLE XVIII—*continued*

Date.	*Day.*	*Cage age in days.*					
		0–9.	*10–19.*	*20–39.*	*40–79.*	*80–149.*	*150 and over.*
1.12.32	755	·007	·101	—	—	·010	—
	6	·013	·101	·021	—	—	·004
	7	·020	·148	·042	—	—	·008
	8	·020	·158	·064	—	—	·012
	9	·013	·154	·061	—	—	·012
	760	·013	·106	·082	—	—	·012
	1	·007	·121	·085	—	—	·008
	2	—	·100	·087	—	·011	·008
	3	·007	·127	·109	—	·012	·008
	4	·020	·128	·163	·016	·024	·013
	5	·020	·145	·184	·016	·024	·017
	6	·027	·162	·177	·016	·026	·017
	7	·034	·111	·200	·016	·013	·013
	8	·034	·069	·154	·016	·014	·013
	9	·021	·064	·167	—	—	·017
	770	·041	·085	·125	—	—	·018
27.12.32	1	·035	·098	·120	—	—	·022
	2	·029	·146	·083	—	—	·023
	3	·036	·156	·125	—	—	·018
	4	·056	·157	·042	—	—	·009
	5	·036	·152	·042	—	—	·009
1.1.33	6	·036	·143	·040	—	—	·009
	7	·036	·100	·039	—	—	·024
	8	·021	·111	—	—	—	·024
	9	·028	·145	—	—	—	·034
	780	·014	·119	·030	—	—	·030
	1	·021	·103	·029	—	—	·031
	2	·021	·102	·026	—	·012	·021
	3	·020	·100	·024	—	·012	·022
	4	·021	·094	·024	—	·013	·011
	5	·014	·132	·024	—	·013	·011
	6	·020	·129	·023	—	·014	·011
	7	·027	·157	·022	—	—	·005
	8	·027	·169	·021	—	·015	·005
	9	·034	·145	·040	—	·030	·011
	790	·034	·150	·059	—	·048	·016
	1	·027	·175	·082	—	·050	·022
	2	·020	·130	·087	—	·053	·033
	3	·020	·107	·119	—	·037	·034
	4	·007	·102	·111	·019	·019	·035
	5	·007	·078	·065	·019	·019	·036
	6	·027	·085	·033	·040	·036	·057
	7	·047	·092	·035	·043	·036	·053
	8	·055	·152	·036	·070	·036	·077
	9	·063	·160	·039	·049	·038	·075
	800	·071	·145	·040	·050	·039	·073
	1	·043	·169	·044	·026	·021	·060
	2	·021	·167	·048	·026	·044	·063
	3	·021	·123	—	—	·048	·047
	4	·014	·121	—	—	·053	·058
	5	·007	·117	—	—	·029	·080
	6	·014	·065	·037	—	·031	·065
1.2.33	7	·014	·087	·036	·022	—	·067
	8	·007	·067	·071	·022	—	·060

TABLE XVIII—*continued*

Date.	*Day.*	*Cage age in days.*					
		0–9.	*10–19.*	*20–39.*	*40–79.*	*80–149.*	*150 and over.*
1.2.33	809	·014	·084	·111	·022	—	·050
	810	·021	·091	·125	·021	—	·039
	1	·014	·087	·150	·021	—	·040
	2	·021	·074	·177	—	—	·056
	3	·043	·092	·133	—	—	·059
	4	·043	·124	·067	—	—	·094
	5	·043	·141	·063	—	·035	·086
	6	·051	·186	·056	—	·035	·076
	7	·044	·208	·050	—	·035	·041
	8	·029	·200	·046	—	·035	·043
	9	·028	·155	·044	·024	·035	—
	820	·036	·125	·039	·025	·036	—
	1	·036	·093	—	·026	·037	·021
	2	·035	·096	—	·027	·040	·043
	3	·028	·115	·024	·056	·087	·044
	4	·028	·157	·021	·029	·100	·044
	5	·014	·200	·020	·061	·056	·047
	6	·014	·225	·060	·063	·059	·024
	7	·035	·200	·085	·065	·063	—
	8	·036	·170	·071	·033	—	—
	9	·029	·132	·077	·033	—	·025
	830	·028	·151	·081	—	—	·026
	1	·028	·132	·028	—	—	·026
	2	·014	·148	—	—	—	·027
	3	·027	·185	·026	—	—	·028
	4	·034	·189	·054	—	—	—
1.3.33	5	·034	·154	·086	—	—	—
	6	·028	·132	·094	—	—	—
	7	·021	·130	·100	—	—	·029
	8	·007	·109	·067	—	—	·029
	9	·014	·083	·032	—	—	·030
	840	·028	·062	—	—	—	·031
	1	·028	·073	—	—	—	·032
	2	·028	·097	—	—	—	—
	3	·028	·082	—	—	—	—
	4	·014	·081	—	·032	—	—
	5	—	·130	—	·033	·031	—
	6	·007	·130	—	·035	·031	—
	7	·013	·132	—	·037	·030	—

The 10–20 group also lost some lives in the heat wave; the subsequent increase did not begin for 40 days, then the rate of mortality began to mount and climbed to a primary peak indicating a rate of some three times the average pre-August rate by the 719th day; among other peaks there are seen crests at the 748th and 765th, 775th, 791st, 817th, 826th and 834th days; at the end of the period of observation, the mortality rate is still far above the old level. One has the impression of a series of pulses, with little tendency to die out. The story of the 20–40 group is similar, but the movement began earlier and at the end of the period shows

indications of settling down. In both 40–80 and 80–150 groups one has a weakened reproduction of these changes and so one comes to the oldest age group. Here the primary epidemic disturbance is trivial, it is not until the 790th day—that is to a time 100 days after the epidemic began to rage in the younger age groups, a time when survivors of that period would be entering the oldest age—that a distinct wave of epidemic mortality affects the seniors, and one which never approaches the maxima of earlier ages.

If one compares the age composition at the end of the observations with its composition at the day of maximum population, day 560, this is the result. Under 10 we have 28 against 30; from 10 to 20, 16 against 25; 20 to 40, 6 against 37; 40 to 80, 15 against 49; 80 to 150, 17 against 39, and over 149, 23 against 82. A quarter of the mice are less than 10 days old and only 21·7 per cent. are more than 149 days old as compared with 11·5 per cent. and 31·3 per cent. This result is due not primarily to a higher rate of mortality in the oldest age group but to failure to enter that age group owing to heavy mortality in the younger groups, but this primary effect is reinforced by a delayed higher mortality in the oldest group itself. Returning to the secular history of the herd from the acute epidemic period to the end of our observations, the facts are found to be as follows. Population continued to decline down to 24th February, 1933, when the total strength was about 60. A recovery then began and by the middle of June the population was 100; it increased through another month and touched 125 on 13th July, 1933. There was then a decline, but down to the end of March, 1934, when the experiment was closed, the population was fairly stable, fluctuating but little about a figure of 100. The smoothed daily death rate remained at a higher level than in the pre-August, 1932, epoch and was more variable than in the Saturnian age of the herd, the mid-1932 age of high population and low mortality, to which the herd has shown no sign of reverting. Stabilization has occurred but at a lower level of population and a higher level of mortality.

We shall now compare the mortality rates before and after August, 1932, in a different way, viz. on the basis of life tables.

To that end we shall use the following tables (they are collected at the end of the section): (1) A table based on the experience from 18.11.30 to 31.8.32, viz. the whole pre-September, 1932, experience. (2) A table based on the data of 1.3.32–31.8.32, viz. the last six months of the pre-September, 1932, experience. (3) A table based on the experience from 1.9.32 to 28.2.33, viz. the period of very high mortality. (4) A table based on the experience from 1.3.33 to 31.8.33, viz. after the subsidence of the acute mortality which we associate with the events of August, 1932. (5) A table based on the data from 1.7.33 to 31.12.33 and lastly, (6) A table covering the experience from 1.9.33 to 28.2.34. To the tables appended some necessary information as to the days of life available is added. Only in the first table are data available for calculation of mortality

at ages beyond 200 days sufficient to be of much statistical value and at lower ages the data of the six months' tables are sometimes scanty. The standard errors inserted in the summarizing table about to be discussed are based on the smallest absolute number involved.

In Table XIX we have set out the probabilities of survivorship from leading ages corresponding to groupings used above. If we compare the whole pre-September, 1932, period with the six months immediately before September, 1932, there is an indication of some improvement in mortality at early cage ages. If we compare the six months which included the immediate high mortality with the following six months it will be seen that early-age mortality increased slightly while at later ages there was a substantial increase. Comparing the period of high mortality, 1.9.32 to 28.2.33, with its predecessor, 1.3.32 to 31.8.32, the respective probabilities of surviving are as percentages of the predecessors, 89, 58, 76, 70, 48 and 78. It is in the youngest *available* age group and the most senior but one of the groups that the difference is greatest. We speak of the group 10–20 as the youngest *available* group, since the period of effective exposure of mice under 10—bearing in mind that infection must precede death by some finite interval—is short, and in all the compared tables more than three quarters of the entrants do survive the 10th day of residence.

As some independent test of stability we show in Table XX the experience of the Ectromelia 2 herd, inaugurated on 17th January, 1931, and brought to an end on 20th October, 1932 (the full tables are given with those of Ectromelia 1 at the end of the text). As already remarked this herd also suffered from the heat wave and 20 days later its rate of mortality began to rise, but too little time was allowed it (the material was required for another experiment) for mortality to gather head and so the epidemiological conditions of the whole experiment were similar to those of Ectromelia 1 in the period before August, 1932. As will be seen, except possibly in the age group 40–80, where the difference is thrice its standard error, there is little to choose between the two experiences.

The Ectromelia 1 experiment covers a longer period of continuous observation under unchanged conditions of immigration than any other herd we have formed. In secular length it invites comparison with the herd reported on in our 1925 memoir, to which three mice were admitted daily for rather more than two years and then one mouse daily for 16 months. Here also a phase was attained during which mortality was almost constant at a relatively low level so that the herd population increased from 60 to 182; here too a subsequent phase of high mortality was experienced having a certain likeness to the post-August, 1932, experience of Ectromelia 1. But, since in the herd just mentioned the exciting cause of the acute phase was not an intensification of the original infection (*Pasteurella*), but the casual importation of *Bact. aertrycke*, the two experiences are not properly comparable.

TABLE XIX

Probabilities of survival in the Ectromelia 1 *herd at different epochs.*

Probability of surviving from day–	*Period.*				
	18.11.30–31.8.32.	*1.3.32–31.8.32.*	*1.9.32–28.2.33.*	*1.3.33–31.8.33.*	*1.7.33–31.12.33.*
0–10	0·913 ± 0·007	0·951 ± 0·009	0·844 ± 0·017	0·777 ± 0·019	0·770 ± 0·020
10–20	0·662 ± 0·014	0·782 ± 0·020	0·457 ± 0·034	0·391 ± 0·038	0·419 ± 0·038
20–40	0·596 ± 0·019	0·630 ± 0·031	0·477 ± 0·044	0·518 ± 0·055	0·487 ± 0·052
40–80	0·562 ± 0·027	0·527 ± 0·045	0·367 ± 0·057	0·689 ± 0·067	0·653 ± 0·064
80–150	0·708 ± 0·031	0·641 ± 0·047	0·307 ± 0·082	0·785 ± 0·086	0·670 ± 0·075
150–200	0·851 ± 0·028	0·860 ± 0·039	0·673 ± 0·076	0·764 ± 0·134	0·909 ± 0·054

TABLE XX

Probabilities of survival in the Ectromelia 2 *herd from* 16.1.31–20.10.32.

Probability of surviving from day–	*Period. 16.1.31 to 20.10.32.*
0–10	0·904 ± 0·007
10–20	0·648 ± 0·014
20–40	0·612 ± 0·019
40–80	0·673 ± 0·023
80–150	0·786 ± 0·024
150–200	0·836 ± 0·024

Of the Pasteurella series, the experiment designated P 3 N is alone strictly comparable, and this covered only a year's experience. The fundamental difference between this experience and that of Ectromelia 1 in its pre-September, 1932, phase is the lower mortality of the latter at later cage ages, as the comparison of the limited expectations of life (limited to 60 days) of Table XXI makes plain.

TABLE XXI

	$_{60}E_x$				
Cage age in days.	*E1 18.11.30–31.8.33.*	*E1 18.11.30–31.8.32.*	*E1 1.9.32–28.2.33.*	*P 3 N (specific deaths.*	*Normal mice.*
0	28·9± ·35	32·6± ·44	23·7± ·81	29·5± ·54	56·8± ·60
5	25·2± ·35	29·2± ·44	19·6± ·81	26·2± ·56	57·4± ·60
10	24·0± ·37	27·5± ·46	17·9± ·88	24·1± ·59	57·1± ·60
15	27·6± ·43	29·5± ·51	22·1±1·09	22·2± ·62	57·1± ·61
20	31·4± ·49	32·7± ·57	26·2±1·27	24·3± ·72	56·8± ·61
25	34·2± ·54	35·2± ·63	29·1±1·41	28·7± ·84	56·7± ·61
30	35·8± ·58	36·5± ·67	30·3±1·49	31·8± ·95	56·7± ·61
35	37·3± ·62	37·7± ·71	32·7±1·59	33·9±1·04	57·7± ·62
40	38·9± ·66	39·6± ·76	32·9±1·64	36·5±1·13	58·3± ·62
45	40·1± ·69	41·3± ·81	32·4±1·67	38·5±1·22	58·4± ·62
50	41·0± ·72	42·6± ·85	33·0±1·74	40·6±1·31	58·4± ·63
55	41·4± ·75	43·6± ·88	31·3±1·77	40·5±1·36	58·5± ·63
60	42·1± ·78	45·3± ·93	29·0±1·77	40·9±1·42	58·5± ·63
65	43·8± ·81	47·1± ·97	30·2±1·87	41·3±1·48	58·4± ·63
70	44·6± ·84	47·6±1·00	31·1±1·98	43·0±1·57	58·6± ·63
80	47·6± ·91	49·9±1·07	34·7±2·23	40·7±1·64	58·8± ·64
90	48·5± ·96	51·1±1·13	33·9±2·26	40·0±1·75	58·5± ·64
100	50·2±1·01	52·8±1·18	34·9±2·49	41·2±1·92	59·1± ·64

At this point it will be convenient to compare a little more attentively the course of events in the pre- and post-August, 1932, phases of Ectromelia 1.

We have plotted on the chart (Fig. 9) the probabilities of dying within the next five days from age points 0, 5, 10, etc., assigned by the life tables constructed from the experiences of 18.11.30.–31.8.32 and of 1.9.32–28.2.33. We also give for comparison the results of the P 3 N experience (data from 24.2.27 to 24.2.28). The first of these is based on 114,565 mouse-days at risk, the second on 28,004 and the third on 39,864. During the periods the numbers of mice involved were 1,956 ; 781 ; 1,095.

The mortality of the post-August, 1932, period has three well-defined peaks at age 10, at age 30 and at age 45 ; these peaks are of decreasing height. The mortality of the pre-September, 1932, period has two peaks at 15 and at 35 of decreasing height. The P 3 N graph has a peak at about 17, another loftier one at about 23 and then mortality declines. Perhaps the points are more clearly brought out by the small table (Table XXII) which records the probabilities not of dying but of surviving for five days.

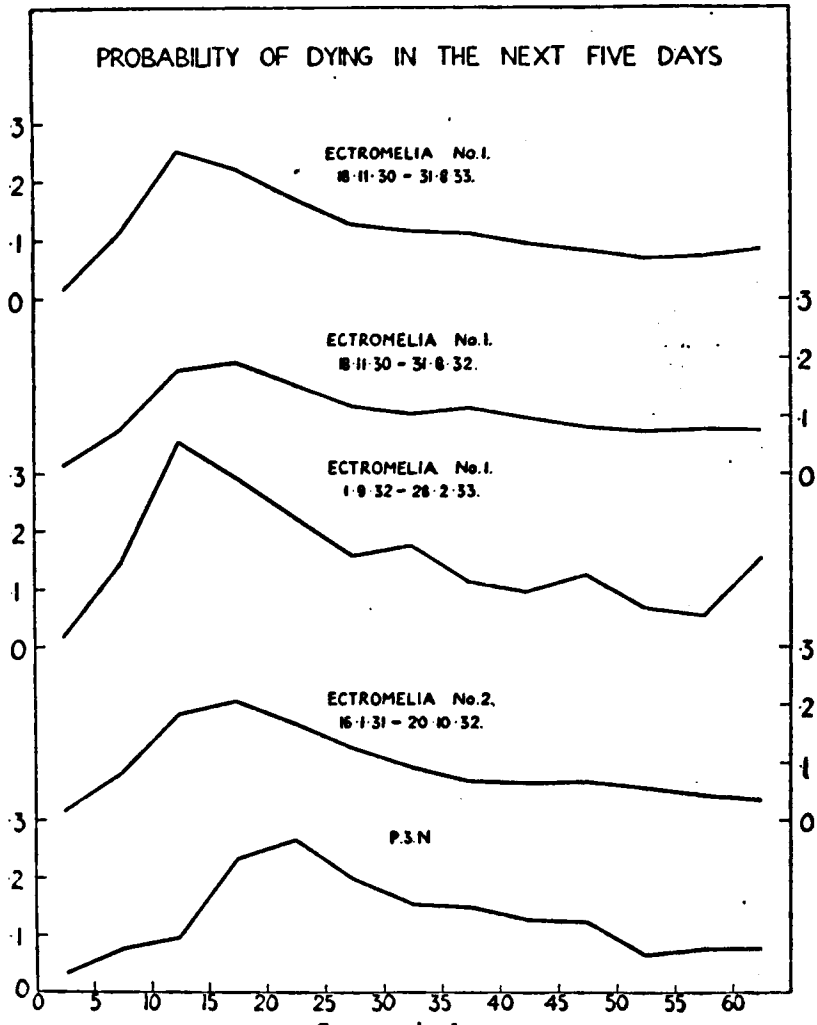


FIG. 9.

After age 50 the P3N mortality is higher and more fluctuating than that of the pre-September, 1932, experience of ectromelia. For reasons set out in our memoir of 1925 no stress can be put on the triple comparison at later ages, since the errors of sampling increase rapidly. We are, however, justified in concluding that the course of mortality with age differs profoundly as between ectromelia on the one hand and pasteurellosis on the other, while in the phases of the ectromelia experiment there is not only a difference in amplitude, of actual severity of mortality, but in the post-September, 1932, period there is a shifting forward in age of the stress of mortality. We may say that in passing through life the member of a herd

infected with ectromelia has to traverse certain danger zones, one about 15 days from entrance, another some 20 days later and probably others in later life (for instance at or about the 65th day of life). Under the post-August, 1932, conditions these crises are somewhat earlier in life.

TABLE XXII

Comparison of the probabilities of surviving five days at different ages between (1) *Ectromelia* 1 *during the period* 18.11.30–31.8.32. (2) *Ectromelia* 1 *observed during* 1.9.32–28.2.33. (3) *The P 3 N herd* (*Pasteurellosis*).

Cage age in days.	*Probabilities of surviving five days from the age stated in the first column.*		
	(1) (*all deaths*)	(2) (*all deaths*)	(3) (*specific deaths*)
5	0·9255±0·006	0·8578±0·016	0·9226±0·006
10	0·8204±0·010	0·6451±0·028	0·9036±0·007
15	0·8068±0·012	0·7077±0·031	0·7689±0·010
20	0·8456±0·012	0·7770±0·031	0·7350±0·012
25	0·8856±0·011	0·8416±0·029	0·8040±0·013
30	0·8979±0·011	0·8220±0·032	0·8492±0·013
35	0·8860±0·012	0·8878±0·028	0·8513±0·014
40	0·9022±0·012	0·9029±0·026	0·8756±0·014
45	0·9197±0·012	0·8749±0·031	0·8791±0·015

We have now described in, we hope, sufficient detail the course of this lengthy experiment. Before passing to a discussion of possible interpretations, it will be convenient to summarize the leading facts. As in all our work there are two aspects to be considered, the special and the general. The special aspect is that concerned with factors of changing secular mortality. How is an epidemic generated? By what mechanisms is a change from a steady, low rate of mortality into a high and varying mortality effected?

The general aspect is that concerned with the factors which, when the results of varying secular mortality are aggregated into, for instance, a life-table form, determine the relation between age and mortality characteristic of the particular type of infection.

As usually, perhaps always, happens in the natural sciences, the distinction between the two classes of phenomena is far from absolute; we have indeed just seen that the relation between age and mortality is not precisely the same at two epochs of the same experiment. Yet for the sake of orderly exposition it *is* convenient to make the separation. The ascertained facts of temporal succession in the ectromelia herd are these.

After the initial fluctuations of mortality usual when an infected herd is started, the herd settled down to a condition in which mortality although not constant exhibited no clearly defined periodicity and was not sufficient to balance the accessions to the

herd, which increased more than threefold in a period of rather more than 20 months. At that point it seemed—but the period of observation was too short to allow us to say more than that it *seemed*—as if equilibrium had been reached. Then, for three or four days, the herd was subjected to unusual heat which directly caused the death of some 10 per cent. of its members. There was a pause; then between the 20th and the 40th day from the catastrophe mortality from ectromelia began to increase, there was a series of waves of specific mortality, not affecting all age groups equally, affecting the very youngest and the very oldest least, but leaving none unaffected.

After the first severity had declined, the herd, now greatly depleted, did not recover its old position. The rates of mortality on the older mice, at ages 40 days and upwards, did indeed recover almost completely, but mortality among the younger mice, particularly in the age group 10–20, continued to be far more severe than before August, 1932. Such being the principal facts, how may they be interpreted?

The first question to be answered was whether the older and, in previous experience, resistant animals were in the period after the heat wave really dying of ectromelial infection. The best evidence available on the point is that afforded by the post-mortem findings. In an earlier section of the report we have recorded the frequency of the characteristic lesions of ectromelia in mice dying at different cage ages from the beginning of the experiment to the occurrence of the heat wave. Table XXIII compares these frequencies with those observed during two subsequent periods, October, 1932, to June, 1933, when the death rate was on the average high and the advantage of the older inhabitants relatively small, and July to December, 1933, when the general death rate was lower and the older inhabitants had regained much of the resistance possessed before August, 1932.

TABLE XXIII

Showing the proportion of mice dying in Ectromelia 1 at different cage ages diagnosed on post-mortem appearances as having died from ectromelia; for three different periods of the epidemic.

	Nov. 1930–Aug. 1932.		*Oct. 1932–June 1933.*		*July–Dec. 1933.*	
Cage age in days.	*No. in group.*	*Per cent. positive.*	*No. in group.*	*Per cent. positive.*	*No. in group.*	*Per cent. positive.*
0–10	154	60·4	113	77·9	79	73·4
11–30	819	67·8	435	81·4	217	78·8
31–50	239	69·0	70	80·0	21	28·6
51–75	137	72·3	58	74·1	19	21·1
76–100	49	46·9	32	62·5	5	20·0
101–200	71	33·8	35	42·9	13	0·0
201 and over	96	13·5	73	19·2	13	7·7
Total ..	1,565	62·1	816	72·3	367	65·7

A glance at the table will show that the period October, 1932, to June, 1933, was marked by a higher percentage of positive findings at necropsy in all age groups, but the frequency of positive findings decreases at the higher cage ages as before. As judged by these findings for the October, 1932, to June, 1933, period just over a quarter of the mice dying after 100 days' residence in the cage succumbed to ectromelia, as against rather over four-fifths of those dying after a residence of 10–50 days. Both these figures are, of course, subject to correction by the additions considered earlier in this paper; but it seems probable that the total death-rate figures are still over-estimating the ectromelia death rate at late cage ages, though to a less degree than during the pre-heat-wave period.

The figures for the final period are curious. The frequency of positive post-mortem findings in mice dying at cage ages under 30 days remains high—higher than during the earliest period and almost as high as in the period of maximal severity—but at later cage ages the frequencies drop sharply and progressively. The numbers in any one group are small, and too much reliance must not be placed on the percentage figures; but there can be little doubt that the differences taken as a whole are significant. It would seem that, during this final phase, very few mice that have survived for 100 days or more have subsequently died of ectromelia.

Interpretation of the Results

An obvious working hypothesis, to account for the relative ineffectiveness of contact immunization during the months following the heat wave of August, 1932—as shown by the greatly increased mortality at later cage ages—and its renewed effectiveness at a later period, would be the assumption of an antigenic variation in the virus. If such a change had occurred in the early autumn of 1932, the old inhabitants, resistant to the virus in its unaltered form, might well have been as susceptible as newcomers to the variant strain. As time went on active immunization against the variant would proceed *pari passu* with infection, and we should reach a new equilibrium similar to that established in regard to the original strain at an earlier epidemic phase. This hypothesis is, however, not in accordance with the experimental evidence. In connection with other investigations, recorded in a later section of this report, we have carried out numerous experiments on the active immunization of mice against ectromelia. If liver filtrates are treated with 0·2 per cent. formol they lose their infectivity within a few days, but retain some immunizing power for a longer, but by no means indefinite period. A considerable series of empirical trials have shown that the injection of 0·1 c.c. of a formolized filtrate that has been stored in the ice chest for ten days, followed one week later by the injection of the same amount of a similar filtrate that has been stored for three days, will usually render a mouse resistant to a massive dose of active virus (0·1 c.c. of an undiluted phenolized filtrate). Using this method we have carried out cross-immunity tests with

the virus isolated from Ectromelia 1 during the post-heat-wave period of high death rate, a second sample of virus obtained from the National Institute for Medical Research, where the original strain isolated by Marchal had been maintained by occasional passage through mice, and a strain kindly sent to us from Manchester by Professor Maitland. In each test 20 immunized and 20 control mice were employed. The results, which appear quite unequivocal, are set out in Table XXIV. Each strain of virus protects as well against each of the two other strains as against itself. There is no evidence of any significant antigenic variation.

TABLE XXIV

Showing the number of mice dying in various groups of 20 immunized or control mice injected with different strains of ectromelia.

Strain used for immunization.	*Strain employed in testing immunity.*		
	Ectromelia 1	*National Institute.*	*Manchester.*
Ectromelia 1	1	1	1
National Institute	1	0	0
Manchester	1	1	1
Nil (controls)	20	20	20

These results do not exclude the possibility of a change in the virus in the direction of increased virulence or infectivity. The virus with which the epidemics were started was, as judged by ordinary standards, of high virulence. The routine method we have employed in testing our phenolized liver filtrates has been to inject 0·1 c.c. of ten-fold increasing dilutions intraperitoneally. Five mice were used for each dosage, the range extending from 10^{-1} to 10^{-5} or 10^{-6}. The larger doses (10^{-1} or 10^{-2}) proved almost uniformly fatal in 4–6 days. Smaller doses (10^{-3} or 10^{-4}) were usually fatal but death was sometimes delayed (7–12 days). A dose of 10^{-5} killed a varying proportion of mice, a dose of 10^{-6} usually none. With the lower doses, however, results have been inconstant and fluctuating when different filtrates were compared. It is clear that with no possibility of securing that one filtrate in the undiluted form contains as many particles of virus as another, this method of titration must be crude. It is indeed a statistically impossible task to test an adequate number of samples of virus drawn from the herd at different stages of the epidemic.

Suppose that both personal survival and infectiveness towards others depend, once personal infection has occurred, on a fluctuating equilibrium between parasite and host. This might be conditioned by the reception of repeated doses of infection from without—too large or too rapidly repeated doses perhaps leading to lowered resistance or even to a " relapse ", smaller or more widely spaced doses to an increase in resistance. It might, if we take the view that those mice that become infected, but do not die, harbour the virus

for long periods within their tissues, be conditioned by some external event that temporarily lowered their general resisting powers, or by some change in the host's tissues not directly connected with any outside happening. In either case our older mice would sometimes be more liable to relapse, sometimes more liable to reinfection, sometimes more liable to infect others because the virus, for the time being, would be multiplying more freely in their tissues and very possibly escaping more freely to the world outside. If we assume that the result of the heat wave was to shift the equilibrium in favour of the virus, without the shift being so decisive that the deaths from ectromelia were immediately increased, the result would be an increase in what Dudley (1929) calls the " infection pressure " within the herd. New entrants to it would be faced with an environment much less favourable than that their predecessors experienced, and mortality at early ages would increase. Actually mortality increased at comparatively late as well as at early ages, but, on the hypothesis of fluctuating resistance, there will be periods of susceptibility dispersed throughout a large part of cage life, and, as we have seen, our figures suggest that such periods of increased susceptibility do in fact occur. The fact that the older mice were affected, *qua* death, only after long delay and then for a shorter period (*see* Table XIX) is explicable on the assumption that the fluctuating equilibrium tends towards, though it never actually reaches, a steady level so high as to exclude the risk of fatal infection. It is, of course, very probable that many of these old inhabitants are liable to minor fluctuations in their infection-immunity equilibrium which render them more liable to harm others, though they are in no greater danger themselves.

We may reach the same result with a change of metaphor. If this is a case of droplet infection and if we suppose that death results on the average from the receipt of more than a certain number of doses in a unit of time and immunity results from receiving less than this number, then the survivors at any age will consist of three categories. (1) The naturally immune. (2) Those who have already received immunizing doses. (3) Those who have only received sub-immunizing doses, or, in a few instances, no dose at all. With the advance of time categories (1) and (2) will increase proportionally to (3). If we suppose that the external factor, the heat wave, so affected all infected mice that their rates of discharge of infective droplets increased, then class (3) will be reduced by deaths from fatal infection or by the passage of mice into class (2), while the new entrants to the herd will be exposed to a more concentrated fire of infection and necessarily succumb at a faster rate.

This seems a reasonable enough interpretation of the facts, but it wholly lacks quantitative precision. We can hardly give it quantitative precision without further *ad hoc* experimentation. For instance, did the relatively high proportion of old mice in the population at the time of the environmental disturbance constitute a favourable or an unfavourable influence on the evolution of the

epidemic? What would be the effect of varying the age constitution? These are questions to which we shall seek by further experiment to find answers. One point of analogy with human experience it is permissible to notice. On the time scale of mice, a day corresponds to at least 30 human days,* so that the period of stability of low regular death rates and growing population, say from the 100th to the 700th day, would be equivalent to a human experience of 49 years, longer than the period of very low death rates from scarlet fever—a period of about 25 years ending in or about 1830—which led our great grandparents to triumph prematurely over this foe. It has been followed by, on the translated time scale, more than 15 years of fierce epidemicity and some 30 or more years of relatively high mortality (the post-1830 epoch of virulent scarlet fever lasted more than 40 years). But in our cages there has been no *new* introduction of disease, there has been no lasting change in the environmental condition, there has been just one heat wave. Our experience down to August of 1932 was so uniform, there was such concordance between two distinct herds, that even sceptical experimenters might have believed that stability had been attained. As the event proved, the equilibrium was extremely unstable.

We pass now to the general problem, viz., of the relation of age in herd to mortality. The most striking apparently decisive result of our experience down to August, 1932, was the very high degree of immunity attained by mice of long seniority. For the first time in our experience, we had reached a population of survivors who died at a rate not widely different from that of unexposed animals.

In the experience of the first 21 months of the Ectromelia 1 experiment, at all ages above 85 days the expectation of life limited to 60 days exceeds 50 days, i.e. is more than five-sixths of its maximum possible value. In Ectromelia 2 (an experience of one year and nine months) the expectation exceeds 50 at every age beyond 65 days and exceeds 53 days at every age beyond 80 days. The experience of the post-heat-wave period was, as we have seen, very different, less favourable than that of the P 3 N experiment at most ages. The fact that the mice in an ectromelia-infected herd, although subjected to no greater, indeed slightly, but only slightly, less mortality in early life than the inhabitants of a *Pasteurella*-infected herd, show at older ages so much greater resistance might at first seem a strong argument against the belief that the decline in rate of mortality with age is due to the elimination by death of the less resistant animals.

* Although this translation seems to have the authority of William James (see W. James, *Principles of Psychology*, Vol. I, p. 639) we must not be taken to regard it as exact. It cannot be true that the *tempo* of all the biological processes in different species of animals varies in exact proportion to the mean duration of life and, as we have pointed out at length, the analogy between the communal lives of mice and men is faint. We think, however, that it is justifiable to hold that our secular experience of mice ought to be measured for epidemiological purposes by some function of the average length of individual life.

A little reflection, however, shows that one can infer no more than that *if* the effects were due to selection, the distribution of innate resistance to ectromelia is different from that of resistance to the other infections. Thus suppose all animals were infected immediately on entrance to a herd and that the time of survival were a function of natural resistance alone (both, of course, extravagantly improbable assumptions). We have seen that in both pasteurellosis and ectromelia infections about 50 per cent. of the entrants survive to day 25. Then in both cases the population surviving to the 25th day consists of the individuals with natural resistance equal to or greater than the natural resistance of the median (we suppose that of 101 individuals, the 50 least resistant are dead by day 25). This equality between the two sets does not imply that the survivors must die out in the same way; it might be, for instance, that the 10 per cent. having the greatest natural resistance to ectromelia can survive much longer in the herd than the 10 per cent. having maximal natural resistance to the other infections.

Of course, in reality, the position could not be so simple. All animals are not infected simultaneously, the chance of becoming infected at any given moment cannot be wholly determined by natural sensitivity—chance, in the colloquial sense of the term, *must* play a part—but the principle would be the same, viz. that ultimate mortality would depend on the distribution of natural resistance.

It is not, we think, possible by analysis of life tables to differentiate satisfactorily between the results of selection and immunization. We can only say that with respect to ectromelia mice have either a different distribution of innate resistance from that which they possess with respect to pasteurellosis or mouse typhoid, or a greater potentiality of becoming immunized. A differentiation of hypotheses must be established by means of other evidence (*see* pp. 139 et seq.).

It is, perhaps, justifiable to regard the observed difference in behaviour between ectromelia on the one hand and mouse typhoid and pasteurellosis on the other as supplying further indirect evidence in favour of the view that immunization is more important than selection, since the acceptance of the full genetic hypothesis requires the assumption, which can be neither proved nor disproved, that genetic resistance against the virus and bacterial infections shows a different kind of distribution, and must hence depend, in part at least, on significantly different factors, while the immunization hypothesis is in entire accord with the general experience that virus diseases, as a whole, tend to confer a more effective immunity than bacterial diseases.

We now return to what we spoke of above as the *general* aspect of this inquiry, viz. the relation between cage age and mortality.

The influence upon the rate of mortality of advancing age and the possibility of testing various biological hypotheses by comparing the numerical consequences of the hypotheses with observed facts are

subjects which have attracted many students of human mortality (cf. Greenwood, 1928). The first attraction was utilitarian, viz. due to a wish to facilitate the computation of annuities on lives, in days before the invention of practical calculating machines. Those who are inclined to take too superior a view of *merely* utilitarian research should reflect that the only " law " of mortality which has had any success was not discovered by a biologist but by an actuary; that " law " was the formula known as the Makeham-Gompertz law, which postulates that the force or intensity of mortality at any age x is the resultant of two factors, one independent of x altogether, the other increasing in geometrical progression as x increases in arithmetical progression. It is now known that human mortality—so far as expressed in the life tables founded on general mortality registers or in the more select experience of Life Assurance Societies—is not capable of adequate representation by the Makeham-Gompertz formula, in the sense that the deviations between the observed rates of mortality and those postulated by the " law " (the constants of the formula are supposed to have been deduced from the data by an efficient method of statistical determination) are very unlikely to have arisen as mere random deviations. Therefore, remembering that, in these times of mechanical and tabular aid to computation, labour-saving formulae are not as important as they were 100 years ago, the once famous " law " has lost a good deal of its practical importance in actuarial circles (except perhaps circles of examiners and circles of students; the deduction of the formula and deductions from it, are of educational value) and " laws " of mortality are abandoned to biologists who sometimes re-discover, if not Gompertz's " law ", some less convenient, but fundamentally similar formula and are gratified because it gives a passable representation of the facts. As one of us pointed out (*see* Greenwood, 1928, *op. cit.* p. 282), had actuaries had to deal with hundreds instead of hundreds of thousands of years of life at risk, then the Makeham-Gompertz formula might have been regarded by them almost as a *real* " law " of nature. What destroyed it was wide experience.

Our problem is in form similar but in substance different from that with which Gompertz was concerned. Gompertz was interested in the problem of physiological senescence. In our infected herds, physiological senescence is of little more importance than it would be in a life table of mortality in the front line trenches during the Great War. Half the entrants to our herds are dead by the 25th day of membership and our experience of the mortality of mice not exposed to infection, scanty as it is, is quite adequate to make certain that at an age which in an infected herd would correspond to the death of more than one in ten, only a tiny fraction of the population in the uninfected herd would have died. In the infected herd the factor of *cage* age is the reflection of wholly different circumstances, viz. some processes of infection, and of selection. These factors are intrinsically, from the epidemiological point of view, of much greater interest than the physiological factors. If we

could discover the "law" of their evolution, we should be much nearer the secret of the evolution of an infective process and of the true nature of herd immunity. Hence an attempt to reach such a "law" is clearly worth making. The process of constructing a life table involves the averaging out of secular variations of mortality. Since the secular rate of mortality varies, it is not true that mice which were aged x days in, say, July suffered the same rate of mortality between the age of x and $x + 1$ days as mice which attained the age of x in August. If we use the experience of August and July for determining the rate of mortality at age x, we are averaging the results of 62 different secular exposures. If, for the compilation, we choose a period throughout which there are no great fluctuations of secular mortality, the average so obtained is more representative of the actual conditions than if we combine experiences under widely different conditions. This is, of course, a truism, but requires stating because, in the special case of Ectromelia 1, as we have seen, there is so great a difference between the experience of the herd before and after August, 1932, that the combination of all the observations in a single life table is, from the present point of view, a doubtfully legitimate operation.

Even when the period of observation is one of reasonably uniform secular mortality, it is evident that as cage age increases the secular range of experience decreases so that the life-table rates of mortality at earlier ages are more representative of average conditions than those of later ages.

Assuming that we have based our life table upon a period of observation which is, from the point of view just explained, adequate, we may be said to be dealing with entrants to an average environment, the conditions fluctuating but little. In these circumstances we may liken the entrants to soldiers passing across a field of fire which is effectively constant. Now if that were really the state of affairs, it would follow that the proportion of unhit members of the herd would decrease in geometrical progression. The "law" applicable would simply be Poisson's limit to the binomial and the proportion of unhit, once hit, twice hit, etc. members of the herd would be given by the terms of $e^{-\lambda}\left(1 + \frac{\lambda}{1!} + \frac{\lambda}{2!} + \frac{\lambda}{3!} + \ldots.\right)$.

Hence it follows that the numbers remaining untouched by the bacterium or virus after one, two, etc., units of time (say days) will be given by the decreasing geometrical series $e^{-\lambda}, e^{-2\lambda}, e^{-3\lambda}$.

If all those receiving one or more doses in the unit of time died within that unit, our life table would have for its l_x's a series of terms decreasing in geometrical proportion and q_x would be constant. That has no resemblance whatever to the facts; the rate of mortality increases rapidly with cage age, but not uniformly, and eventually diminishes to become approximately, very approximately, constant. In Ectromelia 2, for instance, the probability of dying in the next five days is 0·0166 at cage age 0, 0·0811 at cage age 5, 0·1829 at

cage age 10, 0·2067 at cage age 15, 0·1698 at cage age 20 and as little as 0·0371 at cage age 100.

It is not necessary to weary the reader with a detailed account of failures. We have not been able to represent the facts by any function of a single parameter and, from the biological point of view, it would be very unreasonable to expect such a success. Since it is not our intention to attempt any subtle mathematical study—which we do not feel competent to undertake and for which, in our view, the time is not yet ripe—we propose to examine in some detail the biological *quaesita* and to make a few arithmetical suggestions.

Before we do so it is necessary to define our terms; and this is an awkward business, because such terms as "infection" or "infected" have, in common usage, a wide and ill-defined content, which must, for our present purpose, be divided into sub-categories differentiated from one another as precisely as possible. As an alternative to coining new terms we must pay the necessary price in verbal clumsiness.

We have to describe and consider at least three different events—the receipt by an individual host of a dose of the parasite irrespective of whether that dose has any significant effect whatever; the receipt of an effective dose of the parasite, which may result in a fatal infection, or in an alteration, either an increase or decrease, of the host's previous state of resistance; and the induction in the host of an illness leading to death, whether by the receipt of a dose in excess of some lethal limit or by the activation, from any cause whatever, of a pre-existing infection.

It will, perhaps, best avoid confusion if we use the terms "infection" and "infected" to denote any significant change in the host—death, illness or a change in specific immunity—and discuss the problem of the transference of parasites from host to host in terms of the dose transferred. By a "dose" without prefix, we can indicate the receipt by the host of an unspecified number of parasites irrespective of their effect. By a "*sub-effective dose*" we can indicate a dose that is assumed to have no effect whatever on the host's immunological state; by an "*effective dose*", a dose that changes this state in some unspecified way; by a "*sub-lethal dose*," a dose that increases or lowers resistance, and perhaps renders the host liable to the activation of the associated latent infection by non-specific agencies, but does not, in itself, induce an acute fatal infection; by a "*lethal dose*", a dose that initiates an illness that results fatally within the ordinary limits of an acute attack of the disease in question.

It is essential to bear in mind that these are not definitions in terms of size of dose, but of the effect produced. A dose that is sub-effective for one mouse may be lethal for another. It is possible, if improbable, that there may be mice for whom any dose likely to be received will be sub-effective. Moreover, the relation between dose and effect will vary from time to time for any one mouse. In

general, as a mouse extends its experience of life in an infected herd and thus acquires an active immunity, any given dose, specified in terms of size, will, in respect to that mouse, tend to pass from the lethal, through the sub-lethal, to the sub-effective category. But there is good reason to believe that the immunity of any host may wax and wane; so that a given dose may also shift, in respect to a particular host, from the sub-effective to the sub-lethal class, or from the sub-lethal to the lethal, though a shift in this direction is probably less frequent, and perhaps less wide.

We also need a term to describe the event of the parasite reaching the host that carries with it no implication as to the result of such contact, and "dose reception", or "reception", with "dose" understood, would seem to serve our purpose. Similarly an animal that has received a dose may be referred to as a "recipient", with the clear understanding that some recipients may remain quite unaffected.

Thus we should classify our mice as follows:—

Infected.	*Uninfected.*
Animals that have received an effective dose, comprising two categories:—	(1) Animals that have received no dose.
(1) Those that have received a lethal dose.	(2) Animals that have received a sub-effective dose.
(2) Those that have received a sub-lethal dose.	

It seems in the first place probable, perhaps certain that *one* factor of the situation is chance. The entrants to the average herd (the life-table presentation is a presentation of *average* conditions) are all uninfected and for the remainder of their lives in the herd are exposed to a certain average risk of dose-reception which under the present method of consideration we may regard as a constant risk. Hence it seems reasonable to believe that the passage from the non-recipient to the recipient class is effected by a constant multiplier. But (*a*) the entrants differ in innate quality, (*b*) the fact of reception may alter responsiveness in different ways.

We shall first consider some experimental results. Table XXV records the after histories of 66 mice each of which, having been exposed naturally to infection, and having survived an additional period of 63 days in isolation, received 0·1 c.c. of phenolized ectromelia virus.

It will be seen that 12 of these mice died within the first five days after inoculation, 24 in the next five days and only 2 in the next five days. This is direct experimental testing of mice already selected or immunized (or both) by injecting them with a single large dose of virus. Expressed in life-table form it gives a much smaller survivorship through the first two five-day periods and a much larger survivorship through the third than the ectromelia herds. l_5 is only 8,182 and l_{10} 4,545, l_{15} is 4,242. This is what we

should expect because, although these mice have higher resistance, they have all received a large dose and all received that dose at the moment of coming under observation.

TABLE XXV

Mice exposed to infection and subsequently injected with 0·1 c.c. phenolized ectromelia virus.

Out of 66 mice :—			*Per cent.*
No. dying on day	..	1 = 0	—
,, ,,	..	2 = 2	3·0
,, ,,	..	3 = 0	—
,, ,,	..	4 = 4	6·1
,, ,,	..	5 = 6	9·1
,, ,,	..	6 = 11	16·7
,, ,,	..	7 = 8	12·1
,, ,,	..	8 = 5	7·6
,, ,,	..	9 = 0	—
,, ,,	..	10 = 0	—
,, ,,	..	11 = 1	1·5
,, ,,	..	12 = 0	—
,, ,,	..	13 = 1	1·5
,, ,,	..	14–24 = 0	—
,, ,,	..	25 = 2	3·0
,, ,,	..	26 and 27 = 0	—
No. surviving to day		28 = 26	39·4

Table XXVI, based on 554 observations and showing the effect of the inoculation of ectromelia virus into normal mice, is rather striking.

TABLE XXVI

Mice tested with various filtrates from mice suspected to have died from ectromelia. 3–5 mice tested with each filtrate, and only where one or more mice died of ectromelia have the figures been included in this series.

Out of 554 mice :—			*Per cent.*
No. dying on day	..	1 = 1	0·2
,, ,,	..	2 = 3	0·5
,, ,,	..	3 = 12	2·2
,, ,,	..	4 = 61	11·0
,, ,,	..	5 = 146	26·4
,, ,,	..	6 = 88	15·9
,, ,,	..	7 = 59	10·7
,, ,,	..	8 = 38	6·9
,, ,,	..	9 = 31	5·6
,, ,,	..	10 = 21	3·8
,, ,,	..	11 = 14	2·5
,, ,,	..	12 = 9	1·6
,, ,,	..	13 = 9	1·6
,, ,,	..	14 = 4	0·7
,, ,,	..	15 = 2	0·4
,, ,,	..	16 = 1	0·2
,, ,,	..	17 = 1	0·2
,, ,,	..	18 = 2	0·3
,, ,,	..	19 = 0	—
,, ,,	..	20 = 0	—
No. surviving to day	..	21 = 52	9·4

In this case the average inoculum is less potent, but as the data are confined to batches in which at least one inoculee perished of ectromelia, they may be regarded as having received a potentially infective dose of virus and had experienced neither prior selection nor immunization; consequently their mortality rate is enormous, nearly 41 per cent. die within five days and of the survivors nearly 72 per cent. die in the next five days. At the end of 20 days only 52 or less than 10 per cent. of the exposed survive. In Ectromelia 1 in the pre-August, 1932, phase 60 per cent. survive 20 days, and even in the worst period, 1.9.32–28.2.33, nearly 40 per cent. live as long as this. The inoculated survivors (Table XXV), of course, make a more favourable showing; their early mortality is indeed heavier than that of Ectromelia 1 even in its worst phase. Only 46 per cent. survive 10 days compared with 84 per cent. in Ectromelia 1 at its worst. But only two more deaths occurred in the next 10 days, so that 42 per cent. of the exposed lived 20 days, compared with only 38.6 per cent. in Ectromelia 1 at its worst.

The lower rate of mortality in the herd in the earlier days of exposure depends presumably on (1) non-reception of doses, (2) smaller dosage. The subjects of Table XXVI had in common with the herd entrants a lack of immunity due to prior exposure enjoyed by the 66 survivors of Table XXV, but had all received a direct injection of active virus.

The question of mortality in the first five days of herd exposure is important, and we here tabulate the whole of our experience.

TABLE XXVII

Number of experiment.	*Nature of infection.*	*Number out of 10,000 entrants surviving to day 5.*
Normal control ..	None	9,818
P 3 N (specific deaths)	Pasteurellosis	9,675
A 1 „ „	Mouse typhoid	9,798
A 3 „ „	„	9,923
A 6 „ „	„	9,770
A.H. „ „	„	9,937
A.A.D. „ „	„	9,919
A.C. „ „	„ (in immunized mice)	9,979
A.D. „ „	„ „	9,935
A.E. „ „	„ „	10,000
A.F. „ „	„ „	9,935
A.G. „ „	„ „	9,967
A.J. „ „	„ „	10,000
A.K. „ „	„ „	10,000
P 3 (all deaths) ..	Pasteurellosis (some mouse typhoid)	8,031
P 3 (specific deaths) ..	„ „ „	8,567
P 1 (all deaths) ..	„ „ „	8,822
P 1 (specific deaths) ..	„ „ „	9,171
P 6 (specific deaths) ..	„ „ „	8,966

It will be seen that in the last series of Table XXVII, in which pasteurellosis was the reigning infection but occasional deaths occurred from mouse typhoid, the specific mortality of normal

entrants during the first five days of herd life varied between 8 and 14 per cent. In the pure pasteurellosis experiment it was approximately 3 per cent. In mouse typhoid epidemics it has never exceeded 3 per cent.; among three out of five of the mouse typhoid and in all the immunized series the mortality has been less than among mice exposed to no infection at all. Among the latter death has, of course, been due to violence, or to some other non-specific cause. Actually the " specific " death rates at these early cage ages considerably overstate the deaths due to the reigning disease, because (*see* pp. 13–14) those mice that cannot be examined *post mortem* are assigned to the " specific " class. In Ectromelia 1 and 2 the early mortality has been rather less than 2 per cent. The observations recorded earlier in the report make it probable that a proportion of these early deaths are not due to ectromelia at all, but we cannot doubt that some at least are due to the specific infection. What we wish to know is the actual infection rate. Let us return to the inoculation results. (Table XXVI.)

Even if the interval from inoculation to death were constant—which it is evidently not—it would not measure the incubation period alone but be a summation of the interval from inoculation to the commencement of the host reaction and from then to death. All we can conclude is that within 3 days of inoculation about 2·9 per cent. of the inoculees are dead and within 5 days 40·3. That is, that the passing of the life-table generation is much quicker in inoculated mice than in mice exposed to contact with an infected herd. But this we knew already. We *cannot* take these values as approximate measures of what happens to mice who have acquired a casual infection and therefrom determine the unknown constant rate of infection. If we try to do so we merely reach arithmetically ridiculous results.

It is clear that the " law " of mortality in a herd depends upon a number of variables. In the first place, there is a process of passing from the state of non-recipients to recipients; then of the recipients some will die by virtue of the infection, others receiving an effective dose, will recover and when they receive again will react differently from those not previously infected. The process is one of extreme complexity. We decided that it was necessary to form some judgment as to the condition of the population at different periods after entrance to the herd. To that end the following process was adopted. All the mice still living in Ectromelia 1, at the end of the main experiment, 110 in number, were removed and each isolated in a separate cage. We could thus observe the fates of 110 animals, each of which had survived in the herd one or more days. Of these mice 61 died in solitary confinement within 63 days of isolation. The remaining 49 were then inoculated with 0·1 c.c. of a phenolized virus, together with 30 controls, and were observed for 21 days. The first point to notice is that the mortality of the isolated mice over their 63 days of isolation was rather greater than that of mice in herd. For instance, the expected number of survivors to 20 days

of the isolated animals, had they died at the same rate as animals of the same herd age according to the herd life table, would be 73, but in fact only 56 did so survive.

For mice with less than 30 days' herd experience, the survival of the isolated was precisely normal, viz. 27 observed and 27 expected. But isolated mice with more than 30 days, herd experience died faster in solitary confinement than, on the basis of our life tables, they would have done in herd. The survivors numbered 29, when 46 were expected. The explanation of this paradox—apart from a mere chance deviation—is not clear. The standard error of the expectation is of the order $\pm$ 7, so that the deviation although more than twice the standard error is not wildly improbable.

Passing now to a more detailed consideration the following points should be noticed. Since three mice were added daily to the herd, had there been no deaths in herd we should have in the survivors three of each herd age, and there were subject to our observation 15 mice of cage age 1 to 5 days. Of the three of cage age one day, one died on the 9th day of solitary confinement with the stigmata of ectromelia. Of the two who survived 63 days in single cage and were then inoculated, one died on the 8th day after inoculation, the other survived over the period of observation. Therefore, of the three with but a single day's herd experience, one had certainly been infected and another had probably been infected; all the 30 controls died within 9 days of inoculation, therefore the herd survivor who also survived the subsequent test inoculation may be presumed to have acquired an immunity through herd infection. It may even be that the third survivor from the herd had some advantage; it lived 8 days from inoculation, and only 4 of the 30 controls survived so long. Of the three mice with herd membership of two days, one died of ectromelial infection on the 10th day of isolation. The two which survived to be inoculated died respectively on the 6th and 14th day from inoculation. Many controls survived six days so that for one of the three two-day mice there is no presumption that its herd experience had been of importance. The third lived longer than any of the controls but still only 14 days. We may say that certainly one and possibly two of these three animals had been infected in herd. Of the three mice with three days' herd experience all died with signs of ectromelial infection, after respectively 7, 10 and 14 days of life in isolation. All three of the mice with four days' herd exposure survived 63 days in isolation and all three died after inoculation, after 7, 8 and 14 days' survival. They survived longer than the majority of the controls, of which 8 out of 30 lived 7 days or more, 4, 8 days or more, 2, 9 days and none, 10 or more. We cannot therefore say that the three herd survivors were *in pari materia* with the controls, neither can we say that any of them was proved to be an infected animal. Of the three mice with five days' herd experience two died on the 9th day of isolation with stigmata of ectromelial infection; one lived to be inoculated and died on the 8th day. Here certainly two and possibly

all three were infected. Summarizing the experience of these 15 mice, 7 were certainly infected in herd for they died of the herd disease in isolation. Of the remaining 8, 1 survived the test inoculation, 2 lived significantly longer than any of the controls. It seems then certain that nearly half and probable that two-thirds of the animals with no more than five days' herd experience are infected. Taking the 15 mice with 6–10 days' herd experience this is the record; 8 died in isolation with stigmata of ectromelia, 1 died in isolation (after 20 days) without post-mortem evidence of ectromelia, of the 6 that survived 63 days in isolation and were then inoculated 2 survived and the dates of death of the others were 4, 7, 8 and 9 days. In this set then certainly 8, probably 10 and possibly 12 were herd infected. There is little difference between these and the previous results. Of the 15 with herd experience 11–15 days, only 8 survived to be isolated and of these eight 5 died with stigmata of ectromelia in isolation. The remaining 3 lived 3, 8 and 9 days after inoculation. Including those dead in herd among the *infected*, 3 and perhaps only 2 of the 15 can be thought to have escaped. Only 3 of the 15 with herd experience 16–20 days survived, 1 died with stigmata in isolation, the two others survived inoculation 7 and 9 days. Again, on the previous assumption, 2 uninfected survivors. Of the 21–25 day batch, 5 survived to be isolated, three of these died with stigmata, the other two died 5 and 7 days after inoculation. Again 2 in 15 uninfected. Of the 26–30 batch, 9 survived to isolation; 3 died in isolation without stigmata; of the 6 inoculated, 3 survived and the others died after 7, 10 and 12 days. Here as many as 4–6 of 15 had no evidence of infection, but 3 of these died soon in isolation.

Passing forward to the mice with seniority 51–60 days—of the 30 entrants, only 3 survived to isolation, 2 died in isolation, 1 with stigmata of infection and 1 survived the subsequent inoculation test.

As maximum measures of the change in infection with time the Table XXVIII has been constructed. The assumptions made are:—

(1) All mice who died *in herd* were infected.
(2) All mice who died in isolation with stigmata were infected.
(3) All mice who died in isolation within 5 days even without obvious stigmata were infected.
(4) All mice who survived the post-isolation inoculation test were actively immune and therefore infected.

TABLE XXVIII

Cage age in days.	*Not infected.*	*Per cent. infected.*
1–10	12	60·0
11–20	5	83·3
21–30	5	83·3
31–40	3	90·0
41–50	0	100·0
51–60	1	96·7
61–70	3	90·0
71–80	1	96·7
81–90	0	100·0
91–100	0	100·0

Passing to survivors of 100 or more days—between 100 and 230 days there were 14 survivors from herd life; 7 of these were infected, on the assumptions set out above, 7 were not.

TABLE XXIX

Experience at end of various numbers of days.

At the end of	*Uninfected.*	*Surviving (experience 18.11.30–31.8.33).*
0	100	100
10	40·0	87·6
20	16·7	51·0
30	16·7	36·9
40	10·0	29·0
50	0·0	24·2
60	3·3	20·9
70	10·0	17·9
80	3·3	15·5
90	0·0	14·2
100	0·0	13·0

The figures for the first 100 days are tabulated (Table XXIX) against the survivorship of the whole Ectromelia 1 experience. Naturally the figures for the observations just described must be irregular. Not only are they based upon small absolute numbers, but they reflect the conditions of particular periods which are not homogeneous. For instance, the mice with 20 days' experience had their first ten days' experience not contemporaneously with the mice whose experience is listed at ten days, but ten days before. The figures may be compared with those for mouse typhoid of p. 57 which, although based on a small total number of mice, are in life-table form. The ectromelia life tables are, like other life tables, arithmetical constructs reproducing not individual but average results. We can hardly expect the l_x column of such a table to give us more than a faint indication of the " law " of infection.

No doubt, were the environmental conditions absolutely stable and the exposed to risk without individuality, we might expect the survivors to form a decreasing geometrical progression, because, as pointed out before, we might liken the exposed to risk to an army under fire of such intensity that 50 per cent., say, were hit in every unit of exposure. Beginning with 100 per cent. unhit, 50 per cent. would still be unhit at the end of a unit of time, 50 per cent. of these at the end of another unit and so on. Certainly the columns do not conform to any such simple law. Perhaps all we can take as *proven* is that by the 20th day of herd life more than 80 per cent. of the exposed to risk will either be dead or will have their tissue systems modified by a real infection or—perhaps through selection by death—will differ appreciably from the normal in regard to resistance. There is an inherent ambiguity attaching to the results, due to the difficulty, so often mentioned earlier in this report, of differentiating between genetic and acquired immunity. It is

possible that entrants fall into two categories (*a*) the resistant, (*b*) the susceptible, which may be sub-classified as follows :—(*a*) may consist of (a_1) mice with an innate high resistance equal to shielding them against any ordinary dose even when the virus has gained access to their tissues, (a_2) mice that are possessed of some heritable mechanism which renders access of the virus to their tissues difficult. (a_2) differs from (a_1) in that its advantage is solely with respect to that kind of danger experienced in herd; there is no intrinsic humoral or cellular power of superior resistance and on inoculation these animals will react precisely like normal controls. The fire of infection in herd is so intense that, within relatively few days all entrants, whether (*a*)'s or (*b*)'s, will have received doses; the (a_1)'s will be infected but will not die; the (a_2)'s will remain unchanged, the (*b*)'s will be infected and will die after a longer or shorter interval, since, on this hypothesis, active immunization is excluded. Actually of 15 mice with 5 days' exposure 7 died of infection in isolation. Of the remaining 8 one resisted subsequent inoculation and would hence be classed as (a_1); 2 others lived longer than any control and might be classed as near the (a_1) borderline. The remaining 5 might either be non-recipients of infection or, on the present hypothesis, (a_2)'s. We have no means of decision.

If the hypothesis *were* true, it would follow that the ultimate population of the herd must consist of a mixture in unknown proportions of (a_1)'s and (a_2)'s; so far as our record goes, viz. of eight infected and seven uninfected survivors, they would be in about equal proportions. A study of the ultimate mortality in herd may throw some light upon the problem and we will take that up now. In Table XXX we show the probabilities of surviving 5 days from day 50 at intervals of 5 days to 200 for each phase of *Ectromelia* 1. We also show the actual numbers of survivors at each point. A comparison of the three sets of figures shows that in the pre-heat-wave period, the probabilities of surviving were greater than in the other two phases, and a little analysis shows that the differences are greater than can be properly attributed to the fluctuations of small numbers. It is certain that even after 70 days in herd, by when, in the most favourable experience, more than three-quarters of the entrants are dead, we have not reached a population wholly indifferent to the herd conditions.

Further analysis of the 18.11.30–31.8.32 experience suggested that the rate of mortality might continue to improve to a comparatively advanced herd age. We tested the point in the following way. The raw data were roughly smoothed by summation and then graduated on the hypothesis that the survivorship approximated geometrically to unity. The result found was that ${}_5q_x$ could be approximately represented by $0{\cdot}0185/1{\cdot}0373^x$ where the origin of x was cage age 145 and the unit five days. This formula reproduced the observed deaths with reasonable accuracy from day 80 to day 200 (110 expected, 113 observed) without systematic distortion and could, of course, be much improved by a more refined process of

graduation. All we are concerned to show is that the facts are consistent with a continuous but very slow improvement in mortality. This finding neither confirms nor destroys the hypothesis. On the hypothesis it would be produced by the gradual elimination of (a_2). Without the hypothesis—e.g. assuming a steady inflow of new infections, decreasing in volume—the resistance of ultimate survivors would improve. It does not appear that statistical analysis will enable us to reach a conclusion and a little reflection suggests that we could not reach a conclusion.

TABLE XXX

Ectromelia 1.

	Pre-heat wave and heat wave 18.11.30–31.8.32.		*Six months after heat wave 1.9.32–28.2.33.*		*Last six months 1.9.33–28.2.34.*	
Cage age in days.	*No. exposed at cage age.*	*Probability of surviving 5 days.*	*No. exposed at cage age.*	*Probability of surviving 5 days.*	*No. exposed at cage age.*	*Probability of surviving 5 days.*
50	523	·9290	116	·9326	71	·8863
55	482	·9208	112	·9478	63	·9685
60	437	·9239	113	·8448	61	·9186
65	398	·9545	101	·8628	57	·9661
70	375	·9331	90	·8461	59	·9126
75	346	·9621	79	·8532	51	·9427
80	329	·9603	71	·9581	51	·9804
85	311	·9677	71	·8753	51	·9619
90	294	·9625	69	·9137	52	·9615
95	282	·9645	64	·8906	51	·9623
100	271	·9742	57	·8217	51	·9604
105	264	·9735	46	·8696	49	·9800
110	256	·9766	41	·9024	49	·9401
115	250	·9719	37	·9467	48	·9592
120	242	·9752	36	·9722	47	·9357
125	236	·9958	35	·9429	42	·9512
130	234	·9786	34	·9126	39	1·0000
135	227	·9866	32	·9100	39	·9737
140	221	·9910	32	·9687	37	·9452
145	219	·9817	31	1·0000	34	·9706
150	214	·9813	32	·9687	34	·9706
155	208	·9903	33	1·0000	34	·9706
160	203	·9803	36	·9452	33	1·0000
165	194	·9793	38	·8962	33	1·0000
170	188	·9840	36	·9722	33	·9697
175	183	·9890	37	·9197	32	·9082
180	177	·9887	37	1·0000	32	1·0000
185	174	·9770	36	·9444	32	1·0000
190	169	·9763	35	·9714	32	1·0000
195	165	·9939	34	1·0000	30	1·0000
200	160	·9874	38	·8684	31	·9677

The alternative to the hypothesis just suggested (we do not mean an absolute alternative, it cannot be a question of exclusively true descriptions) is that the final relatively high grade of resistance to death is due to active immunization of a fraction of the entering population; some proportion of the entrants receive initial dosages such that their specific resistance is increased. This would mean

simply that a proportion was sorted out from the susceptible—i.e. the doomed-to-death mass of the population—not by *ab initio* superiority but by the chance happening of a favourable effect. As before, we should eventually reach a residual population of high resistance.

It does not seem probable that active immunization affords the *whole* explanation of this gradual increase in average resistance. At every stage of cage age, it will be noted, some proportion of the survivors have failed to show any evidence of infection, either by dying of the disease in isolation or by resisting a subsequent test inoculation. Of 14 mice with over 100 days' residence in herd, half fell into this category. Seven is a small number, but it seems impossible that, with the rate of virus-reception that our life tables demonstrate, *any* mouse can avoid receiving many doses of virus within such a period. There is, we must note, a possible fallacy here. We have set out previously our reasons for believing that either innate or acquired immunity, and especially the latter, may be fluctuating, not steady states.

It might be argued that the mice that survived long periods of herd life, and subsequent life in isolation for 63 days, but succumbed as readily as controls to a test inoculation at the end of that period, had acquired an active immunity at some earlier date in herd life, but had lost it by the time they came to test. This is, indeed, an argument impossible to refute, though it seems to us unlikely, as a complete description of the facts, for the following reasons. If these " non-infected survivors " had lost an acquired immunity while in herd they would probably have contracted another active infection and have either (*a*) died in herd, or (*b*) died in isolation, or (*c*) regained some immunity and proved relatively resistant. That 7 of 14 mice should lose all immunity in 63 days seems unlikely. We postulated a fluctuating immunity partly to account for the fact that *all* mice tend eventually to die of the reigning disease; but the survival time *in herd* of the survivors for 100 days or over approaches that of mice living in uninfected surroundings, and is certainly not compatible with the view that, within 63 days, 50 per cent. of survivors will have lost all effective immunity and become as susceptible as new entrants.

We think, therefore, that there is reason for accepting the existence of mice of the (a_2) class, though probably in relatively small numbers—our seven 100-day survivors represent 1·7 per cent. of the contemporary entrants. We should not, of course, regard them as a separate class, sharply demarcated from their companions. We should suppose that certain non-specific characters, anatomical and physiological, that hinder the access of bacteria or viruses to the tissues are distributed in a certain way among any large sample of mice; just as we believe that other genetic factors, making for resistance to bacteria or viruses that have gained access to the tissues are distributed in various ways among the same sample. Those mice that depart most widely from the mean in the direction of increased resistance to penetration to the tissues will

form our (a_2) class. Of the rest, those that in spite of penetration, resist the resulting infection, will form our (a_1) class. Neither the (a_2)'s nor the (a_1)'s are absolutely resistant. In time all will tend to die of the reigning disease; but as cage age increases there will be a piling-up of both these classes to some limit.

We may then think of the herd phenomenon in the following way. There are steadily separated off from the mass, slices decreasing in size, which contain the members of the herd lethally infected. If the time unit were a day, then in the first day, such and such a percentage of the herd will become fatally infected in the present sense and will die so many on the first day, so many on the second and so on. In the second day a percentage of the remainder passes into this category and begins to die off and so on. We can easily arrange a "law" which will allow these successive quotas to decrease. For instance instead of the geometrical or Poisson terms for survivors, $e^{-\lambda}, e^{-2\lambda}, e^{-3\lambda} \ldots . e^{-n\lambda}$ we might use $e^{-\lambda_1}(1 - e^{-\lambda_2}), e^{-\lambda_1}(1 - e^{-2\lambda_2}), e^{-\lambda_1}(1 - e^{-3\lambda_2}) \ldots . e^{-\lambda_1}(1 - e^{-n\lambda_2})$ which would leave an ultimate residue of $e^{-\lambda_1}$.

For instance, if we accepted the very crude figures of Table XXIX and deduced the constants from the first two observations we should have λ_1 approximately equal to 3·002 and λ_2 approximately equal to 0·4565. The survivors of mortal infection at the end of 10, 20, 30, etc., days would be 33·4 per cent., 16·6 per cent., 10·7 per cent., 8·1 per cent., 6·7 per cent., 6·0 per cent. and so on, they would never fall below about 5 per cent.

Can we with the help of such an hypothesis describe the law of mortality in herd?

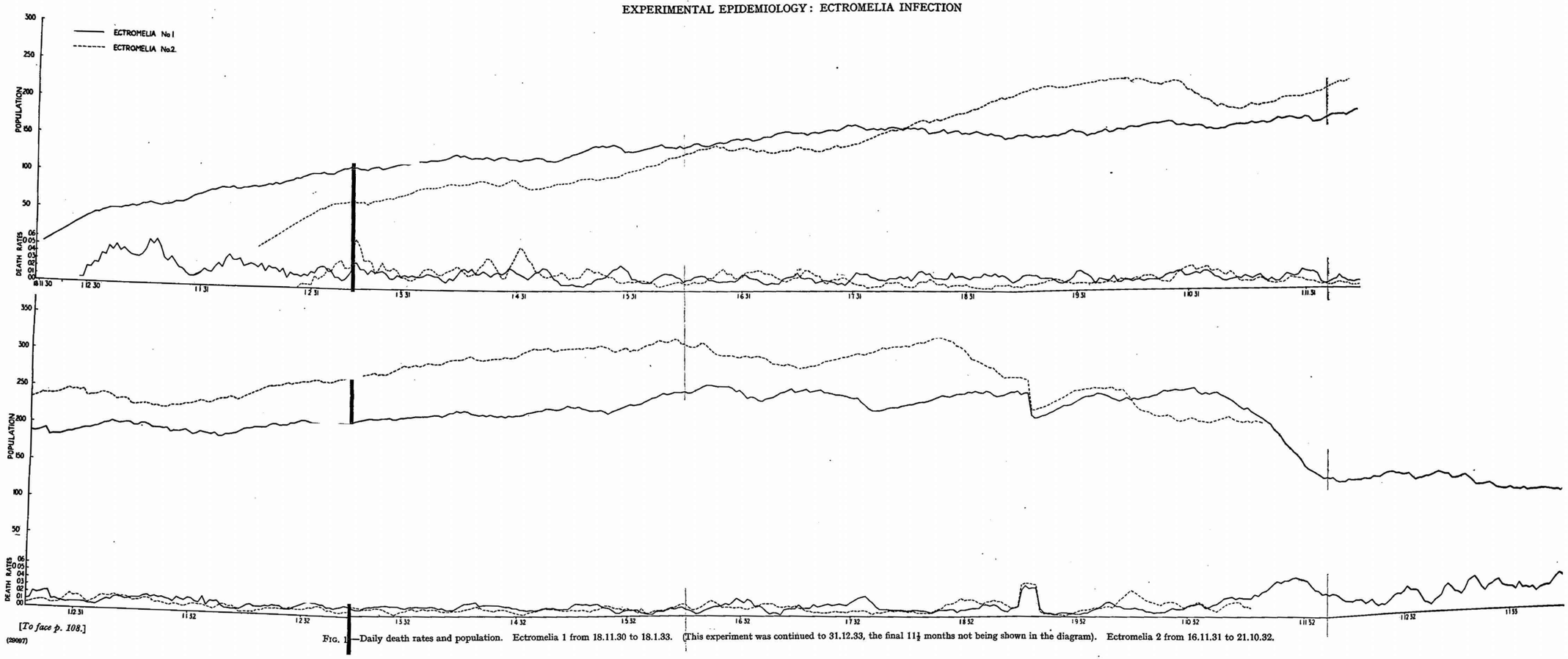
For instance, the following were the successive totals of deaths out of 10,000 for the entire Ectromelia 1 experience.

TABLE XXXI

Cage age.	Deaths.
-10	1,241
-20	3,659
-30	1,410
-40	787
-50	486
-60	326
-70	300
-80	241
-90	129
-100	123
-110	107
-120	76
-130	40
-140	51
-150	33
-160	30
-170	55
-180	37
-190	38
-200	23

What is the law of mortality in this herd?

EXPERIMENTAL EPIDEMIOLOGY: ECTROMELIA INFECTION



[*To face p. 108.*]

(29097)

FIG. 1.—Daily death rates and population. Ectromelia 1 from 18.11.30 to 18.1.33. (This experiment was continued to 31.12.33, the final 11½ months not being shown in the diagram). Ectromelia 2 from 16.11.31 to 21.10.32.

Infection precedes death; if we assume that our previously postulated "law" of lethal infection is true, then all the 1,241 which died in the first ten days are necessarily derived from the mice which entered the infected class within those ten days, viz. 6,667. But of those dying between 10–20 days some will be derived from the 6,667 first-period infections, some from the 1,667 infected in the second interval and so on. Evidently then we can determine a law of interval to death, but unless we know that the law of infection is right, the deduced law has no general validity.

The late Mr. H. E. Soper working on a geometrical law of infection reached a very elegant result. His method was as follows :—

Using the letter A as a mere dummy, the index of which labels the number of unit intervals survived, the lives from entry into the herd until death will be represented by the series $d_0A^0 + d_1A^1 + d_2A^2 + d_3A^3 + \ldots$. Let q be the chance of becoming infected within any interval and $1 - q = p$ that of escaping infection and suppose q and therefore p to be constant; the chances of life before infection must be represented by the series $qA^0 + pqA^1 + p^2qA^2 + \ldots$.

If d_0', d_1', d_2' replace d_0, d_1, d_2, etc., when the origin of measurements is not entry into herd but date of attack, then the series $d_0'A^0 + d_1'A^1 + d_2'A^2 + \ldots$ will denote length of life from infection. But the whole length of life must be the sum of the time lived before and time lived after infection. Therefore the equation :— $d_0A^0 + d_1A^1 + d_2A^2 + \ldots = (qA^0 + pqA^1 + p^2qA^2 + \ldots)(d_0'A^0 + d_1'A^1 + d_2'A^2 + \ldots)$ is an identity. Hence if both sides of it are multiplied by $(qA^0 + pqA^1 + p^2qA^2 + \ldots)^{-1}$ and the coefficients of powers of A equated, we can always determine the d' series from the life-table series of d.

In the special case chosen we have simply $(d_0A^0 + d_1A^1 + d_2A^2 + \ldots)(\frac{1}{q} - \frac{p}{q}A^1) = d_0'A^0 + d_1'A^1 + d_2'A^2 +$

A value of q is then guessed which will satisfy the material conditions (i.e. will not produce negative values in the d' series) and we can see what the result is like. Mr. Soper took our B_6 experience and taking $q = 2/7$ for a two-day unit found that the d' series came to an end at 36 days from entrance. By then 79·5 per cent. of the entrants were dead. In the next 36 days 28·5 per cent. of the survivors would be dead. Apart from the considerations discussed above, the assumption of q constant will not graduate the Ectromelia 1 data because it will not lead to a monomodal d series. But Soper's method does not require a geometrical series at all. For his $(qA^0 + pqA^1 + p^2qA^2 + \ldots)^{-1}$ we can substitute $(a_0 + a_1A + a_2A^2 + \ldots)^{-1}$ and expanding this there is no difficulty at all in expressing the d_x' series in terms of the d_x series.

Thus, writing the expression in the form $\frac{1}{q_0}(A^0 + \alpha_1A^1 + \alpha_2A^2 + \alpha_3A^3 + \alpha_4A^4 + \ldots)^{-1}$ and expanding to terms in A^4 the above equation becomes:—$(d_0A^0 + d_1A^1 + d_2A^2 + \ldots)\frac{1}{q_0}[1 - \alpha_1A + (\alpha_1^2 - \alpha_2)$

$A^2 + (2\alpha_1\alpha_2 - \alpha_3 - \alpha_1^3)A^3 + (\alpha_1^4 + \alpha_2^2 + 2\alpha_1\alpha_3 - 3\alpha_1^2\alpha_2 - \alpha_4)A^4] = d_0'A^0 + d_1'A^1 + d_2'A^2 + d_3'A^3 + \ldots.$

Equating coefficients

$$d_0' = \frac{1}{q_0} d_0$$

$$d_1' = \frac{d_1}{q_0} - \frac{\alpha_1}{q_0} d_0$$

$$d_2' = \frac{d_2}{q_0} - \frac{\alpha_1}{q_0} d_1 + \frac{\alpha_1^2 - \alpha_2}{q_0} d_0$$

$$d_3' = \frac{d_3}{q_0} - \frac{\alpha_1}{q_0} d_2 + \frac{\alpha_1^2 - \alpha_2}{q_0} d_1 + \frac{2\alpha_1\alpha_2 - \alpha_3 - \alpha_1^3}{q_0} d_0$$

$$d_4' = \frac{d_4}{q_0} - \frac{\alpha_1}{q_0} d_3 + \frac{\alpha_1^2 - \alpha_2}{q_0} d_2 + \frac{2\alpha_1\alpha_2 - \alpha_3 - \alpha_1^3}{q_0} d_1 + \frac{\alpha_1^4 + \alpha_2^2 + 2\alpha_1\alpha_3 - 3\alpha_1^2\alpha_2 - \alpha_4}{q_0} d_0 \quad \text{etc.}$$

If therefore we know the law of infection we can determine the law of survival from infection, or if we know the law of survival we can deduce the law of infection. But we cannot advance more than a step unless either one or the other is known. We can indeed be fairly confident that the geometrical law is inapplicable. For if the series were geometrical, $\alpha_2 = \alpha_1^2$, $\alpha_3 = \alpha_1^3, \ldots$ then all the terms on the right vanish except except the first two, and $\alpha_1 = p$. We must so choose q_0 that the d series is never negative. As $d_1 = 3{,}659$ and $d_2 = 1{,}410$, it follows that q_0 cannot be less than 0·61. Take as trial values, 0·65, 0·75 and 0·85. Using these values the deduced d' series are :—

d_0'	1,909	1,655	1,460
d_1'	4,961	4,465	4,085
d_2'	199	660	1,013
d_3'	452	579	677
d_4'	324	386	433
d_5'	240	273	298
d_6'	286	291	295
d_7'	209	221	231
d_8'	69	92	109
d_9'	120	121	122
	etc.	etc.	etc.

It is very improbable that the proportion of distribution of deaths from infection should be bimodal; we may fairly reject this hypothesis.

If, however, we allow ourselves a free choice of constants while we can avoid the improbability just described we shall reach a result the truth of which cannot be tested. Thus if the law of lethal infection proposed above were taken, viz. 66·7% infected in the first, 16·65% in the second, 6% in the third, 2·6% in the fourth interval, etc., the following arithmetical values are reached :—

$\frac{1}{q_0} = 1{\cdot}50$, $\alpha_1 = 0{\cdot}24975$, $\alpha_2 = 0{\cdot}09014$, $\alpha_3 = 0{\cdot}039144$, $\alpha_4 = 0{\cdot}02026$

From these we obtain for the first five terms of the d_x' series 1,861·5, 5,023·6, 692·6, 481·8 and 311·9.

If now we divide by 10,000 and multiply the new infections of each interval, viz. 6,667, 1,665, 600, 261, 135, 70, etc., by 0·1862, 0·50236, etc., the products being set down in columns, the entries in successive rows being one space lower as we pass to the right, i.e. :—

					d_x
1,241					1,241
3,349	310				3,659
462	836	112			1,410
321	115	302	49		787
208	80	42	131	25	486
etc.	etc.	etc.	etc.	etc.	etc.

Then the sums of the rows down to the fifth will merely reproduce the observed d_x's 1,241, 3,659, etc.

Had the computation been carried further, we should have reproduced more of the figures of the original. The process is merely mechanical. *If* 66·7 per cent. of the entrants are infected in the first interval, 16·7 per cent. in the second and so on, then it is *certain* that 18·7 per cent. of the quotas will die within the interval of becoming infected, 50 per cent. in the interval after infection and so on. But the result guarantees in no way that the hypothesis of 66·7 per cent., etc., is correct. All that can be claimed is that it is a trifle more plausible than the hypothesis which killed 19 per cent. in the first interval, 49·6 per cent. in the second and so on.

We do not think any progress can be made on these lines until further experimental work has given stronger justification to the rule of infection tested or replaced it by a more adequate expression. It is, however, evident that on *any* hypothesis a large proportion of deaths must occur within 30 days of infection.

An idea which suggested itself was to continue the series conjecturally. That is to suppose that after, for instance, the fifth computed term, the multipliers followed some simple law. Thus suppose we accept 0·186, 0·502, 0·0693 as determined values. They add up to 0·7573 and the next computed term 0·0482. If the first term of a geometric series is ·0482 and its sum to infinity 0·2427, its ratio is approximately 0·8. We might inquire whether, if the multipliers from the fifth onwards consisted of $0{\cdot}0482 \times 0{\cdot}8$, $0{\cdot}0482 \times (0{\cdot}8)^2$, etc., we should be able by cross summation to reproduce the observed values effectively.

The row for the last completely reproduced series is :—

321	115	302	49		
	80	42	131	25	
		29	18	68	13
			13	9	35
				7	5
					3

The subsequent entries are obtained by use of the calculated coefficients and the table is now to be completed by multiplying the last figure in each row by 0·8, the results so reached again by 0·8 and so on.

Summing the rows we have :—

Observed.	*Calculated.*	*Observed.* (continued)	*Calculated.*
(787)	(787)		
486	535	123	137
326	397	107	110
300	295	76	89
241	213	40	69
129	170	51	56

The total computed mortality is 10 per cent. heavier than the expected and there is no close resemblance between the series.

It is, we think, clear enough, that by arithmetical trial and error we could on these lines probably reach some graduation which would be quite reasonably smooth. *But* we do not see how, by any arithmetical manipulation of such figures as we have, the result reached would satisfy anything but a sense of arithmetical artistry. We do *not* know that the plan of releasing, as a film proprietor might say, 66·7 per cent. of mice in the first ten days, 16·7 per cent. in the next ten days, and compelling our law of survivorship from infection to conform to this prescription is correct. Anybody may object that our experimental data are quite consistent with a release of 66·7 per cent. in the first *five* days and so on. If that were the law, we should obtain different survivorship coefficients.

To progress we must strengthen our experimental foundation. The experiment upon which Tables XIX and XX were based is not adequate. Until we have a better foundation, we do not propose to elaborate arithmetical calculations further.

This is a meagre result of a very large amount of arithmetic (we have made numerous attempts on other data) but our labour has not, we believe, been entirely fruitless.

We think that it is made, if not certain, at least highly probable that quite 60 per cent. of the entrants have passed into the category of the doomed within ten days of entrance to a herd. Put in other terms, we think that if a herd were broken up at the end of ten days' communal life and each individual of it separately confined, that more than half would die of the specific infection. We think it probable that if all who survived 80 days were similarly treated, then their histories would be much more favourable.

We think that the relatively high resistance of survivors in our ectromelia herds is due to the same factors as those that determine the same phenomenon in the case of bacterial infections—in the main to active immunization, in part to an innate resistance that operates against virus that has gained access to the tissues, and in part, probably in very small part, to characters that hinder effective infection of any kind. That the resistance of surviving mice at corresponding cage ages is higher in our ectromelia herds than in our mouse typhoid or pasteurellosis experiments we should attribute to more effective immunization. The observations reported in the next section are, as will be seen, in entire conformity with this view.

LIFE TABLES

ECTROMELIA 1*

Period covered.	*1.3.31–31.8.31.*	*1.9.31–29.2.32.*	*1.3.32–31.8.32.*	*1.9.32–28.2.33.*	*1.3.33–31.8.33.*	*18.11.30–31.8.33.*	*1.9.33–28.2.34.*
No. of mice entering during period ..	552	546	552	543	552	3,051	543
No. of mice alive at beginning of period ..	114	164	218	238	64	—	111
No. of mice who die	502	492	532	717	502	2,937	548
No. of mice surviving at end of period ..	164	218	238	64	114	114	106
No. of mice who survive through whole period	39	40	56	5	12	—	25
No. of mouse days exposed to risk ..	27,967	34,627	44,383	28,004	17,007	159,584	19,645
Expectation of life at entry in days :—							
(*a*) Unlimited	53·12	77·37	81·87	30·15	41·94	55·54	33·98
(*b*) Limited to 60 days	28·22	34·33	38·04	23·66	21·41	28·87	20·21

* In the succeeding life tables relating to experiments Ectromelia 1 and 2 the decimals have been retained merely to secure arithmetical agreement between the columns, not because they have any biological or statistical importance.

The l_x q_x and d_x, columns give the values for single days of cage age ; where, in the later stages of the tables these values are given only at five or ten-day intervals, l_x may continue to decline in the absence of entries against q_x and d_x, for the intervening values for days $x + 1$ to $x + 9$ are not included.

ECTROMELIA 1. Period 18.11.30–31.8.32

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
0	·0030675	10000·00	30·68	73·4	32·4	·0133
1	·0020544	9969·32	20·48	72·6	31·9	·0129
2	·0025773	9948·84	25·64	71·7	31·2	·0166
3	·0025880	9923·20	25·68	70·9	30·6	·0250
4	·0031185	9897·52	30·87	70·1	29·9	·0559
5	·0026110	9866·65	25·76	69·3	29·2	·0745
6	·0057682	9840·89	56·76	68·5	28·5	·1064
7	·0110935	9784·13	108·54	67·9	27·9	·1368
8	·0342429	9675·59	331·32	67·6	27·5	·1594
9	·0227525	9344·27	212·61	69·0	27·6	·1654
10	·0369738	9131·66	337·63	69·6	27·5	·1796
11	·0396450	8794·03	348·64	71·3	27·8	·1838
12	·0370142	8445·39	312·60	73·2	28·2	·1836
13	·0410256	8132·79	333·65	75·0	28·6	·1870
14	·0394649	7799·14	307·79	77·2	29·0	·1946
15	·0418702	7491·35	313·66	79·3	29·5	·1932
16	·0393873	7177·69	282·71	81·8	30·0	·1895
17	·0410334	6894·98	282·92	84·1	30·6	·1816
18	·0500397	6612·06	330·87	86·1	31·1	·1811
19	·0377200	6281·19	236·93	90·2	32·1	·1626
20	·0375546	6044·26	226·99	92·7	32·7	·1544
21	·0300273	5817·27	174·68	95·3	33·3	·1452
22	·0404516	5642·59	228·25	97·3	33·6	·1406
23	·0284872	5414·34	154·24	100·3	34·4	·1272
24	·0283401	5260·10	149·07	102·3	34·8	·1180
25	·0271399	5111·03	138·71	104·2	35·2	·1146
26	·0247578	4972·32	123·10	106·1	35·5	·1103
27	·0254707	4849·21	123·51	107·8	35·8	·1134
28	·0182232	4725·70	86·12	109·6	36·1	·1064
29	·0243902	4639·58	113·16	110·6	36·2	·1134
30	·0226730	4526·42	102·63	112·4	36·5	·1021
35	·0296896	4064·36	120·67	119·9	37·7	·1140
40	·0231839	3601·12	83·49	130·0	39·6	·0978
45	·0138889	3248·94	45·12	138·9	41·3	·0803
50	·0095602	2988·14	28·57	145·8	42·6	·0710
55	·0269710	2776·00	74·87	151·7	43·6	·0792
60	·0160183	2556·05	40·94	159·6	45·3	·0761
65	·0100503	2361·76	23·74	167·6	47·1	·0455
70	·0133333	2254·37	30·06	170·5	47·6	·0669
75	·0057803	2103·58	12·16	177·5	49·4	·0379
80	·0060790	2023·86	12·30	179·4	49·9	·0397
85	·0160772	1943·53	31·25	181·7	50·6	·0323
90	·0102041	1880·72	19·19	182·7	51·1	·0375
95	·0141844	1810·24	25·68	184·7	52·0	·0355
100	·0110701	1745·91	19·33	186·4	52·8	·0258
110	·0078125	1655·71	12·94	186·3	53·6	·0234
120	—	1571·48	—	186·0	54·6	·0248
130	·0085470	1526·02	13·04	181·5	54·6	·0214
140	·0090498	1473·39	13·33	177·8	54·7	
150	—	1433·30	—	172·6	54·5	
160	·0049261	1392·88	6·86	167·5	54·1	
170	·0053191	1337·10	7·11	164·3	54·2	
180	—	1301·23	—	158·7	53·3	
190	·0118343	1256·95	14·85	154·0	52·6	
200	—	1219·67	—	148·6		
210	·0067114	1158·30	7·77	146·1		
220	·0071429	1103·35	7·88	143·2		
230	·0076336	1039·90	7·94	141·6		

ECTROMELIA 1. Period 18.11.30–31.8.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
240	—	975·44	—	140·6		
250	—	942·28	—	135·4		
260	—	907·12	—			
270	·0106383	861·77	9·17			
280	—	824·49	—			
290	—	796·05	—			
300	—	737·75	—			
310	·0144928	717·25	10·40			
320	—	664·44	—			
330	—	618·97	—			
340	—	583·26	—			
350	—	558·96	—			
360	—	496·85	—			
370	—	420·41	—			
380	—	394·93	—			
390	—	381·77	—			
400	—	368·61	—			
410	—	341·30	—			
420	—	312·21	—			
430	—	237·88	—			
440	—	237·88	—			
450	—	237·88	—			
460	—	190·30	—			
470	·0909091	174·44	15·86			
480	—	142·73	—			
490	—	142·73	—			
500	—	142·73	—			
510	—	142·73	—			
520	—	126·87	—			
530	—	126·87	—			
540	·1428571	111·01	15·86			
550	—	95·15	—			
560	—	95·15	—			
570	·2000000	79·29	15·86			
580	—	31·72	—			
590						

ECTROMELIA 1. Period 1.3.32–31.8.32

0	·0090580	10000·00	90·58	81·9	38·0	·0217
1	·0018282	9909·42	18·12	81·6	37·7	·0165
2	·0018315	9891·30	18·12	80·8	37·1	·0147
3	—	9873·18	—	79·9	36·5	·0128
4	·0091743	9873·18	90·58	78·9	35·8	·0275
5	·0037037	9782·60	36·23	78·6	35·4	·0278
6	—	9746·37	—	77·9	34·9	·0502
7	—	9746·37	—	76·9	34·2	·0633
8	·0148699	9746·37	144·93	75·9	33·5	·0950
9	·0094340	9601·44	90·58	76·1	33·3	·1114
10	·0267176	9510·86	254·11	75·8	32·9	·1276
11	·0137525	9256·75	127·31	76·9	33·1	·1211
12	·0337972	9129·44	308·55	76·9	32·8	·1266
13	·0327869	8820·89	289·21	78·6	33·3	·1184
14	·0274262	8531·68	233·99	80·2	33·7	·1053
15	·0195228	8297·69	161·99	81·5	33·9	·1039

ECTROMELIA 1. Period 1.3.32–31.8.32—*continued*

Cage-age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
16	·0199115	8135·70	161·99	82·1	33·9	·1193
17	·0247191	7973·71	197·10	82·8	33·9	·1172
18	·0184758	7776·61	143·68	83·8	34·1	·1088
19	·0258824	7632·93	197·56	84·4	34·3	·1160
20	·0363196	7435·37	270·05	85·6	34·3	·1120
21	·0176322	7165·32	126·34	87·8	34·9	·0964
22	·0154639	7038·98	108·85	88·4	34·8	·0880
23	·0263158	6930·13	182·37	88·8	34·7	·0921
24	·0215633	6747·76	145·50	90·2	35·0	·0838
25	·0193370	6602·26	127·67	91·2	35·1	·0802
26	·0084746	6474·59	54·87	91·9	35·2	·0987
27	·0199430	6419·72	128·03	91·7	34·8	·1253
28	·0173913	6291·69	109·42	92·6	34·9	·1133
29	·0176991	6182·27	109·42	93·2	34·9	·1127
30	·0390390	6072·85	237·08	93·9	34·9	·1059
35	·0307167	5429·85	166·79	99·8	35·8	·1375
40	·0241935	4683·24	113·30	110·3	38·4	·0953
45	·0135747	4283·61	58·15	115·4	39·2	·0952
50	·0151515	3875·72	58·72	122·3	40·6	·0709
55	·0271739	3600·76	97·85	126·5	41·2	·0821
60	·0120482	3305·09	39·82	132·6	42·5	·0909
65	·0194805	3004·79	58·53	140·6	44·4	
70	·0208333	2828·26	58·92	144·3	45·2	
75	·0155039	2591·57	40·18	152·2	47·3	
80	—	2470·07	—	154·6	48·0	
85	·0169492	2351·37	39·85	157·3	48·7	
90	·0181818	2251·55	40·94	159·2	49·4	
95	·0183486	2190·52	40·19	158·6	49·3	
100	·0186916	2110·32	39·45	159·5	49·8	
110	·0196078	1936·33	37·97	163·4		
120	—	1789·88	—	166·5		
130	·0181818	1739·05	31·62	161·2		
140	·0188679	1675·82	31·62	157·2		
150	—	1584·00	—	156·0		
160	·0097087	1538·87	14·94	150·4		
170	—	1446·88	—			
180	—	1430·98	—			
190	·0246914	1396·91	34·49			
200	—	1362·42	—			
210	—	1290·71	—			
220	·0147059	1253·83	18·44			
230	·0151515	1199·06	18·17			
240	—	1125·48	—			
250	—	1086·23	—			
260	—	1022·28	—			
270	—	958·39	—			
280	—	912·75	—			
290	—	866·51	—			
300	—	768·44	—			
310	—	714·47	—			
320	—	631·47	—			
330	—	543·85	—			
340	—	543·85	—			
350	—	483·42	—			
360	—	396·56	—			
370	—	311·58	—			
380	—	289·33	—			
390	—	265·22	—			

ECTROMELIA 1. Period 1.3.32–31.8.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
400	—	243·11	—			
410	—	221·01	—			
420	—	221·01	—			
430	—	128·92	—			
440	—	128·92	—			
450	—	128·92	—			
460	—	116·03	—			
470	·0909091	104·43	9·49			
480	—	85·44	—			
490	—	85·44	—			
500	—	85·44	—			
510	—	85·44	—			
520	—	75·95	—			
530	—	75·95	—			
540	·1428571	66·45	9·49			
550	—	56·96	—			
560	—	56·96	—			
570	·2000000	47·47	9·49			
580	—	18·99	—			

ECTROMELIA 1. Period 1.9.32–28.2.33

0	·0018416	10000·00	18·42	30·2	23·7	·0166
1	·0018450	9981·58	18·42	29·2	22·8	·0240
2	·0018484	9963·16	18·42	28·3	22·0	·0333
3	·0055556	9944·74	55·25	27·3	21·2	·0574
4	·0055866	9889·49	55·25	26·5	20·4	·1006
5	·0093633	9834·24	92·08	25·6	19·6	·1422
6	·0113422	9742·16	110·50	24·9	18·9	·1775
7	·0267686	9631·66	257·83	24·1	18·2	·2368
8	·0510806	9373·83	478·82	23·8	17·8	·2765
9	·0516529	8895·01	459·45	24·0	17·9	·3140
10	·0501089	8435·56	422·70	24·3	17·9	·3549
11	·0825688	8012·86	661·61	24·6	17·9	·3617
12	·0775000	7351·25	569·72	25·7	18·7	·3563
13	·1002710	6781·53	679·99	26·9	19·3	·3370
14	·1081081	6101·54	659·63	28·8	20·5	·3279
15	·0602007	5441·91	327·61	31·2	22·1	·2923
16	·0747331	5114·30	382·21	32·2	22·6	·2711
17	·0498084	4732·09	235·70	33·7	23·6	·2452
18	·0880000	4496·39	395·68	34·5	24·0	·2665
19	·0608696	4100·71	249·61	36·8	25·4	·2454
20	·0319635	3851·10	123·10	38·1	26·2	·2230
21	·0418605	3728·00	156·06	38·4	26·2	·2289
22	·0765550	3571·94	273·45	39·0	26·5	·2229
23	·0618557	3298·49	204·03	41·2	27·9	·1831
24	·0329670	3094·46	102·02	42·9	28·9	·1707
25	·0393258	2992·44	117·68	43·3	29·1	·1584
26	·0344828	2874·76	99·13	44·1	29·5	·1681
27	·0292398	2775·63	81·16	44·6	29·7	·1607
28	·0476190	2694·47	128·31	45·0	29·8	·1755
29	·0186335	2566·16	47·82	46·2	30·5	·1758
30	·0503145	2518·34	126·71	46·1	30·3	·1780

ECTROMELIA 1. Period 1.9.32–28.2.33—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
35	·0287770	2070·03	59·57	50·6	32·7	·1122
40	·0305344	1837·68	56·11	51·7	32·9	·0971
45	·0396825	1659·32	65·85	52·0	32·4	·1251
50	—	1451·77	—	54·1	33·0	·0674
55	·0089286	1353·97	12·09	52·8	31·3	·0522
60	·0265487	1283·24	34·07	50·6	29·0	·1552
65	·0297030	1084·08	32·20	54·4	30·2	
70	·0333333	935·29	31·18	57·7	31·1	
75	·0126582	791·36	10·02	62·8	32·8	
80	—	675·21	—	68·2	34·7	
85	·0422535	646·95	27·34	66·0	32·7	
90	·0434783	566·28	24·62	70·1	34·0	
95	—	517·40	—	71·5	33·9	
100	·0175439	460·81	8·08	75·0	34·9	
110	—	329·26	—	93·2		
120	—	281·29	—	98·2		
130	·0294118	257·85	7·58	96·6		
140	·0312500	214·14	6·69	105·4		
150	—	207·45	—	98·8		
160	·0277778	200·97	5·58	91·9		
170	—	170·23	—			
180	—	152·21	—			
190	—	143·75	—			
200	·0789474	139·65	11·02			
210	·0857143	117·71	10·09			
220	—	104·36	—			
230	—	91·71	—			
240	—	86·06	—			
250	—	72·28	—			
260	·0625000	68·09	4·26			
270	·0303030	63·83	1·93			
280	—	60·08	—			
290	—	58·26	—			
300	—	56·64	—			
310	—	52·17	—			
320	—	45·31	—			
330	·0303030	39·29	1·19			
340	—	35·79	—			
350	·0714286	30·13	2·15			
360	—	25·88	—			
370	—	23·76	—			
380	—	20.06	—			
390	—	16·73	—			
400	—	13·59	—			
410	—	12·62	—			
420	—	9·99	—			
430	—	9·99	—			
440	—	5·18	—			
450	—	5·18	—			
460	—	5·18	—			
470	—	3·70	—			
480	—	2·22	—			
490	—	2·22	—			
500	—	2·22	—			
510	—	1·48	—			
520	—	1·48	—			
530	—	·74	—			
540	—	·74	—			

ECTROMELIA 1. Period 1.3.33–31.8.33

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
0	—	10000·00	—	41·9	21·4	·0091
1	—	10000·00	—	40·9	20·5	·0145
2	·0018116	10000·00	18·12	39·9	19·7	·0235
3	·0036298	9981·88	36·23	39·0	18·8	·0616
4	·0036430	9945·65	36·23	38·2	18·0	·1418
5	·0054845	9909·42	54·35	37·3	17·2	·2155
6	·0091743	9855·07	90·41	36·5	16·5	·3013
7	·0407407	9764·66	397·82	35·8	15·7	·3746
8	·0888031	9366·84	831·80	36·3	15·5	·4196
9	·0891720	8535·04	761·09	38·8	16·1	·4165
10	·1142191	7773·95	887·93	41·6	16·8	·4377
11	·1131579	6886·02	779·21	45·9	18·1	·4185
12	·1097923	6106·81	670·48	50·7	19·5	·3866
13	·0838926	5436·33	456·07	55·8	21·1	·3655
14	·1222222	4980·26	608·70	59·9	22·2	·3558
15	·0840336	4371·56	367·36	67·2	24·4	·3043
16	·0645161	4004·20	258·34	72·3	25·9	·2731
17	·0792079	3745·86	296·70	76·3	27·0	·2581
18	·0698925	3449·16	241·07	81·8	28·5	·2165
19	·0520231	3208·19	166·90	86·9	30·0	·2233
20	·0429448	3041·29	130·60	90·6	31·0	·2120
21	·0451613	2910·69	131·45	93·7	31·7	·2354
22	·0275862	2779·24	76·67	97·1	32·6	·2198
23	·0780142	2702·57	210·83	98·8	32·9	·2190
24	·0381679	2491·64	95·10	106·1	35·1	·1914
25	·0714286	2396·54	171·18	109·3	35·9	·1835
26	·0256410	2225·36	57·06	116·7	38·1	·1466
27	·0265487	2168·30	57·57	118·7	38·5	·1510
28	·0454545	2110·73	95·94	121·0	39·1	·1548
29	·0288462	2014·79	58·12	125·7	40·4	·1332
30	·0294118	1956·67	57·55	128·4	41·2	·1266
35	·0219780	1708·94	37·56	141·7	44·8	·0779
40	·0238095	1575·76	37·52	148·5	46·6	·0600
45	—	1481·27	—	152·8	47·9	·1033
50	·0270270	1461·52	39·50	149·9	47·0	·0541
55	—	1382·52	—	153·3	48·2	·0448
60	—	1320·61	—	155·5	49·1	·0317
65	·0327869	1278·69	41·92	155·4	49·2	
70	·0178571	1215·80	21·71	158·4	50·5	
75	·0192308	1107·66	21·30	168·7	54·4	
80	—	1086·36	—	167·0	54·7	
85	—	1064·63	—	165·3	55·0	
90	—	1041·49	—	164·0	55·3	
95	—	1016·09	—	163·0	55·8	
100	—	1016·09	—	158·0	54·9	
110	·0294118	1016·09	29·88	148·0		
120	—	953·33	—	147·4		
130	—	920·45	—	142·6		
140	—	920·45	—	132·4		
150	—	852·27	—	132·8		
160	—	813·53	—	128·8		
170	—	776·55	—			
180	·0588235	737·73	43·40			
190	—	650·93	—			
200	—	650·93	—			
210	—	650·93	—			
220	—	578·61	—			
230	—	506·28	—			

ECTROMELIA 1. Period 1.3.33–31.8.33—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
240	—	506·28	—			
250	—	361·63	—			
260	—	361·63	—			
270	—	361·63	—			
280	—	271·22	—			
290	—	271·22	—			
300	—	271·22	—			
310	—	271·22	—			
320	—	271·22	—			
330	—	271·22	—			
340	—	271·22	—			
350	—	271·22	—			
360	—	271·22	—			
370	—	271·22	—			
380	—	271·22	—			
390	—	271·22	—			
400	—	135·61	—			
410	—	135·61	—			
420	—	135·61	—			
430	—	135·61	—			

ECTROMELIA 1. Period 1.7.33–31.12.33

0	·0036232	10000·00	36·23	38·4	21·4	·0163
1	—	9963·77	—	37·5	20·6	·0273
2	·0018182	9963·77	18·12	36·5	19·8	·0418
3	·0036430	9945·65	36·23	35·6	18·9	·0820
4	·0073126	9909·42	72·46	34·7	18·1	·1481
5	·0147330	9836·96	144·93	34·0	17·4	·2172
6	·0149533	9692·03	144·93	33·5	16·7	·2766
7	·0436433	9547·10	416·67	33·0	16·1	·3439
8	·0753968	9130·43	688·41	33·5	16·0	·3723
9	·0879828	8442·02	742·75	35·2	16·4	·3713
10	·0894118	7699·27	688·41	37·5	17·0	·3709
11	·1064935	7010·86	746·61	40·1	17·8	·3888
12	·0850440	6264·25	532·74	43·9	19·1	·3515
13	·0739550	5731·51	423·87	46·9	20·0	·3465
14	·0874126	5307·64	463·96	49·6	20·7	·3572
15	·1153846	4843·68	558·89	53·3	21·9	·3341
16	·0519480	4284·79	222·59	59·2	23·9	·3045
17	·0779817	4062·20	316·78	61·4	24·5	·3221
18	·0891089	3745·42	333·75	65·6	25·8	·3148
19	·0546448	3411·67	186·43	70·9	27·6	·3132
20	·0760234	3225·24	245·19	74·0	28·4	·2909
21	·0759494	2980·05	226·33	79·1	30·1	·2752
22	·0680272	2753·72	187·33	84·5	31·9	·2367
23	·0869565	2566·39	223·17	89·6	33·4	·2092
24	·0240000	2343·22	56·24	97·1	36·1	·1722
25	·0569106	2287·98	130·15	98·5	36·4	·1833
26	·0254237	2156·83	54·83	103·4	38·1	·1587
27	·0344828	2102·00	72·48	105·1	38·5	·1452
28	·0442478	2029·52	89·80	107·8	39·4	·1413
29	·0370370	1939·72	71·84	111·8	40·7	·1015
30	·0285714	1867·88	53·37	115·1	41·7	·0858
35	·0102041	1707·61	17·42	120·7	43·3	·0799
40	·0108696	1571·19	17·08	125·9	44·8	·0766

ECTROMELIA 1. Period 1.7.33–31.12.33—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
45	—	1450·87	—	131·2	46·4	·0230
50	·0232558	1417·51	32·97	129·2	45·4	·0698
55	—	1318·62	—	133·7	46·8	·0250
60	·0253165	1285·65	32·55	132·0	46·0	·0641
65	·0138889	1203·19	16·71	135·9	47·1	·0581
70	·0158730	1133·31	17·99	139·1	48·0	
75	—	1061·35	—	143·4	49·4	
80	·0178571	1025·38	18·31	143·3	49·5	
85	—	988·75	—	143·5	49·8	
90	—	970·78	—	141·1	49·2	
95	—	952·80	—	138·7	48·7	
100	·0200000	915·80	18·32	139·2	49·1	
110	·0217391	860·46	18·71	137·9		
120	—	786·86	—	140·6		
130	—	704·15	—	146·7		
140	—	704·15	—	136·7		
150	—	686·98	—	129·9		
160	—	653·06	—	126·4		
170	—	635·41	—			
180	—	561·65	—			
190	—	542·93	—			
200	—	542·93	—			
210	—	504·84	—			
220	—	484·65	—			
230	—	436·19	—			
240	·0769231	436·19	33·55			
250	—	369·08	—			
260	·0909091	369·08	33·55			
270	·1250000	301·97	37·75			
280	—	226·48	—			
290	—	188·73	—			
300	—	188·73	—			
310	—	141·55	—			
320	—	141·55	—			
330	—	141·55	—			
340	—	141·55	—			
350	—	141·55	—			
360	—	141·55	—			
370	—	141·55	—			
380	—	141·55	—			
390	—	141·55	—			
400	—	141·55	—			
410	—	141·55	—			
420	—	141·55	—			
430	—	141·55	—			
440	·5000000	94·37	47·18			
450	—	47·18	—			
460	—	47·18	—			
470	—	47·18	—			
480	—	47·18	—			
490	—	47·18	—			

ECTROMELIA 1. Period 1.9.33–28.2.34

0	·0036832	10000·00	36·83	34·0	20·2	·0110
1	—	9963·17	—	33·1	19·4	·0222

ECTROMELIA 1. Period 1.9.33–28.2.34—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
2	—	9963·17	—	32·1	18·5	·0407
3	·0018484	9963·17	18·42	31·1	17·6	·0944
4	·0055556	9944·75	55·25	30·2	16·8	·1818
5	·0148976	9889·50	147·33	29·3	16·0	·2593
6	·0189394	9742·17	184·51	28·8	15·3	·3163
7	·0559846	9557·66	535·08	28·3	14·7	·3916
8	·0981595	9022·58	885·65	29·0	14·7	·4190
9	·0997733	8136·93	811·85	31·1	15·3	·4057
10	·0906801	7325·08	664·24	33·5	16·1	·4126
11	·1270718	6660·84	846·41	35·7	16·8	·4483
12	·0984127	5814·43	572·21	39·9	18·4	·4027
13	·0774648	5242·22	406·09	43·2	19·5	·3863
14	·1102661	4836·13	533·26	45·7	20·3	·3912
15	·1459227	4302·87	627·89	50·4	21·9	·3453
16	·0550000	3674·98	202·12	57·9	24·9	·3027
17	·0736842	3472·86	255·90	60·2	25·5	·3197
18	·0847458	3216·96	272·62	64·0	26·8	·3212
19	·0432099	2944·34	127·22	68·8	28·5	·3009
20	·0903226	2817·12	254·45	70·9	29·1	·2884
21	·0780142	2562·67	199·92	76·9	31·3	·2736
22	·0757576	2362·75	179·00	82·4	33·3	·2348
23	·0573770	2183·75	125·30	88·1	35·4	·2046
24	·0260870	2058·45	53·70	92·4	36·9	·1736
25	·0714286	2004·75	143·20	93·9	37·3	·1691
26	·0288462	1861·55	53·70	100·1	39·6	·1147
27	·0392157	1807·85	70·90	102·0	40·2	·0884
28	·0206186	1736·95	35·81	105·2	41·3	·0714
29	·0208333	1701·14	35·44	106·4	41·6	·0725
30	·0106383	1665·70	17·72	107·6	42·0	·0738
35	—	1542·80	—	110·9	42·7	·0568
40	·0120482	1455·14	17·53	112·4	42·8	·0608
45	—	1366·61	—	114·5	43·1	·0274
50	·0281690	1329·17	37·44	112·7	41·9	·1137
55	—	1177·99	—	121·8	44·9	·0315
60	·0491803	1140·88	56·11	120·7	44·2	·0814
65	—	1048·00	—	126·2		
70	—	1012·48	—	125·5		
75	—	923·95	—	132·3		
80	·0196078	870·99	17·08	135·2		
85	·0196078	853·91	16·74	132·9		
90	—	821·37	—	133·0		
95	—	789·78	—	133·2		
100	·0196078	759·98	14·90	133·3		
110	·0204082	715·27	14·60	131·4		
120	—	644·93	—	135·4		
130	—	574·01	—			
140	·0270270	558·91	15·11			
150	—	512·73	—			
160	—	483·01	—			
170	—	483·01	—			
180	—	425·38	—			
190	—	425·38	—			
200	—	425·38	—			
210	—	398·38	—			
220	—	384·64	—			
230	—	356·15	—			
240	·0416667	356·15	14·84			
250	—	296·79	—			

ECTROMELIA 1. Period 1.9.33–28.2.34—*continued*

Cage-age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
260	·0555556	281·17	15·62			
270	·0714286	247·85	17·70			
280	—	185·97	—			
290	—	159·41	—			
300	—	159·41	—			
310	—	127·52	—			
320	—	127·52	—			
330	—	127·52	—			
340	—	127·52	—			
350	—	127·52	—			
360	—	127·52	—			
370	—	127·52	—			
380	—	127·52	—			
390	—	127·52	—			
400	—	127·52	—			
410	—	127·52	—			
420	—	127·52	—			
430	—	127·52	—			
440	1·000000	85·02	85·02			

ECTROMELIA 2.

Period covered	*16.1.31–20.10.32*	*1.5.31–31.10.31*	*1.11.31–30.4.32*	*1.5.32–20.10.32*
No. of mice entering during period	1,929	552	546	519
No. of mice alive at beginning of period.	—	110	203	315
No. of mice who die	1,718	459	434	623
No. of mice surviving at end of period.	211	203	315	211
No. of mice who survived through whole period.	—	64	92	89
No. of mouse days exposed to risk	138,700	33,903	48,712	48,455
Expectation of life at entry in days—				
(*a*) Unlimited	86·26	71·55	125·80	64·79
(*b*) Limited to 60 days ..	32·29	33·01	35·60	30·37

ECTROMELIA 2. Period 16.1.31–20.10.32

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
0	·0041472	10000·00	41·47	86·3	32·3	·0166
1	·0020855	9958·53	20·77	85·6	31·7	·0168
2	·0010466	9937·76	10·40	84·8	31·1	·0242
3	·0031480	9927·36	31·25	84·3	30·8	·0379
4	·0063258	9896·11	62·60	83·2	29·7	·0588
5	·0042508	9833·51	41·80	82·7	29·2	·0811
6	·0096205	9791·71	94·20	82·0	28·6	·1019

ECTROMELIA 2. Period 16.1.31–20.10.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
7	·0151351	9697·51	146·77	81·8	28·2	·1313
8	·0247389	9550·74	236·27	82·1	27·9	·1611
9	·0299266	9314·47	278·75	83·1	27·8	·1699
10	·0268065	9035·72	242·22	84·7	28·0	·1829
11	·0419916	8793·50	369·25	86·0	28·0	·1899
12	·0489028	8424·25	411·97	88·8	28·5	·1910
13	·0349835	8012·28	280·30	92·3	29·3	·1865
14	·0451436	7731·98	349·05	94·6	29·7	·2066
15	·0351759	7382·93	259·70	98·1	30·4	·2067
16	·0432191	7123·23	307·86	100·6	30·8	·2152
17	·0436817	6815·37	297·71	104·2	31·5	·2111
18	·0587755	6517·66	383·08	107·9	32·3	·2062
19	·0452174	6134·58	277·39	133·6	33·7	·1837
20	·0455373	5857·19	266·72	118·0	34·7	·1698
21	·0382044	5590·47	213·58	122·6	35·8	·1513
22	·0378109	5376·89	203·31	126·4	36·6	·1306
23	·0321243	5173·58	166·20	130·3	37·5	·1245
24	·0289389	5007·38	144·91	133·7	38·2	·1191
25	·0242826	4862·47	118·07	136·6	38·8	·1262
26	·0147392	4744·40	69·93	139·0	39·2	·1261
27	·0310702	4674·47	145·24	140·1	39·3	·1339
28	·0261593	4529·23	118·48	143·6	40·1	·1217
29	·0367197	4410·75	161·96	146·4	40·6	·1128
30	·0242038	4248·79	102·84	151·0	41·7	·0943
35	·0197183	3848·09	75·88	161·5	43·8	·0691
40	·0075758	3582·34	27·14	168·3	45·0	·0669
45	·0147059	3342·75	49·16	175·1	46·2	·0673
50	·0141593	3117·76	44·15	182·6	47·7	·0550
55	·0056497	2946·32	16·65	188·1	49·0	·0434
60	·0039604	2818·49	11·16	191·5	49·7	·0377
65	·0082474	2712·36	22·37	193·9	50·4	·0456
70	·0043668	2588·64	11·30	198·0	51·5	·0395
75	·0046083	2486·46	11·46	201·0	52·5	·0301
80	·0047847	2411·73	11·54	202·2	53·2	·0312
85	·0024876	2336·56	5·81	203·6	54·0	·0249
90	—	2278·30	—	203·8	54·5	·0078
95	·0078125	2260·64	17·66	200·3	54·0	·0182
100	·0185676	2219·43	41·21	199·0	54·1	·0371
110	—	2101·53	—	200·0	55·6	·0085
120	—	2053·83	—	194·5	55·1	·0175
130	—	2011·86	—	188·5	54·6	·0090
140	—	1950·98	—	184·2	54·4	
150	·0098684	1895·31	18·70	179·5	54·1	
160	·0034247	1832·84	6·28	175·5	54·1	
170	·0070922	1770·07	12·55	171·5	54·1	
180	·0037175	1707·26	6·35	167·7	54·1	
190	·0077519	1668·96	12·94	161·4	53·3	
200	·0040816	1584·87	6·47	159·8		
210	·0042553	1539·45	6·55	154·3		
220	—	1479·72	—	150·3		
230	·0048309	1425·26	6·89	145·8		
240	—	1362·71	—	142·3		
250	·0108108	1334·39	14·43	135·2		
260	—	1262·26	—			
270	—	1210·32	—			
280	—	1141·47	—			
290	·0149254	1086·51	16·22			
300	·0165289	1012·33	16·73			

ECTROMELIA 2. Period 16.1.31–20.10.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
310	—	953·77	—			
320	—	909·90	—			
330	—	890·64	—			
340	—	829·44	—			
350	—	778·24	—			
360	·0140845	757·34	10·67			
370	—	692·40	—			
380	—	670·42	—			
390	—	623·11	—			
400	—	611·12	—			
410	·0217391	574·46	12·49			
420	—	474·55	—			
430	—	424·60	—			
440	—	424·60	—			
450	—	399·62	—			
460	—	324·69	—			
470	—	273·18	—			
480	—	234·15	—			
490	—	221·14	—			
500	—	141·91	—			
510	—	113·53	—			
520	—	99·34	—			
530	—	66·23	—			
540	·6666667	66·23	44·15			
550	—	22·08	—			
560	—	22·08	—			
570	—	22·08	—			
580	—	22·08	—			
590	—	22·08	—			

ECTROMELIA 2. Period 1.5.31–31.10.31 inclusive

0	·0036232	10000·00	36·23	71·6	33·0	·0218
1	—	9963·77	—	70·8	32·4	·0273
2	·0018182	9963·77	18·12	69·8	31·7	·0418
3	·0072860	9945·65	72·46	68·9	31·3	·0492
4	·0091743	9873·19	90·58	68·4	30·5	·0624
5	·0092593	9782·61	90·58	68·1	30·1	·0741
6	·0149533	9692·03	144·93	67·7	29·6	·0841
7	·0094877	9547·10	90·58	67·7	29·4	·0986
8	·0210728	9456·52	199·28	67·4	28·9	·1397
9	·0215264	9257·24	199·28	67·8	28·8	·1584
10	·0199608	9057·96	180·80	68·3	28·7	·1760
11	·0305499	8877·16	271·20	68·7	28·6	·1817
12	·0546218	8605·96	470·07	69·8	28·7	·1960
13	·0424107	8135·89	345·05	72·8	29·6	·1832
14	·0419580	7790·84	326·89	75·0	30·2	·1867
15	·0267640	7463·95	199·77	77·3	30·8	·1854
16	·0475000	7264·18	345·05	78·4	31·0	·2064
17	·0395778	6919·13	273·84	81·3	31·8	·1963
18	·0465753	6645·29	309·51	83·6	32·5	·1910
19	·0521739	6335·78	330·56	86·7	33·4	·1808
20	·0400000	6005·22	240·21	90·4	34·6	·1574
21	·0353698	5765·01	203·91	93·2	35·4	·1386
22	·0333333	5561·10	185·37	95·5	36·1	·1205
23	·0344828	5375·73	185·37	97·8	36·7	·1252

ECTROMELIA 2. Period 1.5.31–31.10.31 inclusive—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
24	·0250896	5190·36	130·22	100·3	37·4	·1159
25	·0185874	5060·14	94·06	101·9	37·8	·1193
26	·0151515	4966·08	75·24	102·8	37·9	·1294
27	·0384615	4890·84	188·11	103·4	37·9	·1276
28	·0241935	4702·73	113·78	106·5	38·9	·1048
29	·0289256	4588·95	132·74	108·1	39·3	·1034
30	·0297872	4456·21	132·74	110·3	39·9	·0895
35	·0046948	4057·58	19·05	115·9	41·1	·0560
40	·0100503	3830·21	38·49	117·7	40·9	·0905
45	·0166667	3483·65	58·06	124·1	42·4	·0719
50	·0178571	3233·14	57·73	128·6	43·4	·0595
55	·0063291	3040·81	19·25	131·6	44·0	·0633
60	·0068027	2848·35	19·38	135·3	44·9	·0474
65	·0212766	2713·22	57·73	136·9	45·2	
70	·0150376	2463·94	37·05	145·5	48·0	
75	·0077519	2317·95	17·97	149·5	49·6	
80	—	2229·22	—	150·4	50·0	
85	—	2141·87	—	151·4	51·2	
90	—	2055·38	—	152·7	52·3	
95	·0163934	2004·14	32·85	151·5	52·7	
100	·0256410	1922·10	49·28	152·9	54·1	
110	—	1824·00	—	151·0		
120	—	1739·70	—	148·0		
130	—	1722·65	—	139·5		
140	—	1686·57	—	132·3		
150	—	1667·83	—	123·8		
160	—	1628·35	—	116·6		
170	—	1628·35	—			
180	—	1628·35	—			
190	—	1602·90	—			
200	—	1549·79	—			
210	—	1549·79	—			
220	—	1513·75	—			
230	—	1513·75	—			
240	—	1513·75	—			
250	—	1513·75	—			
260	—	1513·75	—			
270	—	1513·75	—			
280	—	1513·75	—			

ECTROMELIA 2. Period 1.11.31–30.4.32

0	·0036630	10000·00	36·63	125·8	35·6	·0183
1	·0055147	9963·37	54·95	125·3	35·1	·0147
2	—	9908·42	—	125·0	34·7	·0148
3	·0018484	9908·42	18·31	124·0	34·0	·0222
4	·0074074	9890·11	73·26	123·2	33·4	·0333
5	—	9816·85	—	123·1	33·1	·0504
6	·0055970	9816·85	54·94	122·1	32·4	·0765
7	·0075047	9761·91	73·26	121·8	32·5	·0958
8	·0132325	9688·65	128·21	121·7	32·1	·1267
9	·0249042	9560·44	238·10	122·3	31·4	·1378
10	·0275591	9322·34	256·92	124·4	31·5	·1550
11	·0263158	9065·42	238·56	126·9	31·8	·1572
12	·0414079	8826·86	365·50	129·4	32·0	·1674
13	·0258621	8461·36	218·83	133·9	32·8	·1615

ECTROMELIA 2. Period 1.11.31–30.4.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
14	·0442478	8242·53	364·71	136·5	33·1	·1855
15	·0300926	7877·82	237·06	141·8	34·0	·1775
16	·0380952	7640·76	291·08	145·2	35·5	·1802
17	·0346535	7349·68	254·69	149·9	35·3	·1721
18	·0537084	7094·99	381·06	154·2	36·1	·1780
19	·0349462	6713·93	234·63	162·0	37·6	·1634
20	·0332410	6479·30	215·38	166·8	38·4	·1580
21	·0286533	6263·92	179·48	171·5	39·3	·1547
22	·0414201	6084·44	252·02	175·6	40·0	·1385
23	·0369231	5832·42	215·35	182·2	41·2	·1225
24	·0287540	5617·07	161·51	188·1	42·4	·1141
25	·0294118	5455·56	160·46	192·7	43·2	·1171
26	·0100671	5295·10	53·31	197·5	44·1	·1003
27	·0236486	5241·79	123·96	198·5	44·2	·1113
28	·0276817	5117·83	141·67	202·3	44·9	·0966
29	·0320285	4976·16	159·38	207·0	45·8	·0780
30	·0109890	4816·78	52·93	212·9	47·0	·0621
35	·0232558	4517·75	105·06	221·8	48·5	·0665
40	·0042017	4217·37	17·72	232·4	50·6	·0339
45	·0088496	4074·25	36·06	235·5	51·2	·0398
50	·0140845	3911·92	55·10	240·2	52·3	·0427
55	—	3744·82	—	245·8	53·7	·0306
60	·0052910	3630·28	19·21	248·5	54·7	·0160
65	·0054945	3572·13	19·63	247·5	54·9	
70	—	3513·04	—	246·6	55·2	
75	—	3433·19	—	247·2	56·0	
80	·0060606	3351·32	20·31	248·2	57·0	
85	—	3269·32	—	249·4	58·0	
90	—	3227·80	—	247·6	58·5	
95	—	3227·80	—	242·4	58·1	
100	·0070423	3227·80	22·73	237·6	57·6	
110	—	3159·44	—	232·6		
120	—	3135·69	—	224·6		
130	—	3135·69	—	214·4		
140	—	3077·61	—	208·3		
150	·0208333	3045·88	63·46	200·3		
160	—	2885·48	—	201·4		
170	—	2740·65	—			
180	—	2628·02	—			
190	—	2590·48	—			
200	—	2552·38	—			
210	—	2552·38	—			
220	—	2515·92	—			
230	—	2515·92	—			
240	—	2479·46	—			
250	—	2406·00	—			
260	—	2337·69	—			
270	—	2278·13	—			
280	—	2191·98	—			
290	·0129870	2137·52	27·76			
300	·0136986	2053·50	28·13			
310	—	1911·65	—			
320	—	1854·58	—			
330	—	1824·67	—			
340	—	1699·34	—			
350	—	1603·15	—			
360	—	1569·04	—			
370	—	1496·06	—			

ECTROMELIA 2. Period 1.11.31–30.4.32—*continued*

Cage age in days.	q_x	l_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
380	—	1450·73	—			
390	—	1336·81	—			
400	—	1336·81	—			
410	—	1336·81	—			
420	—	1188·27	—			
430	—	1029·84	—			
440	—	1029·84	—			
450	—	1029·84	—			
460	—	1029·84	—			

ECTROMELIA 2. Period 1.5.32–20.10.32

0	·0077071	10000·00	77·07	64·8	30·4	·0193
1	·0019417	9922·93	19·27	64·3	29·8	·0175
2	·0019455	9903·66	19·27	63·4	29·1	·0253
3	·0019493	9884·39	19·27	62·5	28·4	·0429
4	·0058594	9865·12	57·80	61·7	27·7	·0703
5	·0058939	9807·32	57·80	61·0	27·0	·0963
6	·0098814	9749·52	96·34	60·4	26·4	·1106
7	·0199601	9653·18	192·68	60·0	25·9	·1456
8	·0305499	9460·50	289·02	60·2	25·6	·1669
9	·0336134	9171·48	308·28	61·1	25·6	·1742
10	·0216920	8863·20	192·26	62·2	25·8	·1801
11	·0487805	8670·94	422·97	62·5	25·6	·1930
12	·0443925	8247·97	366·15	64·7	26·1	·1843
13	·0391198	7881·82	308·34	66·7	26·5	·1854
14	·0405063	7573·48	306·77	68·4	26·8	·2081
15	·0370370	7266·71	269·14	70·3	27·2	·2120
16	·0385675	6997·57	269·88	71·9	27·5	·2259
17	·0457143	6727·69	307·55	73·8	27·9	·2265
18	·0658683	6420·14	422·88	76·3	28·5	·2195
19	·0451613	5997·26	270·84	80·7	29·8	·1839
20	·0540540	5726·42	309·54	83·5	30·5	·1724
21	·0392857	5416·88	212·81	87·2	31·5	·1500
22	·0371747	5204·07	193·46	89·8	32·2	·1375
23	·0232558	5010·61	116·53	92·2	32·7	·1350
24	·0316206	4894·08	154·75	93·4	32·9	·1457
25	·0284553	4739·34	134·86	95·4	33·3	·1624
26	·0252101	4604·48	116·08	97·2	33·7	·1628
27	·0343348	4488·40	154·11	98·7	33·9	·1796
28	·0353982	4334·29	153·43	101·2	34·5	·1769
29	·0504587	4180·86	210·96	103·9	35·2	·1648
30	·0289855	3969·90	115·07	108·4	36·5	·1443
35	·0388889	3396·96	132·10	121·3	39·8	·0984
40	·0117647	3062·73	36·03	129·3	41·8	·0875
45	·0189873	2794·86	53·07	136·4	43·5	·0966
50	·0070922	2524·80	17·91	145·7	46·0	·0703
55	·0074074	2347·27	17·39	151·5	47·6	·0442
60	—	2243·47	—	153·4	48·2	·0600
65	—	2108·80	—	158·0	49·6	
70	—	2024·84	—	159·4	50·2	
75	·0085470	1925·66	16·46	162·5	51·4	
80	·0090090	1859·25	16·75	163·2	52·9	
85	·0091734	1809·30	16·60	162·7	52·1	
90	—	1760·40	—	162·1	52·3	
95	·0087719	1760·40	15·44	157·1	51·0	

ECTROMELIA 2. Period 1.5.32–20.10.32—*continued*

Cage age in days.	q_x	L_x	d_x	*Expectation of life unlimited.*	*Expectation of life limited to 60 days.*	*Probability of dying in the next 5 days.*
100	·0256410	1729·78	44·35	154·9	50·6	
110	—	1567·43	—	160·6		
120	—	1540·63	—	153·3		
130	—	1466·59	—	150·8		
140	—	1397·21	—	148·1		
150	·0081301	1319·96	10·73	146·4		
160	·0076923	1288·02	9·91	140·0		
170	·0150376	1230·57	18·50			
180	·0076336	1167·04	8·91			
190	·0153846	1131·27	17·40			
200	·0079365	1046·38	8·30			
210	·0080645	989·54	7·98			
220	—	933·75	—			
230	·0091743	870·90	7·99			
240	—	806·06	—			
250	·0215054	789·61	16·98			
260	—	721·97	—			
270	—	678·38	—			
280	—	623·62	—			
290	·0172414	575·74	9·93			
300	·0204082	514·71	10·50			
310	—	493·91	—			
320	—	446·70	—			
330	—	432·74	—			
340	—	402·90	—			
350	—	376·49	—			
360	·0357143	363·51	12·98			
370	—	312·00	—			
380	—	300·45	—			
390	—	281·02	—			
400	—	272·51	—			
410	·0357143	246·96	8·82			
420	—	194·41	—			
430	—	177·50	—			
440	—	177·50	—			
450	—	164·82	—			
460	—	129·50	—			
470	—	108·58	—			
480	—	93·07	—			
490	—	87·90	—			
500	—	56·40	—			
510	—	45·12	—			
520	—	39·48	—			
530	—	26·32	—			
540	·6666667	26·32	17·55			
550	—	8·77	—			
560	—	8·77	—			
570	—	8·77	—			
580	—	8·77	—			
590	—	8·77	—			

SECTION V

THE EFFECT OF VACCINATION ON HERD MORTALITY

The knowledge that has been gained with regard to the behaviour of infected herds when they are recruited by daily additions of susceptibles, has afforded a basis for the study of the effect of interfering with the natural course of events by actively immunizing some, or all, of the immigrants that gain access to the infected herd.

It is clear—and we would stress the point—that the conditions in our cages differ widely from those to which human herds are subjected under natural conditions. Neither war, nor the absence or break-down of adequate sanitary control among a civilian population, is at all likely to produce conditions so severe as those to which our herds of mice are continuously exposed. We should, therefore, discount to some extent any relative failures and emphasize the significance of any striking successes. Should it prove possible to reduce the mortality in our herds to negligible proportions by any method of prophylactic immunization, we have no doubt at all that an equally efficient method, employed under any probable field conditions, would be an effective check to the natural spread of an epidemic.

Experiments with Mouse Typhoid

The available data in regard to the active immunization of individual mice against experimental infection with *Bact. aertrycke* make it clear that, even under these conditions, it is not possible, by any method as yet available, to induce a uniformly effective immunity (*see* Loeffler, 1906; Wolf, 1908; Yoshida, 1909; Brückner, 1911; Webster, 1922; Ornstein, 1922; Neufeld, 1924; Lange and Yoshioka, 1924; Topley, Wilson and Lewis, 1925 a; Topley, 1926 a). The mortality among the immunized mice is significantly lower than among the non-immunized controls, and most of those that die succumb to a subacute or chronic infection; but there is always some mortality among the immunized, and of those that survive many, when killed and examined *post mortem*, are found to be harbouring *Bact. aertrycke* in the spleen. It was, then, hardly likely that a similar procedure would protect more than a proportion of the immunized mice against the risks of prolonged residence in our infected herds; but it was obviously desirable to determine whether the proportion of mice that withstood the herd conditions was of the same order as the proportion that proved resistant to a single injection of virulent bacteria. Were this the case we might reasonably expect a significant slowing-down of herd mortality, though not its entire suppression. So far as we are aware the only data in regard to the influence of vaccination on an epidemic of mouse typhoid recorded by other workers are those reported by Lynch (1922), and these are not of a kind to allow of any statistical analysis.

Experiment Vac. I.—Our first experiment (Topley and Wilson, 1923) was, as subsequent experience has shown us, carried out on far too small a scale to yield significant results. The suggestion afforded was that the immunization of all mice at risk afforded protection against a relatively minor risk of infection, while immunization of half the mice at risk had little effect, the immunized animals, once the infection had developed in the small herd at risk, suffering from a mortality only slightly less than that of their susceptible companions.

Experiment Vac. II.—A later series of experiments (Topley, 1926 a) were designed to test the efficacy of two injections of *Bact. aertrycke* vaccine given (*a*) 21 and 14 days before exposure to the risk of infection, (*b*) 14 and 21 days after the period of exposure began, and (*c*) 42 and 49 days after the same date, when the epidemics in these two herds were well under way. The mice composing all these groups were, at the start of the experiment, housed together for 14 days together with 83 mice among which mouse typhoid was slowly spreading; a sufficient excess, both of previously immunized and of normal mice, was allowed to provide at least 100 in each group at the end of the 14 days' exposure. During these 14 days several deaths from mouse typhoid occurred, showing that the infection was spreading. At the end of this period 100 of the previously vaccinated mice were placed in a separate cage (Herd A), and three lots, each of 100 mice, were placed in separate cages (Herds B, C and D). Of these, the mice of Herd B were vaccinated on the day they were separated and again 7 days later, the mice of Herd C were vaccinated 28 days after separation (42 days after first exposure) and again 7 days later, while the mice of Herd D received no vaccine. Each herd was observed for 84 days. The results may be summarized as follows:—

Herd A (vaccinated 14 and 21 days before exposure).	Mortality = 29 per cent.
Herd B (vaccinated 14 and 21 days after exposure).	" = 48 " "
Herd C (vaccinated 42 and 49 days after exposure).	" = 57 " "
Herd D (unvaccinated)	" = 49 " "

The mortality among the non-immunized mice was, as will be seen by comparison with the happenings in similar experiments described elsewhere in this report, not of a high order. The figures suggest that the mortality among an immunized herd will be significantly less than that among a non-immunized herd when (*a*) the infecting organism is not possessed of high virulence and high infectivity, (*b*) the mice are immunized before exposure to infection, and (*c*) a high level of epidemicity is not maintained by the admission of susceptible mice to the infected herd. Vaccination practised (*a*) after exposure to risk but before any significant epidemic spread has occurred, or (*b*) on the surviving mice during the

rise of an epidemic wave, confers no benefit. These findings should be compared with those of the following experiment.

Experiment Vac. III.—Our third experiment (Greenwood, Topley and Wilson, 1931 a) was carried out on a larger scale, using the method of the long-continued epidemic. The opportunity was also taken to check the observations of other workers (Felix, 1924; Arkwright, 1927; Ibrahim and Schütze, 1928; Schütze, 1930) on the importance of the O somatic antigen as an immunizing agent, and to determine the possible effect of a non-specific response to the stimulus provided by the injections of our vaccines.

The experiment was carried out as follows :—

An epidemic of mouse typhoid was started on 4.i.28 by infecting 50 mice with *Bact. aertrycke*, adding to them 50 normal mice, and adding to the herd so constituted 3 mice a day until 17.ix.29, when the experiment terminated.

To this infected herd 10 mice of each of 7 immunized groups (Groups A, B, C, D, E, F and G) and 10 non-immunized controls were added once a week from 1.v.28 to 27.xi.28, the number of mice belonging to each group added during this period being 300. From 4.xii.28–5.ii.29, 20 C mice, 20 J mice, and 20 normal mice were added to the cage at weekly intervals—180 mice of each group. From 19.ii.29–9.vii.29, 20 C mice, 20 K mice and 20 normal mice were added weekly—200 mice of each group.

These different groups had been treated as follows :—The mice of Groups C and E had received vaccines prepared in different ways from a smooth strain of *Bact. aertrycke*. Those of Groups F and G had received vaccines prepared from two different smooth strains of *Bact. paratyphosum B*, which has the same O antigens as *Bact. aertrycke*. The mice of these four groups should, therefore, have been effectively immunized. The mice of Group D received a vaccine prepared from a motile strain of *Bact. aertrycke* that appeared to have become completely rough, and thus to have lost its O (somatic) antigen while retaining its H (flagellar) antigen. In accord with the view that the H antigen has no immunizing value, in the sense of inducing an effective active immunity, the mice of this group would be expected to show little if any advantage over the controls. The mice of Group B received a vaccine prepared from a rough strain of *Bact. typhosum*, those of Group A a vaccine of *Staph. aureus*. In each case any increase in resistance would have to be ascribed to a non-specific immunizing response, the relationship of *Bact. typhosum* to *Bact. aertrycke* being a little less distant than that of *Staph. aureus*, in that the former organisms possess a common antigen in the rough state. The mice of Group J received a vaccine prepared from a strain of *Past. pseudotuberculosis* kindly supplied to us by Dr. Schütze. This strain (Schütze, 1928) shows an antigenic similarity to *Bact. aertrycke*, but the results of cross-absorption tests render the exact nature of this relationship obscure. The mice of Group K received a vaccine exactly similar to that given to Group C, except that it was prepared some eight months later. It was tested

against the latter vaccine to determine the keeping qualities of this preparation, which was a saline suspension from an agar culture, killed by adding 0·25 per cent. formalin and heating at 55° C. for 30 minutes.

Each vaccine employed was standardized to contain $1{,}000 \times 10^6$ bacilli per c.c., and two doses, each of 0·5 c.c., were injected intraperitoneally 14 and 7 days before the mice were added to the infected herd.

The results may be adequately summarized by giving the expectation of life limited to 60 days ($_{60}E_x$) for the different groups. These are set out in Table XXXII.

TABLE XXXII

Showing the expectation of life, limited to 60 days, of mice treated with different vaccines before exposure to risk of infection with mouse typhoid. (Specific deaths.)

Group.	*Vaccine.*	$_{60}E_x$	*S.E.*
C	*Bact. aertrycke* (smooth)	32·1	0·43
E	*Bact. aertrycke* (smooth)	35·0	0·85
F	*Bact. para. B* (smooth)	35·1	0·85
G	*Bact. para. B* (smooth)	33·7	0·85
K	*Bact. aertrycke* (smooth)	30·8	0·65
D	*Bact. aertrycke* (rough)	29·4	0·77
B	*Bact. typhosum* (rough)	29·3	0·73
A	*Staph. aureus*	29·9	0·71
J	*Bact. pseudotuberculosis*	25·9	0·75
Controls (added in batches with vaccinated)		26·4	0·35
Controls (added daily)		27·1	0·29

The figures in the table are not, in all cases, strictly comparable *inter se*, because they do not all refer to the same period of the epidemic. The figures for the mice of Group C, and for the controls, added in batches and added daily, are based on happenings for the whole period of the epidemic. The mice of Groups A, B, D, E, F and G were added between 1.v.28 and 27.xi.28, those of Group J between 4.xii.28 and 5.ii.29, and those of Group K between 19.ii.29 and 9.vii.29. Had the mortality rate in the herd as a whole fluctuated widely during the 21 months over which this experiment lasted, these differences in the period of exposure might have led to significant differences in the mortality rates of the groups concerned. Such wide fluctuations did not, in fact, occur, and the comparison of the $_{60}E_x$ figures of Groups A, B, D, E, F, G, J and K with $_{60}E_x$ figures for the C mice, the controls added in batches, and the added-dailies calculated for the corresponding epidemic periods (*see* original paper) show differences that do not differ significantly from those set out in Table XXXII.

It is clear that the mice immunized with the vaccines containing the smooth O antigen (Groups C, E, F, G, and K) live significantly

longer than the non-immunized controls added with them, or than the non-immunized mice added daily to the cage. This difference becomes more striking if (*see* Table XXXIV, p. 139) we compare the limited expectation of life not from the day of entry, as we have done in Table XXXII, but from the day of herd life when this expectation is at a minimum for any group, i.e. from the time when the majority of the mice have contracted an active infection. In this experiment the period of minimal ${}_{60}E_x$ fell between the 20th and 30th day for all groups. Thus, for day 23, the figures are: Group C 15·4 days, Group E 17·9 days, Group F 19·1 days, Group G 18·8 days, Controls 9·8 days.

The effect of the vaccines that did not contain the smooth O antigen (Groups D, B, A and J) is, Table XXXII shows, on the average, much less; but there is a suggestion that vaccines D, B and A induced a trivial and transient increase in resistance.

The immunity conferred by the more effective vaccines is, however, of little value from the epidemiological point of view. There is, indeed, no significant difference between the ultimate fate of the immunized and non-immunized mice. The immunized die of mouse typhoid, but they do not die so soon.

It is of some interest to compare these figures with those obtained in tests in which the immunity conferred by vaccines such as C or K is determined by injecting the immunized mice with a single dose of virulent bacilli. Table XXXIII shows the results obtained in three different experiments of this type. In the first vaccines C and K were employed, in the second and third two other vaccines prepared in exactly the same way. In each experiment groups of 50 mice were employed, the two doses of vaccine were of the same size as used in the main experiment (500×10^6 bacilli) and the test dose of 1,000 virulent *Bact. aertrycke* was given intraperitoneally 7 days after the second injection of vaccine. All mice were observed for 28 days.

TABLE XXXIII

Showing the effect of injecting immunized mice with a dose of living Bact aertrycke.

Experiment.	*Vaccine.*	*No. mice.*	*No. surviving 28 days.*	*No. survivors negative P.M.*
I	C	50	23	16
	K	50	20	8
	Controls (no vaccine)	50	1	0
II	As C and K	50	21	9
	Controls (no vaccine)	50	0	—
III	As C and K	50	19	4
	Controls (no vaccine)	50	0	—

It will be seen that 38–46 per cent. of the immunized mice survived the test injection, and that 8–32 per cent. of them resisted it completely, in that, when killed in apparent health on the 28th day, no *Bact. aertrycke* could be recovered from their tissues. Had the immunized groups of our epidemic withstood the prolonged risk of infection to which they were subjected as well as they withstood a single test inoculation of living bacilli there would certainly have been a wider difference between their fate and that of the controls. It is clear that the testing of immunity by a single experimental injection of living organisms, though serving as an indication of the probable effectiveness of any given vaccine under field conditions, cannot be accepted as a substitute for a trial in which the immunized animals are subjected to a prolonged risk of contact infection. It seems probable, also, that only a vaccine giving a very high degree of immunity as determined by inoculation tests will withstand the far more rigorous trial of life in an infected herd. We may note in passing that in this respect, as in others, there would seem to be a rather sharp difference between antibacterial immunity, as exemplified by the experiments under review, antitoxic immunity, as exemplified in the well-documented fields of diphtheria and scarlet fever prophylaxis, and antiviral immunity as exemplified by a variety of data from the field, and by the experiment on ectromelia described on pp. 139–143.

Experiment Vac. IV.—Since the method of vaccination *per os* has been recommended by various workers, especially in prophylaxis against intestinal infections, it seemed desirable to determine how this form of immunization would compare with the intraperitoneal injection of vaccine employed in the preceding experiments.

Preliminary trials (Greenwood, Topley and Wilson, 1931 c) showed that the administration *per os* of two large doses ($5{,}000 \times 10^6$) of a killed suspension of *Bact. aertrycke*, introduced into the stomach through a fine ureteric catheter, induced an O-antibody response of the same order as that induced by the intraperitoneal injection of two doses, each of 500×10^6 bacilli. The production of H antibodies was found, in conformity with the results recorded by Pijper and Dau (1930), to be far less after the administration of vaccine *per os*, than after its injection into the tissues.

On 3.x.29 25 mice were injected, each with 1,000 *Bact. aertrycke*, and were added to 100 normal mice. Three normal mice were added daily to the herd until 12.xii.29. Thereafter two normals were added each day, and at weekly intervals three batches of mice were added—10 mice vaccinated *per os*, 10 mice vaccinated intraperitoneally, and 10 non-immunized controls. On 24.iv.30 the last of the weekly batches was added, giving a total of 200 mice in each of the three groups. On 22.vi.30, the last daily additions were made and all survivors were killed on the following day.

The results may be adequately summarized by noting the expectation of life on day of entry to the herd, limited to 60 days, in the different groups.

	e_0E_s	S.E.
Immunized by intraperitoneal injection ..	37·3	1·15
,, *per os*	34·5	1·20
Non-immunized controls added in batches ..	30·2	1·02
,, mice added daily	30·2	0·92

The effect of immunization *per os* would appear to be somewhat inferior to the disappointing results obtained with parenteral immunization (though the difference is not more than might be due to chance).

Experiment Vac. V.—In the preceding experiments we were concerned with a comparison of the behaviour of immunized and non-immunized mice in an infected herd recruited by the daily additions of normal animals. The immunized mice were thus subjected to a continuous risk of infection due to the spread of disease among their normal companions. It seemed possible that more encouraging results might be obtained if an infected herd were recruited entirely from immunized mice. Even with a resistance of a relatively low order, the frequency of severe infection in the herd might be lowered to a point at which the average risk was significantly less than in Experiments Vac. III and Vac. IV, so that the total effect of immunization might be cumulative.

An experiment on these lines was, therefore, carried out. On 14.iii.29 25 mice, each inoculated with 1,000 *Bact. aertrycke*, were added to 100 normal mice, and thereafter until 12.v.29 3 normal mice were added daily to the cage. From 13.v.29 to 20.x.29 3 immunized mice were added each day instead of the 3 normals. From 21.x.29 to 29.vi.30 only one immunized mouse was added each day, since earlier experiments had shown that the reduction of the immigration rate to this level tended to induce a more fluctuating level of mortality.

The results are sufficiently summarized by giving the expectation of life at entry, limited to 60 days, for the three periods.

	e_0E_s	S.E.
15.iii.29–12.v.29—Three normal entrants a day ..	26·4	1·00
13.v.29–20.x.29—Three immunized entrants a day	32·1	0·66
21.x.29–29.vi.30—One immunized entrant a day	35·4	0·93

These results are no more encouraging than those of Experiment Vac. III. The immunized mice show just about the same advantage, as regards their length of life, but that is all. Though no non-immunized mouse was added to the herd during the 13 months 13.v.29–29.vi.30 mouse typhoid was spreading and killing as effectively at the end as at the beginning. An immunity of the level attained in these experiments would seem to offer no hope of effective control when contact within the herd is free and continuous.

Summary of Results of Prophylactic Vaccination against Mouse Typhoid

Little can be hoped from prophylactic immunization against mouse typhoid under the conditions obtaining in these experiments.

The factors that determine this lack of success would appear to be :—(*a*) the low average level of immunity induced in the vaccinated mice, and (*b*) the severe and continuous risk of infection to which these mice are exposed. In any attempt to argue from mice to men, these limiting factors must be kept constantly in mind.

In regard to (*a*) it seems to us highly desirable that attempts should be made to induce a far more effective level of immunity. In this connection we may note that studies are being undertaken (*see* Raistrick and Topley, 1934; Delafield, 1934; Martin, 1934) with the object of isolating from *Bact. aertrycke* the chemical components responsible for an effective immunizing response, and so placing this problem on a more satisfactory and scientific basis. If a high average level of immunity could be attained, it is at least possible that prophylactic vaccination might yield results of a very different order from those recorded above. This possibility is, we think, greatly strengthened by the results obtained in infectious ectromelia (pp. 139–143).

In regard to (*b*)—the severe and continuous risk of infection to which our mice were exposed—we feel that this factor makes it impossible to draw definite conclusions as regards the probable effect on human herds, and perhaps on animal herds under field conditions, of a method of prophylactic immunization yielding a relatively low average level of immunity.

We must emphasize the importance of the point just made, viz., the severe and *continuous* risk run in our herds. The advantage enjoyed by the inoculated for a limited period is very great, In mouse typhoid the period of maximal mortality is, roughly, from the 10th to the 35th day of exposure. Between these days the controls, whether added in batches or added daily are reduced from approximately 9,600 (out of 10,000 entrants) to from 1,500 to 1,800. that is more than 80 per cent. die. But of the immunized E, F and G batches, out of approximately 9,900, from 3,400 to 4,000 survive at the end of this period, that is some 60 per cent. die ; the survivors therefore are twice as numerous as among the controls.

There is a temptation to stress the analogy with enteric fever in man. We do not know the level of immunity induced by the ordinary typhoid or T.A.B. vaccine. Experimental trials are clearly impossible and statistical data are difficult to obtain. It is, unfortunately, still true that the data published nearly a quarter of a century ago by the Anti-typhoid Committee are, statistically, the best available, and of these the only quite unequivocal figures relate to short periods of exposure, e.g., periods of one or two years (i.e., very short periods, having regard to the time scales of men and mice). Thus an analysis of the figures relating to troops having equal time-exposure and, as appears, equal risks of infection, showed 70 cases of enteric fever for 611 man-years of exposure of uninoculated, but only 2 cases of enteric in 465 man-years of exposure of inoculated. This is a stricter comparison than that afforded by the summarized data of the Committee (56 cases

among 10,378 inoculated, 272 among 8,936 uninoculated) because in the latter there are difficulties as to precise length of exposure, but either set of figures shows an advantage on the side of the inoculated very unlikely to be a mere freak of chance distribution. It will be noticed, however, that these figures do not suggest an absolute immunity; the attack rate on the inoculated, using the massed figures, is 0·54 per cent. or rather more than a sixth of that on the uninoculated (3·04 per cent.).

The data published in the *History of the Great War—Medical Services. Diseases of the War* (Vol. I, p. 57) are unsatisfactory. In 1915 the incidence rate of typhoid on protected men is stated as 0·93 per 1,000, upon unprotected men 8·1 per 1,000. In 1916, the respective figures are 0·57 and 0·51, in 1917, 0·10 and 1·09, in 1918, 0·02 and 0·19. For paratyphoid A, the figures in 1916 were 0·21 and 3·19, in 1917, 0·07 and 1·12, in 1918, 0·01 and 0·04. There was not absolute protection; 1,520 cases of typhoid fever were recorded among vaccinated men and 1,267 cases of paratyphoid fever among men who had received T.A.B. vaccine. Protected men formed an immense majority of the exposed to risk and the incidence of typhoid during the campaign was much less than in peace time, or *a fortiori* in the Boer War. We see no reason to doubt that protective inoculation was one factor of this great improvement. We see no ground for making it responsible for the whole improvement; the whole system of hygienic organization was far superior in the western theatre of war in 1914–18 than in the South African campaign of 16 years before.

This, in fact, is the lesson that we should be disposed to draw from a consideration of our experimental results taken in conjunction with the available data of typhoid inoculation in man. Given transient or intermittent exposure to infection, there seems good reason to expect that the immunity induced—even if far less than absolute —will reduce the incidence of the disease to relatively small proportions. Even if the exposure to risk is severe and continuous the vaccinated will, on the average, fare slightly better than the non-vaccinated. There would, on the other hand, seem to be no justification for the view that, in the vaccines at present available, we have an instrument that will afford protection against all probable risks, and will so allow us to disregard or relax our measures of general hygiene. Whether a more effective method of immunization would allow us to control infection by this means alone we cannot tell. In the particular case of enteric infections the problem does not arise under civilized conditions of general sanitary control, and in areas where such control is grossly defective communal energies will certainly be better employed in an attempt to establish a higher standard of general hygiene, with a consequent fall in *all* intestinal infections, than in any attempt to escape this primary and essential method of prophylaxis by the immunization of the population at risk. In other infections, however, and particularly in those conveyed by the respiratory tract, the problem does

arise, and in an acute form, for under the present conditions of civilized life general hygiene has no solution to offer.

That an intensive study of the antigenic structure of bacteria offers a reasonable hope of improving our present methods of antibacterial immunization is attested by such recent work as that of Grinnell (1932), Perry, Findlay and Bensted (1933, 1934), and Felix and Pitt (1934) on typhoid infection, or by that of Gardner and Leslie (1932), and Sauer (1933, 1935) on whooping-cough.

TABLE XXXIV

Effect of vaccination in mouse typhoid.

Expectation of life limited to 60 days. (Specific deaths.)

	Vaccinated.				*Controls (added in batches).*	*Controls (added daily).*
Day.	*C*	*E*	*F*	*G*		
0	32·1	35·0	35·1	33·7	26·4	27·1
1	31·2	34·1	34·3	32·9	25·4	26·2
2	30·3	33·2	33·5	32·0	24·5	25·3
3	29·4	32·3	32·6	31·1	23·5	24·4
4	28·5	31·4	31·7	30·4	22·5	23·5
5	27·6	30·5	30·9	29·5	21·6	22·6
6	26·7	29·6	30·1	28·7	20·7	21·6
7	25·7	28·8	29·2	27·8	19·7	20·8
8	24·9	27·9	28·3	26·9	18·8	19·9
9	24·0	27·0	27·4	26·2	18·0	19·2
10	23·2	26·3	26·6	25·4	17·2	18·4
11	22·4	25·4	25·8	24·5	16·4	17·7
12	21·6	24·6	25·0	23·9	15·6	17·0
13	20·8	24·0	24·4	23·1	15·0	16·3
14	20·1	23·2	23·6	22·3	14·3	15·6
15	19·3	22·7	23·0	21·6	13·5	15·2
16	18·7	21·9	22·3	21·2	13·0	14·6
17	18·0	21·2	21·5	20·9	12·4	14·2
18	17·4	20·4	21·2	20·1	11·6	13·7
19	16·8	19·8	20·6	19·3	11·1	13·4
20	16·4	19·0	20·1	18·9	10·9	13·0
21	16·0	18·5	19·8	18·8	10·6	12·7
22	15·8	18·4	19·2	18·5	10·1	12·7
23	15·4	17·9	19·1	18·8	9·8	12·8
24	15·0	18·2	18·7	19·1	9·7	12·9
25	14·6	17·9	18·4	19·1	9·3	13·3
26	14·8	17·8	18·8	19·2	9·3	13·8
27	15·1	18·8	19·3	20·2	9·7	14·5
28	15·9	18·6	19·4	21·5	9·9	15·1
29	16·5	18·7	19·4	22·2	10·3	15·8
30	16·8	19·0	19·5	23·4	10·5	16·9
35	20·0	21·4	24·5	27·9	13·0	21·8
40	23·9	22·3	25·5	32·1	15·1	26·4
45	32·1	27·5	28·5	36·6	18·0	30·5
50	35·5	31·1	30·8	41·8	25·3	36·9

PROPHYLACTIC IMMUNIZATION AGAINST ECTROMELIA

By the immunization of mice with two injections of formolized virus, the second at least of which is living but attenuated, it is possible to induce a level of immunity against a subsequent massive injection of fully virulent virus far higher than can be attained in

vaccination against mouse typhoid. Reference to Table XXIV, p. 91 will indicate how effective this immunity may be. Of 180 mice included in the immunized groups of that table, only 7 succumbed to the injection of virulent virus, while 60 non-immunized controls all died.

The much greater advantage of old survivors in our ectromelia herds than in our herds infected with mouse typhoid or with pasteurellosis, indicating a greater effectiveness of natural immunization by latent or minor infections, also encouraged us to hope that prophylactic immunization against ectromelia might give results far more satisfactory than those described in the preceding sections. This proved to be the case.

On 21.x.32 100 surviving mice from the second experiment described in Section IV of this report were selected as the basis of a new herd. Among these 100 mice ectromelia was still actively spreading. On that day, and on every seventh day thereafter for 70 days, 30 immunized mice and 30 non-immunized controls were added to the herd. From 21.x.32 to 27.ii.33 three normal mice were also added daily. The experiment was terminated on 16.iii.33.

Table XXXV gives the expectation of life limited to 60 days for each of the three groups, immunized mice, non-immunized controls and non-immunized mice added daily, for the day of entry (day 0), for each day thereafter till the 30th day, and then at five-day intervals until the 50th day of herd life.

Taking the figures for the day of entry the advantage of the immunized mice is obvious and striking. Not only did they live on the average nearly two and a half times as long as the controls, but their ${}_{60}E_x$ figure is not greatly less than that of old residents in an infected herd, when selection as well as natural immunization has played its part, and not inordinately less than that of mice living in a non-infective environment.

This superiority of anti-ectromelia as compared with anti-typhoid vaccination is strikingly confirmed by a more detailed examination of this table and a comparison with Table XXXIV which gives corresponding figures for the more effective of the *Bact. aertrycke* vaccines used in Experiment Vac. III, p. 132. Taking the latter table first it will be noted that the expectation of life of the non-immunized controls falls progressively from the day of entry to the herd (day 0) to day 26, when it has reached approximately one-third of its initial value. Each group of immunized mice it will be noted shares in this progressive fall of the ${}_{60}E_x$ value during the early days of residence in the herd, though the minimal values obtained are here approximately one-half of the figures for the day of entry. Turning to the ectromelia figures (Table XXXV) we note that the control mice, and the normal mice added daily, show a similar fall in the ${}_{60}E_x$ figure during the early days of herd life; though here the minimal value is attained earlier (8th or 9th day), and the difference between the initial and the minimal ${}_{60}E_x$ is not so great. When we turn to the immunized mice we

find that this fall in the ${}_{60}E_x$ value is barely perceptible; the figure for day 0 is 49·1 days, while the minimal value on day 8 is 48·0 days. The immunized mice would seem to be almost indifferent to the spread of infection within the herd.

TABLE XXXV

Effect of vaccination in ectromelia.

Expectation of life limited to 60 days.

Cage age in days.	*Immunized mice (added in batches).*	*Control mice (added in batches).*	*Controls (added daily).*
0	49·1	20·8	21·4
1	48·8	20·0	20·6
2	48·8	19·2	19·7
3	48·9	18·4	19·1
4	48·6	17·7	18·4
5	48·6	16·9	17·6
6	48·2	16·1	17·0
7	48·2	15·5	16·2
8	48·0	15·3	15·7
9	48·2	15·9	15·7
10	48·5	17·1	16·3
11	48·5	18·1	17·4
12	48·3	19·8	19·3
13	48·3	20·5	21·3
14	48·7	21·8	23·2
15	49·2	23·4	24·9
16	49·4	27·0	28·3
17	49·8	29·8	31·1
18	50·6	35·6	33·6
19	51·3	38·3	35·4
20	51·2	40·5	38·4
21	50·8	44·4	40·3
22	50·9	44·7	42·5
23	51·4	46·6	45·1
24	51·6	47·8	46·9
25	51·5	48·2	46·5
26	51·6	50·5	46·0
27	51·2	52·1	48·0
28	50·8	52·8	47·5
29	50·4	52·4	51·3
30	50·0	52·1	50·9
35	48·4	51·9	50·2
40	47·8	50·7	47·6
45	45·5	48·2	44·4
50	43·2	45·4	43·9

They were not, however, entirely indifferent, and the termination of the experiment did not justify the conclusion that immunization could be relied on to reduce herd mortality from ectromelia to negligible proportions. It will be seen (Table XXXV) that after attaining maximal values between the 24th and 29th days of herd life the ${}_{60}E_x$ figures for each group show a steady, though not great, decline. This is due to the fact that, about the 100th day of the experiment—30 days after the addition of immunized mice had ceased—a considerable wave of mortality occurred, killing many vaccinated mice as well as controls. Had this not occurred, the figures for the immunized mice throughout would have been even more favourable than those recorded in the table.

The problem of determining the proportion of deaths actually due to ectromelia, which has been discussed in detail on pp. 64 to 70, becomes particularly acute in relation to the fate of the immunized mice. The figures of post-mortem findings for the whole period of the experiment are set out in Table XXXVI and may be compared with those recorded in Table XII, p. 65. Mice that were eaten, and so could not be examined *post mortem*, are omitted. The figures for positive post-mortem findings are given as actual numbers instead of percentages, since in the later cage-age groups the numbers are too small to justify the inclusion of a percentage figure for the individual group.

TABLE XXXVI

Showing the number of mice in the immunized and control groups dying at different cage ages and showing the post-mortem appearances of ectromelia.

Cage age days at death.	*Immunized.*		*Non-immunized (added in batches).*		*Non-immunized (added daily).*	
	No. examined.	*No. positive.*	*No. examined.*	*No. positive.*	*No. examined.*	*No. positive.*
0–10	16	5	68	47	52	40
11–30	38	9	161	121	195	147
31–50	17	6	4	1	19	7
51–75	33	10	8	3	8	6
76–100	51	19	11	4	8	2
101–200	49	13	7	3	7	1
201 +	1	1	0	0	0	0
Total ..	205	63 (30·7%)	259	179 (69·1%)	289	203 (70·2%

The total percentages of positive post-mortem findings in the two non-immunized groups are a little higher than those recorded for the two epidemics of Table XII, but the figure for the immunized mice is much lower. Moreover, here again the immunized mice behave alike at all cage ages, giving approximately one-third of positive post-mortem findings, while the non-immunized mice, so far as can be judged from the small figures available at the later cage ages, show the usual tendency to give a decreasing proportion of positive post-mortem findings with increasing length of survival of the herd. A combination of groups gives the percentages shown in Table XXXVII.

In basing our comparison between the fate of immunized and non-immunized mice on all deaths, we are overestimating the ectromelia death rate among the immunized mice, just as we are overestimating the ectromelia death rate among the old survivors; and this makes the comparison of Table XXXV insufficiently favourable to the immunized, since the weight that should be applied for non-specific deaths affects all the mice in the vaccinated group, while in the control groups it affects only that small minority of mice that live beyond the 30th–50th day.

TABLE XXXVII

Cage age in days at death.	Percentage positive. Immunized.	Percentage positive. Non-immunized (added daily and in batches).
0–10	31·3	72·5
11–30	23·7	75·3
31–75	32·0	43·6
75 +	32·7	30·3

As regards the wave of mortality that set in towards the end of the experiment it is extremely difficult to determine whether the deaths were due entirely to ectromelia, or in part to some other condition.

We may note that no other recognizable infection gained access to the herd at any time.

We are, then, left in considerable doubt as to the actual cause of death in the majority of those vaccinated mice that died. We did, for interest, apply to a proportion of the immunized mice that died after the 100th day with negative post-mortem findings the liver filtrate test that we employed in our earlier experiments, but the significance of the results is uninterpretable. Of 54 liver filtrates obtained in this way 18 gave rise to ectromelia in one or more of the mice into which they were injected. Reference to Table XIV, p. 67, will show that of 127 liver filtrates obtained from non-immunized mice dying in our earlier epidemics without post-mortem evidence of ectromelia only 12 were infective. This difference cannot, however, be regarded as evidence that the proportion of deaths due to ectromelia in the absence of positive post-mortem lesions was higher in the vaccinated than in normal mice. The immunized mice had been injected with living and attenuated virus, and, as the results recorded in the following section will show, it is very probable that this virus persists in the tissues for considerable periods of time.

SUMMARY OF RESULTS OF PROPHYLACTIC IMMUNIZATION AGAINST ECTROMELIA

Taken as a whole, the results of this experiment appear to us to be highly encouraging. They accord with the general experience that anti-viral immunity is, as a rule, far more effective than anti-bacterial immunity; and they suggest that, even under the severest conditions of prolonged exposure, a relatively high degree of protection may be attained.

We have as yet made no attempt to determine, in the case of ectromelia, the effect of recruiting an infected herd entirely from immunized immigrants. This experiment we hope to carry out in the near future.

SECTION VI

THE EFFECT OF VARIATIONS IN MICROBIAL VIRULENCE AND INFECTIVITY AND THE POSSIBLE DEVELOPMENT OF "EPIDEMIC" STRAINS

That variations in the biological characters of a microbial parasite may play an important part in the initiation and progress of an epidemic prevalence, and in the striking differences in morbidity or mortality that may distinguish different epidemics of the same disease, is a possibility so obvious that it has engaged the attention of most workers who have made any serious study of these problems, whether their approach has been from the demographical, the statistical, or the experimental side.

This is, indeed, one of those questions that tends to be answered before it has been asked. The assumption that microbial parasites are subject, under natural conditions, to changes in virulence and infectivity offers so convenient an explanation of many observed epidemic happenings, and seems so well justified by our knowledge of the variability of bacterial parasites under many different conditions, that attempts to support this particular working hypothesis with adequate experimental proof have been relatively scanty. Nor has this neglect been wholly due to inattention or indolence, since the technical difficulties that hinder the solution of this problem are, as will be seen, unusually formidable.

Before we describe the results that we have ourselves obtained, and compare them with those recorded by other workers, it will be necessary, as it has been in previous sections of this report, to discuss and define our terms.

The term "virulence" has been employed by some workers to denote all those potentialities of the parasite that determine its behaviour in a herd exposed to risk. Such a definition will not serve our purpose, for we cannot assume that the characters that determine the death of an individual host, following the administration of a measured dose of the microbial parasite by one or other route, are the same as those that determine its ready spread from host to host under natural conditions. The presence or absence of such a correlation is, indeed, one of the problems that we desire to solve. It would seem better, therefore, to limit the term virulence to the power of any particular strain of bacterium or virus to cause a fatal infection in an individual animal. This is, indeed, the sense in which the term is commonly employed by those workers whose universe of study has been the individual rather than the herd, and we shall not be departing from common usage if we restrict our definition in this way. If we do so, we shall need a term to express the power of a particular strain of bacterium or virus to spread readily from host to host, inducing some degree of infection in the hosts to which it spreads. The term "infectivity," suitably defined, would seem to meet our requirements.

There is another terminological usage that has been employed not infrequently in recent literature—the reference to " epidemic strains " of particular bacterial parasites, with the implication that such strains are peculiarly potent in inducing severe epidemics of disease, presumably because they are at the same time highly infective and highly virulent. Defined in this sense, this term would seem to serve a useful purpose.

We should, then, define our three terms as follows :—

VIRULENCE, *as applied to any particular strain of a given microbial species, denotes the potentiality of that strain for producing a fatal infection in individuals of a given host species, irrespective of the mechanisms by which such infection is produced, or of the route by which the parasite is introduced into the host's body.*

INFECTIVITY *denotes the power of any particular strain of a given species of microbial parasite to spread readily from one individual to another among a herd composed of a given species of host, inducing in the hosts to whom it spreads a significant infection, which may take the form of a fatal or non-fatal illness, or a latent infection associated with some change in resistance.*

AN EPIDEMIC STRAIN *of a given species of microbial parasite denotes a strain that possesses both relatively high infectivity and relatively high virulence, in relation to a given host species, and therefore tends to induce in a herd composed of animals of that species a relatively severe epidemic of disease.*

It should, perhaps, be noted that we have given to the term " virulence " the broadest significance that has been attributed to it in ordinary bacteriological literature. We have not used it, as some workers have done, to denote only the power of invasiveness and multiplication of a parasite after it has been introduced into the tissues, excluding the poisonous action of a toxin diffusing from some relatively non-invasive bacterium. Nor have we specified the route of introduction into the body. To have done so would have involved the coining of an undesirable number of terms to cover each of the possible routes of administration. It will, however, be desirable to qualify our estimates of virulence in certain instances by adding a statement as to the route of administration employed, since it seems quite possible that this factor may influence the correlation between any given measurements of virulence and infectivity.

With our terms thus clarified we may proceed to a discussion of the experimental evidence.

Webster and his colleagues have, throughout their investigations, adopted the view that changes in the characters of the microbial parasites are of little if any significance in the evolution or progress of an epidemic. They base this conclusion in part on their failure to exalt the virulence of *Bact. aertrycke* or *Bact. enteritidis* by serial passage through mice, in part on their failure to demonstrate any differences in the virulence of strains isolated at different stages of an epidemic prevalence. Their findings in these respects with organisms of the Salmonella group have been paralleled in other

experiments with *Pasteurella lepiseptica* in rabbits, *Pasteurella* infections in fowls, and *Pasteurella* and Friedländer infections in mice. (*See* Webster, 1923 a, b, c, 1924 c, d, e, f, 1925 b, 1926, 1930 a, b, c, d; Webster and Burn, 1926 a, b, 1927 a, b, c.) They have, however, recorded differences in virulence among strains of the same bacterial species isolated from different epidemic prevalences of the same disease (Webster, 1930 a, Hughes, 1930, Pritchett, Beaudette and Hughes, 1930 a, b), and they would regard these differences in virulence as determining certain observed differences in behaviour between one epidemic and another. But they believe that once a given strain of a pathogenic bacterium has been introduced into a susceptible population its virulence remains constant indefinitely, or for so long a period of time that the virulence factor may be regarded as constant in any study of the fluctuations in mortality that occur during an epidemic prevalence, these fluctuations being always referable to other causes, such as changes in host resistance, variations in the distribution or concentration of the parasite, seasonal, dietetic, or other factors. It is true that they have found that virulent strains of *Past. lepiseptica* may be replaced in the nasal passages of rabbits by relatively avirulent strains of the same organism (Webster, 1926)—an observation that has many analogies in bacteriological records—but to variations of this kind they attach little, if any, epidemiological importance (Webster, 1932).

Recently (Webster and Clow, 1933) they have made an interesting series of observations on pneumococcal infections in mice. They find that different strains of pneumococci vary widely in their virulence for these animals as judged by the intranasal instillation of graded doses, and that these differences are in many instances not paralleled by the relative virulence of the strains as judged by intraperitoneal inoculation, a high intraperitoneal virulence being often associated with a low intranasal virulence, and a high intranasal virulence being sometimes associated with an intraperitoneal virulence of only moderate degree. Serial passage, from nose to nose, consistently failed to raise the intranasal virulence of the initially lowly virulent strains. Serial intranasal passage of the strains of high initial intranasal virulence, on the other hand, reduced the intranasal virulence almost to zero, without inducing any fall in the intraperitoneal virulence. This interesting observation did not, however, lead the authors to modify in any way their previous conclusions.

It should, perhaps, be added that most of the virulence tests referred to in the papers of Webster and his colleagues were carried out by the injection of bacteria of the Salmonella group directly into the stomach by a catheter, or by the instillation of bacteria of the *Pasteurella* group into the nose, i.e. by the administration of the parasite via the natural route of entry and not by direct inoculation into the tissues.

Before describing our own experiments it will be convenient to refer briefly to the technical difficulties to which we have drawn attention above.

If the character that we wish to measure is virulence, in the sense in which it is here defined, the only available method is to inoculate a given number of animals by some predetermined route, either with a constant dose of measured size or with a series of graded doses. The statistical difficulties involved in such tests have been discussed by Lockhart (1926) and need not be recapitulated here. It is sufficient to say that it is impossible, as a practicable procedure, to determine with any accuracy the average lethal dose of any particular strain—to do so would involve the use of many hundred mice for each strain tested. By using 20–30 mice for each strain it is usually possible to compare two strains in such a way as to detect moderate differences in virulence; but, with groups of 20 mice, there must be a difference of 5–8 mice in the numbers of deaths in the two groups before we can assume that there is any significant difference in the virulence of two strains, apart altogether from assessing how great that difference is. There is, of course, little difficulty in detecting a difference so great that one strain kills none or few of the mice inoculated, while another kills almost all; but when this is the best that can be done it is hardly possible to exclude the existence of differences that may be significant in relation to the course of events in an infected herd, though too small to be detected by the very unsatisfactory inoculation tests that are practicable.

Accepting these statistical difficulties as unavoidable, what is the technical position? We can form a rough estimate of the initial virulence of any given strain of a bacterial parasite by sacrificing 30–100 mice. If our subsequent procedure is to pass this strain artificially through a series of mice, determining at intervals the virulence of the recovered strain, we can detect gross changes in virulence, in either a plus or minus direction, by the use of similar numbers of mice at each successive test, our degree of accuracy varying according to the square root of the number of animals employed. But the method of experimental passage by inoculation represents very imperfectly the complex course of events in an infected herd; and the conditions may well be far more favourable to bacterial variation when our simple experimental passage is replaced by the random to-and-fro transit of natural contact infection. If we desire to carry out our tests under these conditions our task is formidable. Having initiated our epidemic with a strain of approximately known virulence we must withdraw our strains from the infected herd at random. If variation has occurred we have no means of knowing the relative proportions of the variants, or their distribution among the herd. We cannot tell whether any one infected animal is likely to harbour only one strain, or strains of differing virulence. Our method of isolation—from the tissues of dead mice, from the spleen or other tissues of mice killed while in apparent health, or from the excreta of living mice—may well result in selective sampling. If we are to use 30 mice for each strain tested, and especially if we are to test more than one strain from

any one member of the herd, our sampling will be very imperfect unless we are prepared to sacrifice many thousands of mice in solving this single problem. To determine the frequency of the variants at any one time, apart altogether from tracing their possible fluctuations during successive epidemic phases, appears an impossible task. Here again gross changes will be detectable with relative ease and certainty. The virtual replacement in the herd of a strain of low virulence by another of much higher virulence, or an equally wide change in the reverse direction, should not escape our notice.

When we try to measure infectivity our difficulties are even greater. The only method known to us is that which we have referred to as the " closed epidemic ". A certain number of mice, usually 25, are infected by administering to them a constant dose of the organism under study. These 25 mice are then housed in a single composite cage of the standard type employed in these experiments together with 100 normal uninfected mice. The course of events is observed over some arbitrarily fixed period, usually 60 days. All mice that die are examined *post mortem* and, at the end of the experimental period, the survivors are killed and cultures are taken from heart and spleen. In this way it is possible to obtain a rough measure of an organism's capacity to spread under conditions of natural contact; and experiments with a single strain carried out on as many as five herds simultaneously, and successive trials of the same bacterial strain made at different times, have yielded results sufficiently consistent to justify the conclusion that strains of significantly different infectivity can be differentiated from one another with a reasonable degree of probability. The use of 125 mice for the testing of each strain, and the multiplication of this figure by two or three whenever confirmatory tests are required, is not, however, a practicable procedure when large numbers of strains are concerned, and the limitations imposed on the detection of variant strains among samples drawn at random from an infected herd are even greater when infectivity is the character in question, than when we are concerned with virulence.

We are, then, almost compelled to rest satisfied with a very imperfect survey, and to postpone any attempt to measure the frequency distribution of different variants among a herd at risk until we have discovered some easily determinable character so highly correlated with virulence or infectivity that we can safely use it as an indicator.

With these preliminary remarks we may proceed to a description of those of our own results that bear upon the problem at issue, and it will be convenient to consider separately experiments on mouse typhoid and experiments on mouse pasteurellosis.

Experiments with Mouse Typhoid

We may commence by a reference to the only instance in which our observations have suggested that an epidemic wave of mouse typhoid has been initiated by the emergence of a virulent and

infective variant from a relatively avirulent strain. The series of experiments in question (Topley, Greenwood, Wilson and Newbold, 1928) consisted in the testing, by the closed epidemic method, of the infectivity of five different strains of *Bact. aertrycke*, each strain being tested on five different groups of 125 mice—25 infected by intraperitoneal injection and 100 exposed to contact infection.

In this particular series of experiments we were interested rather in the epidemicity of our strains—their power to induce fatal contact infection—than in their infectivity *per se*; and the figure that we employed as a measure of this epidemicity was the mean expectation of life of the 100 mice exposed to contact infection limited to 60 days, the arbitrary period of observation. The same figure ($_{60}E_x$) for the 25 inoculated mice of each experiment will clearly give us some measure of the virulence of the strain employed to initiate the epidemic, though not an exact measure because there is a possibility of contact infection added to the effect of the initial inoculation. The actual figures for these five series of epidemics, each series consisting of five experiments, arranged in ascending order of the mean survival time of the mice submitted to the risk of contact infection, are set out in Table XXXVIII.

TABLE XXXVIII

	Mice submitted to contact.		*Mice inoculated.*	
Experiment.	$_{60}E_x$	*S.E.*	$_{60}E_x$	*S.E.*
2	31·3	1·09	6·3	0·26
3	37·2	1·33	44·3	1·66
1	42·4	1·24	19·8	1·56
5	52·2	1·50	38·9	1·55
4	52·6	0·40	38·1	1·21

It may be noted that the standard errors given are calculated from the five separate means recorded in each experiment, and the differences, except the trivial difference between Experiments 4 and 5, are in all cases statistically significant—though those between Experiments 1 and 3 and 2 and 3 can only be regarded as suggestive.

It will be seen that there is a general concordance in the order of epidemicity, as shown in column 1, and the order of virulence, as shown in column 3; but that Experiment 3 stands out in striking contrast. Not only does the $_{60}E_x$ figure for the inoculated mice fall out of its proper order, but the $_{60}E_x$ for the inoculated mice is in this case significantly greater than the $_{60}E_x$ of the normal mice placed in contact with them, while in every other instance the inoculated mice, as one would expect, died far more rapidly than those that contracted the disease from them by natural contact.

The determination of virulence, in this series of tests, was not, however, confined to the calculation of the $_{60}E_x$ of the inoculated mice. In each experiment but that labelled Experiment 1 the virulence of the initiating strain was determined by injecting 1,000 bacilli intraperitoneally into each of 30 mice (except in a

few instances in which 25 mice were used), housing the mice in separate cages, and observing them for 14 days. Strains were also isolated from mice dying during the epidemic, and from the spleens of survivors killed at the end of the 60 days' observation, and examined by the same method. In all experiments except Experiment 3 the results were concordant. The strain employed to start Experiment 2 killed all of 25 mice within 14 days. Each of three strains isolated from survivors killed on the 60th day of one or other of the five epidemics of Experiment 2 killed all of 30 mice within 14 days. The strain used to initiate Experiment 4 was of low virulence killing none of 30 mice. Fourteen strains recovered from mice dying during the group of epidemics belonging to this Experiment 4, or killed on the 60th day, killed 0–7 of the 30 mice inoculated with each of them. The strain used to initiate the epidemics of Experiment 5 killed 7 of 30 mice. Nine recovered strains killed 0–5 of 30 mice.

The results obtained in Experiment 3 differed sharply. The strain employed to initiate the epidemics was of low virulence, killing 6 of 25 mice. Three strains isolated from contact mice dying during the epidemics, and three isolated from contact mice killed on the 60th day, were of high virulence, killing 28–30 of 30 mice. Three strains were isolated from inoculated mice that had survived throughout the epidemic period. One of these was of high virulence, killing 29 of 30 mice. Two were of low virulence, each killing 5 of 30 mice. No strains of intermediate virulence were encountered. To sum up, this particular group of five epidemics in Experiment 3 was started with a strain of low virulence, yet in each case a severe and fatal epidemic was induced. The strains isolated from the contact mice dying during the epidemic phase were of very high virulence, but two out of three strains isolated from inoculated mice that lived throughout the whole period of one or other epidemic, showed the same low virulence as the initiating strain. The inoculated mice—inoculated with the bacillus in its relatively avirulent form—lived on the average significantly longer than the mice placed in contact with them, in sharp contradistinction to the relation observed in all the other epidemics of this series.

An explanation of this series of events in accordance with all these observed happenings would be as follows. The initiating strain, though of low virulence, was relatively unstable, and rapidly gave rise when passed from mouse to mouse by natural contact to a highly virulent variant that initiated a fatal epidemic among the normal mice exposed to risk. The inoculated mice lived longer than their normal companions because, by the time the epidemic caused by the virulent variant was under way, they had acquired an immunity from the sub-lethal infection induced by the bacterium in its non-virulent form. That this explanation is, in its main outline, correct we have no doubt. The experiment to which we shall next refer affords striking confirmatory evidence of this working

hypothesis in relation to the behaviour of the inoculated mice; but in view of the relatively rapid appearance of the virulent form in each of the five epidemics of Experiment 3 it seems at least possible that the initiating strain, though of low virulence, was incompletely degraded, and contained a few virulent bacilli. Though few of the 125 inoculated mice in the five epidemics initiated with this strain received sufficient virulent bacilli to cause their death, the virulent form might well spread rapidly among the contacts. We do not think that it is possible to decide between these alternative hypotheses; and for this reason we should not regard this experiment as affording definite evidence of variation from low to high virulence during epidemic spread.

It may be noted that, in this series of trials, the results suggested a high correlation between virulence and infectivity.

Taking the experiments again in order of the descending severity of the epidemic, that is in ascending order of the ${}_{60}E_x$ figures, the percentages of surviving contacts giving positive spleen cultures were as follows:—

	Contact mice.	
Experiment.	${}_{60}E_x$	*Infected survivors per cent.*
2	31·3	79·2
3	37·2	79·8
1	42·4	70·1
5	52·2	9·0
4	52·6	9·7

The virulent strains were also infective; the avirulent strains used in initiating the epidemics of Experiments 5 and 4 were clearly of low infectivity as well as of low virulence; but, as will be seen from the series of experiments described later in this section, these relations do not always hold.

A strain of *Bact. aertrycke* used to initiate a series of closed epidemics had shown an unusual combination of properties (Topley, Greenwood and Wilson, 1931 b). It had a moderately high virulence when tested by the injection of 1,000 bacilli intraperitoneally into mice, killing 64–74 per cent. of the injected mice within 14 days in a series of tests carried out over many months. When used to initiate closed epidemics, however, it had failed to induce more than a trivial mortality among the contact mice. The ${}_{60}E_x$ values of the contacts in two such epidemics were 56·9 and 56·0 days respectively. When the surviving mice from these two epidemics were killed and examined on the 60th day, 68 of 173 survivors, or 39 per cent., yielded cultures of *Bact. aertrycke* from their spleens, showing that, in spite of the low mortality, there had been a considerable spread of infection. This combination of characters—moderately high virulence, low epidemicity, and relatively high infectivity—was so unexpected that it seemed desirable to examine

this strain in greater detail. It was tested on four other closed herds, and three strains isolated from mice dying in the epidemics thus initiated were tested each on two closed herds; so that this strain, or strains derived from it by natural contact passage, were tested on 10 further herds, making 12 tests in all. In spite of minor differences the general behaviour of the strain, or of its derivatives, never altered significantly. The ${}_{60}E_x$ value of the contact mice in the 10 additional tests varied from 47·0 days to 53·9 days, and the mortality among the contact mice during the 60 days of the epidemic varied from 23 per cent. to 40 per cent., mortalities which, though relatively severe when judged by any ordinary standard, and somewhat higher than those recorded when this strain was first tested, indicate only a moderate epidemicity so far as mouse typhoid is concerned.

In 6 of these 10 closed epidemics the surviving contact mice, numbering 416 in all, were killed on the 60th day and examined *post mortem*; 31·0 per cent. gave positive spleen cultures. The mean ${}_{60}E_x$ for these epidemics was 51·3 days. These figures may be compared with the two series of mild epidemics induced by the avirulent strains referred to above—one series showing a mean ${}_{60}E_x$ of 52·6 days with an infection rate of 9·7 per cent. among the surviving contacts, the other a mean ${}_{60}E_x$ of 52·2 days and an infection rate of 9·0 per cent. It is clear that the strain of *Bact. aertrycke* studied in this series of tests, while approximating to the relatively avirulent strains in its low epidemicity, as judged by the death-rate among the mice exposed to risk, possessed an infectivity of a far higher order, as judged by its capacity for inducing latent infections.

It was of obvious interest to enquire whether mice that had passed through an epidemic caused by this strain had acquired an active immunity. For this reason the surviving contacts from certain of the epidemics referred to above were not killed on the 60th day, but were retained for this additional test. We had also available survivors from three closed epidemics of much greater severity, initiated with a highly virulent and infective strain, and a sample of mice immunized by two injections of a killed *Bact. aertrycke* vaccine. With these mice four closed epidemics were initiated, one with survivors from the relatively mild epidemics, one with survivors from the severe epidemics, and two with vaccinated mice. Three control epidemics were initiated with normal mice. The 25 infecting mice added to each of these groups were injected with a strain of high virulence and infectivity. The ${}_{60}E_x$ figures, taking the mean value where more than one epidemic was included in a single group, were as follows:—

	${}_{60}E_x$	*S.E.*
Survivors of mild epidemics ..	48·8	1·86
Survivors of severe epidemics ..	48·3	1·84
Vaccinated mice	42·0	1·53
Normal mice	37·5	2·61

It will be seen that the survivors of the mild epidemic had a slightly, but insignificantly, greater advantage over the normal mice than the survivors of the severe epidemics—among whom, it may be noted, the selective elimination of susceptibles by death had been much more extensive. Both groups of survivors had a significantly greater advantage than the vaccinated mice.

It seems clear that the strain of relatively low epidemicity, spreading among the mice at risk, had induced a significant degree of active immunity at a relatively low price in mortality. If a strain with these characters were produced as a variant during a prolonged epidemic prevalence it would almost certainly exert a significant effect on the subsequent course of events. For this reason we cannot agree with Webster's opinion that the appearance of strains of lowered virulence among an infected herd has little if any epidemiological significance.

Finally we may note that, though we have never yet demonstrated a clear and indubitable variation in virulence or infectivity in *Bact. aertrycke* during an epidemic prevalence, we have never carried out a series of tests on a scale that would ensure the detection of such variants unless they constituted a high proportion of the total bacterial population. We should, however, agree with Webster that the evidence at present available suggests that *Bact. aertrycke* tends to retain a constant virulence under a variety of different conditions. We have, for instance, carried out a large number of tests of the virulence of strains isolated from the tissues of survivors from closed epidemics. With the single exception noted above we have never found these strains to differ significantly in virulence from the strain with which the epidemic was started. We have also tested, on many occasions, strains recovered from vaccinated mice that had survived the injection of a virulent strain. No strain that we have yet isolated under these conditions has shown any significant diminution in virulence. This, as will be seen from the description of hitherto unpublished results in the following section, is not true of bacteria in general.

Experiments with Mouse Pasteurellosis

The first indication that we obtained of in-vivo variations in virulence of *Pasteurella muriseptica* emerged during a series of experiments on the infection rate among the survivors of vaccinated and normal mice after the intraperitoneal injection of a relatively virulent strain of the organism. The dose administered was 100,000 bacilli, and the vaccinated and control mice were observed for 28 days. Since spleen and heart cultures from survivors from epidemics of pasteurellosis had, on the average, given rather fewer positive results than we usually obtained with *Bact. aertrycke* infections, we thought it possible that latent foci might more commonly be found in the lungs or mediastinal glands than in the spleen. In this particular experiment, therefore, we dealt with the survivors on the 28th day by removing the thoracic organs

and the spleen, mincing and grinding them with saline, and injecting the extract so obtained from each survivor into a normal mouse.

Of the 50 vaccinated mice injected with virulent bacilli only 2 died during the observation period. Tissue extracts from the 48 survivors were injected into 48 normal mice. Of these, 9 died from pasteurellosis. The remaining 39 were killed 21 days after the injection of the tissue extract and examined *post mortem*. Cultures from the heart or spleen yielded a culture of *Pasteurella* in seven instances.

Of the 50 controls, 37 died. Tissue extracts from the 13 survivors were injected into 13 normal mice. Of these, 8 died of pasteurellosis, and of the 5 survivors only one yielded a culture of *Pasteurella post mortem*.

It seemed desirable to determine whether the organisms that had been recovered from the vaccinated mice by passage through normal mice, but had failed to kill the mice through which they were passed, had retained the full virulence of the parent strain. Five of these seven strains were therefore injected intraperitoneally into 20 mice in a dose of 100,000 bacilli, the original strain being injected in the same dose into another group of 20 mice as a control. All mice were observed for 21 days. The deaths in the various groups were as follows:—

Original strain	14
From immunized Survivor 1	15
,, ,, ,, 2	0
,, ,, ,, 3	1
,, ,, ,, 4	2
,, ,, ,, 5	0

Thus only one of the five strains recovered from the vaccinated mice had retained the virulence of the original strain, the other four having become almost avirulent. These four strains differed in other ways from the parent strain. They fermented maltose but not mannitol, whereas the parent strain and the virulent passage strain fermented mannitol but not maltose. In growth in broth and in colony form on agar they also departed from the typical smooth form of the strain from which they were derived. They could not, on these tests, be classed as rough variants, but they certainly deviated from the parent form in this direction, and their almost complete loss of virulence seemed to mark the variation as approximating to the S⟶R type. We may add that, in many further experiments with this organism, we have found that a strain that ferments maltose but not mannitol is always of low virulence, while only once have we encountered an avirulent strain fermenting mannitol but not maltose.

This experiment was repeated with very similar results. Fifty vaccinated and 50 control mice were injected with a virulent strain of *Pasteurella*. Of the 50 vaccinated, 7 died, of the 50 controls, 42. In this instance we did not prepare tissue extracts from the survivors, but took the usual cultures from the heart and spleen. Five of 43

vaccinated survivors, and 4 of 8 controls, gave cultures of *Pasteurella*. Six strains from vaccinated mice, including two strains from a single mouse, one isolated from the heart, the other from the spleen, three strains from control mice, and the original strain as a control were tested for virulence in the usual way by injecting 100,000 bacilli intraperitoneally into groups of 20 mice. The deaths in these groups were as follows :—

		Deaths
Original strain		18/20
Strains from :—		
Immunized Survivors	1	1/20
	2 (Heart)	0/20
	2 (Spleen)	10/20
	3	13/20
	4	14/20
	5	17/20
Non-immunized Survivors	1	19/20
	2	17/20
	3	18/20

Of the two avirulent strains, that recovered from the heart of vaccinated survivor 2 fermented maltose but not mannitol, that recovered from vaccinated survivor 1 fermented mannitol but not maltose—forming the single exception referred to above. All the virulent strains were mannitol fermenters.

In this instance, it will be noted, only 2 of 6 strains isolated from immunized survivors proved avirulent. The other 4 strains from immunized survivors, and all 3 strains from non-immunized survivors retained the virulence of the parent strain (with the possible exception of spleen No. 2).

These experiments demonstrate quite clearly that *Past. murisep-tica*, when vegetating in the tissues without causing a fatal infection, tends to undergo variation with the production of avirulent forms. They suggest—and this suggestion accords well with our knowledge of the influence of anti-bodies on variation *in vitro*—that this change is more likely to occur in the tissues of immunized than in those of normal mice. This suggestion is, as will be seen, strengthened by the experience described below, but even with this added support it falls far short of proof.

It is quite certain that this loss of virulence is not an invariable concomitant of latent infection in immunized mice. The figures quoted above show this clearly enough, and we may add that in several other experiments carried out on identical lines we have found all the strains isolated from immunized survivors to have the same high virulence as the strain used for inoculation.

Before we can accept the view that these happenings in artificially immunized and infected mice have any significance in relation to the natural epidemic process, it is clearly necessary to show that they occur in an infected herd. The observation that our avirulent strains fermented maltose but not mannitol, while our virulent

strains fermented mannitol but not maltose—only a small minority of strains fermented both substances—seemed to offer a hope of sampling the bacterial population of some of our infected herds without an impossible sacrifice of mice. We therefore tested a number of strains isolated from mice dying during a continuous additive epidemic, and from mice dying during closed epidemics, or killed and examined *post mortem* at their close. The selection of strains from dead mice clearly restricted our chances of recovering avirulent strains from the additive epidemic, but to withdraw mice and kill them would have disturbed our general records of mortality, and we have not found the isolation of *Pasteurella* from the nasal passages or throats of living mice an easy or successful procedure. Moreover, it seemed at least possible that a proportion of the mice dying after long residence in an infected herd might have succumbed to a variety of causes of which a latent or sub-lethal *Pasteurella* infection might be only one. We may note that the additive epidemic selected for this test was one in which both normal and immunized mice were being added at regular intervals to a herd, so that we had another opportunity of comparing the behaviour of these two classes of mice.

Our method consisted in testing each strain examined in maltose and mannitol, and confirming the avirulence of the maltose-fermenting strains by injecting 5 mice intraperitoneally with 100,000 bacilli. In order to confirm the reliability of this fermentation test as a rough sorting method we also tested a number of mannitol-fermenting strains on groups of 5 mice. The results of these comparative tests, grouping the strains isolated from additive and closed epidemics together, were as follows. Of 105 mice injected with one or other of 21 mannitol-fermenting strains all died. Of 45 mice injected with one or other of 9 maltose-fermenting strains 5 died. Thus the correlation between mannitol-fermentation and virulence seemed to hold.

During the additive epidemic 100 strains from non-immunized mice were examined by the fermentation test. All fermented mannitol but not maltose. Twenty-eight strains from immunized mice were tested in the same way; 26 fermented mannitol but not maltose, 2 fermented maltose but not mannitol. These two strains, and a selection of the mannitol fermenters, were tested for virulence, and are included in the strains referred to above.

The remaining avirulent, maltose-fermenting strains that we have encountered have been recovered from mice dying during another additive epidemic (2 strains), mice dying during a closed epidemic that produced a low mortality although induced with a virulent strain (4 strains), and from a mouse killed at the termination of another closed epidemic (1 strain). All these strains were recovered from non-immunized mice, so that it is clear that artificial immunization is not an essential factor in inducing this change. It would be strange if it were, since the majority of mice become actively immunized after long survival in an infected herd.

It is clear then that avirulent strains do arise by variation during the spread of epidemic infection, and it seems very probable that if a more thorough and efficient method of sampling had been employed, including the isolation of strains from living and apparently healthy mice, the frequency of such strains would have been much higher.

Wide differences have been observed in different experiments in regard to the relative frequency of avirulent variants among the survivors. In one experience we have the recovery of 4 avirulent strains from a small number of mice dying in a single small closed epidemic, with only 100 mice at risk, which must be compared with the extreme rarity of such strains in other much larger epidemics initiated with other strains. This striking difference suggests that the frequency of occurrence of such variants is dependent in large part on the nature of the initiating bacterial strain, and that one of the factors determining the epidemic behaviour of any given bacterial strain may be the relative frequency with which it gives rise to avirulent variants.

An Increase in the Infectivity of *Past. muriseptica* during its Epidemic Spread

We have had an opportunity of observing a sequence of events that appears to afford a clear demonstration of an increase in the infectivity of *Past. muriseptica* during epidemic spread.

On 6.iv.32 we inoculated 25 mice with 100,000 *Pasteurella* from a strain ($P.M._3$) that killed about 20 of 25 mice when injected intraperitoneally in this dose. To these we added 100 normal mice with the intention of starting a continuous epidemic. On 15.iv.32 one added mouse died of pasteurellosis, but as no further spread had occurred by 22.iv.32 we added another 25 mice injected with 100,000 $P.M._3$ By 4.vi.32 11 added mice had died, but only from 6 of them was *Past. muriseptica* recovered. From this date onwards, however, there was a small wave of pasteurellosis, and between 4.vi.32 and 13.vi.32 30 added mice succumbed to the disease. We then began adding 3 normal mice a day, according to our usual practice, but the little wave of deaths soon died down, and during the two months 15.vii.32–15.ix.32 only 18 mice died in the cage, most of which were eaten by their companions and so could not be submitted to post-mortem examination. As the cage population was rapidly increasing we then stopped the daily additions. On the next day a mouse died of pasteurellosis. The strain recovered from this mouse was injected into 20 mice on 23.ix.32, in the usual dose of 100,000 organisms, and these 20 mice were added to the herd. On 1.x.32 the epidemic began to spread again and continued to do so. On 28.x.32 we again started adding 3 normal mice a day, and from that time onwards the epidemic continued, until the experiment was discontinued many months later, the mortality curve showing only those minor fluctuations to which all our previous experience had accustomed us.

This long delay in the spread of the initial strain, followed by a short abortive spread, and a second effective and continuous spread some two months later, gave us an opportunity to test the infectivity of strains of *Past. muriseptica* isolated from the herd during well-differentiated phases of the epidemic.

The strains we employed for this series of tests were as follows :—

P.M.$_3$ The strain with which the epidemic was initiated, and which had been maintained in stab-agar culture in the ice chest during the intervening period.

P.M.$_3$ (2) The same strain after repeated subculture.

P.A.39 A strain isolated from a contact mouse dying in the herd during the period of abortive spread.

P.A.39(2) The same strain after repeated subculture.

P.62 A strain isolated from the herd during the final period of effective and continuous spread.

With each of these strains we initiated a closed epidemic of the type previously described, 25 inoculated mice being herded with 100 normal contacts and the whole community being observed for 60 days. On the 60th day the survivors were killed, and cultures were taken from heart and spleen. All mice dying during the epidemic were examined *post mortem*, except when cannibalism rendered this impossible. The results are summarized in Table XXXIX.

TABLE XXXIX

	Strain of Pasteurella.				
	P.M.$_3$	*P.M.$_3$(2)*	*P.A.39*	*P.A.39(2)*	*P.62*
No. inoculated	25	25	25	25	25
No. inoculated that died ..	20	20	25	21	25
No. contacts	100	100	100	100	100
No. contacts that died ..	12	3	13	13	62
No. contacts that died showing *Past.* P.M.	0	0	6	3	50
No. surviving contacts ..	88	97	87	87	38
No. survivors showing *Past.* P.M.	0	0	0	1	7

The results of this series of tests seem quite definite. P.M.$_3$ and P.M.$_3$ (2), in conformity with the behaviour of this strain in the experiment described above, showed no tendency to spread, although they produced a high mortality in the inoculated mice. P.A.39 and P.A.39 (2) behaved alike, and differed little from the original strain P.M.$_3$. There is a suggestion that they were slightly more infective, but the difference is trivial. Strain P.62, in conformity with its behaviour in the main herd, combines high virulence with high infectivity. There can, we think, be little doubt that the onset of effective and continuous spread in the original herd was due to the appearance of a variant that had gained infectivity and retained, or perhaps increased, its original virulence.

Another experiment, of an exactly analogous kind, gave results that, so far as they went, were in entire conformity with those recorded above. A strain that gave an initial but transitory spread in an ordinary additive epidemic, and a remote subculture of this strain, showed relatively high virulence but relatively low infectivity when tested by the closed epidemic method. A strain recovered from the first of these closed epidemics showed similar characters. A strain recovered from the original additive epidemic many months later, when effective spread was occurring, showed an increased infectivity when tested in a closed epidemic. Unfortunately three of these four closed epidemics were cut short in the seventh week by the accidental spread of ectromelia to the herds. We have, therefore, no satisfactory records beyond the 42nd day. The original strain and its derivatives showed no significant difference in virulence—the numbers of inoculated mice dying varied from 21 to 24 out of 25. As regards infectivity, and taking the records to the 42nd day, the number of contacts dying in the epidemics started by the original strain and its derivatives were 15, 10 and 16 respectively with 10, 5, and 7 isolations of *Pasteurella post mortem*, while the figures for the epidemic started with the more infective strain were 34 deaths, of which 18 were proved *post mortem* to be due to pasteurellosis; the rather low proportion of post-mortem isolations of *Pasteurella* in this series of tests was due almost entirely to the relatively large number of mice partially eaten by their companions. Owing to the advent of the ectromelia infection we were unable to determine the frequency of infection in surviving mice. This was an unsatisfactory series of experiments, and the results should be regarded as compatible with those recorded in the earlier series of tests, rather than definitely confirmatory of them.

The demonstration, in these two series of tests, that a relatively high virulence, as judged by direct injection into the tissues, might be associated with low infectivity, even in the case of a natural parasite of the mouse, led us to compare somewhat more carefully the virulence and infectivity of certain of our strains, using graded doses for the virulence tests, and the intraperitoneal route of inoculation. The results of a series of such tests are set out in Table XL. In the first two columns are shown the results of infectivity tests, expressed as the proportion of deaths among 100 contacts in a closed epidemic, and the proportion of infected mice among the survivors, and in the last five columns the results of the virulence tests in which five mice were injected with each of the graduated doses.

Clearly we must regard such virulence tests as these, with no more than five mice to a group, as the roughest of comparisons. But we need have no hesitation in classing strains P.64, P.62, P.29 and P.A.39 as highly virulent, and strain P.58 as much less virulent. Of these it will be noted that the virulent strains P.64 and P.62 are highly infective as well as highly virulent—they are typical epidemic strains, in the sense in which we have defined that term. But

strain P.29, though highly virulent, has relatively little infective power, and strain P.A.39 combines high virulence with the lowest infectivity of the series, at least as low as that of the relatively avirulent strain P.58.

TABLE XL

Strain.	*Infectivity.*		*Virulence.*				
			Deaths among 5 mice per dose.				
	Contact deaths.	*Survivors infected.*	10^5	10^4	10^3	10^2	10
P.64	73/100	6/27	5	5	5	5	4
P.62	62/100	7/38	5	5	4	4	2
P.29	26/100	0/74	—	5	4	4	2
P.58	18/100	1/82	3	1	0	1	0
P.A.39	13/100	0/87	5	4	5	4	2

This series of tests demonstrates quite clearly that virulence and infectivity are not synonymous terms, and that the two characters must be studied and considered separately in any attempt to analyse the epidemic process from this angle.

SUMMARY

We think that it has been demonstrated—

(1) That virulence and infectivity are separable bacterial characters that must be studied separately. They certainly vary independently—a highly virulent strain may have little infectivity, and strains of relatively low virulence may spread readily by contact—but we have not yet encountered a strain of very low virulence that has the power of producing frequent contact infection, as infection is here defined.

(2) That these differences are significant from the epidemiological point of view. A strain of high virulence and high infectivity—a typically "epidemic" strain—spreads readily and kills many while immunizing few. A strain of lower virulence, but retaining a relatively high infectivity, immunizes many while killing few.

(3) That, at least in pasteurellosis a variation from a virulent to an avirulent form occurs in the tissues of the host, both after artificial infection and during natural epidemic spread. There is a suggestion that this change occurs more frequently in the tissues of immune, or partially immune, hosts than in those of completely susceptible animals.

(4) That, again in pasteurellosis, an epidemic may fail to develop in an infected herd because the infecting parasite, while possessing an adequate virulence, lacks infectivity; and that, during the natural spread of contact infection,

an " epidemic " variant may be produced which, combining these two essential characters, induces in a herd a continuous epidemic spread associated with a high mortality.

Taking these observations as a whole, they seem to us to lend strong experimental support to the view that variations in the character of a bacterial parasite may well be of decisive importance in the secular history of any epidemic disease, and in the determination of the differences that have so often been noted in the behaviour of epidemics of the same disease occurring in different places during a relatively short period of time. We do not think that our evidence supports the view that a variation in the characters of the parasite is an essential, or even a frequent, concomitant of the evolution of a single epidemic wave, with its characteristic rise and fall. It is in the long-distance development of the epidemic process, or in the introduction or evolution of epidemic strains among particular herds, that this factor would seem to play its most significant part.

SECTION VII

THE INFECTIVITY OF MICE IMMUNIZED AGAINST ECTROMELIA, BEFORE AND AFTER THE FURTHER INJECTION OF FULLY ACTIVE VIRUS

In any attempt to estimate the probable effectiveness of a given method of prophylactic immunization as a means of controlling herd infection, it is necessary to enquire whether the immunized individuals may be regarded as resistant to infection in the widest sense, and hence as innocuous to their herd companions, or whether they must be considered to be potential carriers, though not potential cases. When the method of immunization adopted involves the injection of a living bacterium or virus, we must also consider the possibility that we are ourselves creating transient or persistent foci of infection. The risk, to the herd at large, of the presence of any considerable quota of immune but potentially infective individuals might well be serious, even though the infectivity of any one immunized animal were much less than that of an infected non-immune. The importance of this factor in the control of infection among human herds is clearly illustrated in the well-documented history of diphtheria prophylaxis (Dudley, 1923, 1926; Dudley, *et al.*, 1934; Lee, 1931; Godfrey, 1932). The account by Dudley and his colleagues of carrier epidemics among an adolescent human herd, fully immunized against diphtheria as a clinical disease, leaves no doubt as to the true position of affairs in this particular instance; and it may be noted that here, as in our mouse-typhoid experiment, immunization of every member of the herd may fail to suppress the overt disease under conditions of unusual stress. We have no such complete and detailed data in regard to the immunization of animal herds under field conditions, and in its absence we must do the best we can with arguments from analogy based on laboratory experiments. The problem is of obvious importance from the agricultural point of view; and we may note that virus diseases bulk largely in the infections that menace our live-stock, and that it is in these diseases that the use of a living vaccine is a common procedure. The possibility, for instance, of substituting a policy of immunization for a policy of slaughter in the control of foot-and-mouth disease must depend in part on the potential infectivity of the immunized animals.

The fact that we could induce an effective active immunity in mice to the injection of large doses of potent ectromelia virus seemed to offer an opportunity for the study of this problem under experimental conditions. It was realized that the technical difficulties were formidable. It was necessary to work with large numbers of mice, divided into many different groups; and our experience had taught us that it was extremely difficult, in this disease, to prevent the accidental spread from group to group when an experiment had to be continued over a long period of time. Our

expectations were fulfilled. It proved impossible, working with fifty or more cages distributed in a single experimental room, and cared for by the same assistants, to maintain effective isolation over periods of many weeks. Attempts at improving the isolation by the use of glass or gauze screens were unsuccessful. The problem is, of course, an entirely different one from that presented by the segregation of a single large herd, or of two large herds that can be separated more widely in space, where the only risk of infection is from one herd to the other. It became clear that the avoidance of the multiplied risks, inherent in the particular study that we wished to undertake, demanded a number of separate experimental rooms and of different groups of assistants that were not at our disposal. We have had, therefore, to be content with results that are clearly open to criticism. They seem to us to give a fairly definite answer to the main question at issue; but that, perhaps, is a matter of opinion.

Experiment 1.—On 17.vii.33 the following groups of mice were assembled in experimental cages:—

(*a*) Fifty mice that had received an injection of a ten-day-formolized virus on 30.vi.33, and of a three-day-formolized virus on 7.vii.33, were divided into 10 groups of 5 mice, and to each group were added 5 normal mice.

(*b*) Fifty mice that had received an injection of a ten-day-formolized virus on 23.vi.33 and of a three-day-formolized virus on 30.vi.33, followed by an injection of a potent phenolized virus on 7.vii.33, were also divided into 10 groups of 5, each group being caged with 5 normal mice. These 50 immunized and infected mice came from a group of 76 immunized mice that had been given a massive dose of a potent phenolized virus. During the 10 days from 7.vii.33 to 17.vii.33 two of these mice had died, one of them with the post-mortem appearances of ectromelia.

(*c*) Fifty normal mice were injected on 17.vii.33 with the same dose of phenolized virus that had been given to the immunized mice 10 days earlier. These 50 infected non-immunes were divided among 10 cages, 5 to a cage, and to each cage 5 normal uninfected mice were added.

(*d*) There were available at this time 14 surviving immunized mice from the epidemic described in the preceding section. They had been exposed to risk in the original herd for varying periods of time, and had been isolated in single cages for 34 days prior to 17.vii.33. These 14 mice were placed in 3 cages (5, 5 and 4 mice to a cage) and to each cage 5 normal mice were added.

(*e*) Fifty normal uninfected mice were placed in 10 cages, 5 mice to a cage. These 10 cages were dispersed at regular intervals among the 33 cages described under (*a*) and (*d*) above, in order that we might detect the occurrence of any accidental spread of infection. The experiment was continued for 84 days.

The results are summarized in Table XLI. The deaths in each main group are set out in columns under headings giving the treatment to which the infected mice had been subjected. Each column is divided into two sub-columns, the deaths among the infected and potentially infective mice, and among the normal mice exposed to risk being given separately. The table is divided into ten rows, each row in each column corresponding to an arbitrarily numbered cage containing 5 infecting and 5 normal mice. The mice in the ten cages of the last column were normal controls. The deaths listed are those from ectromelia, i.e. those in which the post-mortem examination revealed the lesions associated with this disease. In a proportion of cases the naked-eye post-mortem diagnosis was checked by injecting a liver filtrate into normal mice. As always happens when several hundred mice are observed over a period of three months, a certain number of deaths occurred in which the post-mortem examination revealed no abnormalities. Deaths of this kind are not included in the table. Again, when mice are housed together in groups a certain number of the dead are always partially eaten by their companions, so that a post-mortem is impossible. In such cases some arbitrary rule must be adopted. In recording the deaths of these partially-eaten mice, we have regarded them as dying from ectromelia if any other death from ectromelia occurred in that cage. If no death from ectromelia occurred in a cage during the 84 days of observation, a partially-eaten mouse from that cage has been regarded as dying from some other cause. We may perhaps add that no deaths occurred from any other diagnosable type of infection.

TABLE XLI

Showing deaths from ectromelia among infecting mice (5) *and mice exposed to risk* (5) *in various groups and sub-groups. Figures in brackets refer to the day on which the first death from ectromelia occurred in that sub-group.*

Sub-group No.*	*Immunized (living virus).*		*Immunized and infected.*		*Infected non-immunized.*		*Survivors from epidemics.*		
	Infecting.	*Exposed.*	*Infecting.*	*Exposed.*	*Infecting.*	*Exposed.*	*Infecting.*	*Exposed.*	*Controls.*
1	0	0	0	3 (22)	5 (4)	0	0	1 (79)	2 (43)
2	0	0	1 (45)	3 (26)	5 (4)	1 (49)	0	0	0
3	0	0	0	0	5 (4)	1 (28)	0	0	0
4	1 (32)	0	1 (33)	3 (16)	5 (4)	1 (15)			0
5	0	3 (10)	0	2 (21)	5 (4)	3 (13)			1 (84)
6	0	0	0	1 (16)	5 (4)	0			0
7	1 (52)	0	0	1 (53)	5 (4)	3 (22)			1 (68)
8	1 (43)	4 (13)	0	1 (16)	5 (4)	1 (28)			5 (38)
9	0	0	1 (8)	4 (24)	5 (4)	0			1 (47)
10	0	4 (34)	0	3 (16)	5 (4)	3 (12)			0

* In each series, corresponding to each double column, the cages containing the groups of 10 mice were numbered 1 to 10 at random, so that, for instance, Sub-Group No. 1 of the immunized and infected is no more comparable with Sub-Group No. 1 of the immunized (living virus) than with any other Sub-Group of the latter.

The figures in brackets refer in each case to the day on which the first death from ectromelia occurred in that group of 5 mice, infective or exposed to risk. Thus, taking the top row of the second double column, in one of the 10 cages containing 5 immunized and infected and 5 normal mice, no death from ectromelia occurred among the immunized and infected mice during the 84 days of the experiment, but 3 deaths from ectromelia occurred among the 5 normal mice exposed to contact infection, the first death occurring on the 22nd day. Considering the results on this basis, we may take first the very unfortunate history of the controls. Deaths from ectromelia occurred in 5 of these 10 cages. We had failed to prevent accidental cage-to-cage infection, and the mice in any cage must be regarded as having been exposed to this risk, as well as to the risk of infection from their companions in the same cage. It will, however, be noted that these accidental infections occurred relatively late in the experiment. Had the period of observation been limited to 5 weeks no ectromelia deaths would have occurred among the controls; had it been limited to 6 weeks only one control cage would have been regarded as infected. If we turn to the other groups of normal mice, exposed to contact with potentially infective mice in the same cage, it will be seen that, of 20 groups among which deaths from ectromelia occurred, the first ectromelia death occurred in under 5 weeks in 17 instances, in 16 instances it occurred within 4 weeks, and in 10 instances within 3 weeks.

It seems very unlikely that this consistent difference in the date of spread of infection is fortuitous. Taking the figures as they stand, spread occurred in 3 of 10 cages in which immunized mice were housed with normal mice, in 9 of 10 cages in which immunized and infected mice were housed with normal mice, in 7 of 10 cages in which infected non-immunes were housed with normals, in 1 of 3 cages in which vaccinated survivors from an epidemic were housed with normal mice, and, accidentally, to 5 of 10 cages of normal mice containing no infected companions.

Limiting ourselves to deaths within a 4 weeks' period, spread occurred in 2 of 10 cages containing immunized and normal mice, in 8 of 10 cages containing infected immunes and normals, in 6 of 10 cages containing infected non-immunes and normals, in none of 3 cages containing survivors from an epidemic and normals, and to none of 10 cages containing normal controls. Accepting the view that these results cannot reasonably be assigned to accidental cage-to-cage infection and that they must, therefore, be regarded, at least during the earlier stages of the experiment, as indicating contact infection within the various cages, we may examine each group in greater detail.

Taking first the group in which normal mice were exposed to the risk of infection from immunized and infected mice, it will be seen that spread occurred in 9 of 10 cages, or in 8 of 10 if we confine ourselves to the 28-day period. In 5 of these 8 groups no deaths occurred among the immunized and infected mice. There can, we

think, be little doubt that these results indicate that immunized and infected mice, even after they have survived in apparent health for 10 days or more after receiving a dose of potent virus, are dangerous carriers and readily pass the disease to susceptible companions by contact infection. In three instances a late death occurred among the immunized and infected mice, and in these instances it might be argued that spread occurred from these particular mice and not from the apparently healthy carriers. We do not think that it is possible to arrive at any sound conclusion on this point. There is, in the first place, the difficulty of arriving at a correct diagnosis of the actual cause of death in the immunized mice. These deaths are recorded as due to ectromelia because the dead mice showed certain of the lesions associated with this disease. But they were known to have received a large dose of potent virus, and it would be in no way surprising if they had developed recognizable lesions during a sub-lethal infection and had subsequently died from some other cause—there were, it will be noted, only 3 deaths of this kind among 50 immunized and infected mice observed for 3 months. In the second place it will be seen that, in 2 of the 3 instances in which deaths among the normal mice were associated with a death among the immunized and infected mice, the deaths among the normal mice occurred first, the interval between the first normal death and the death in the infected group being longer than the period usually covered by a fatal attack of the disease. It is, of course, possible that a partially immune mouse, harbouring a virus that eventually causes its death, will be more infective during the weeks or months that precede the fatal issue than another mouse whose immunity is of a higher or more permanent order. Our whole experience with immunized and infected mice, both in bacterial and virus diseases, would indeed lead us to the view that a proportion—and often a high proportion—of such mice are in a condition of unstable equilibrium, harbouring the parasite in their tissues for many weeks or months, and liable to succumb to its activities under various non-specific stresses.

Turning to the immunized but not infected mice—the first double column of Table XLI—we may first note that the heading is somewhat misleading. These mice had, indeed, never received a dose of potent virus, but the three-day-formolized virus that formed the second immunizing injection was certainly not dead, and was sufficiently potent to kill an appreciable proportion of mice not previously immunized with a ten-day-formolized virus. Ectromelia has proved, in our hands, to be one of those virus diseases in which an effective immunity cannot be induced by the use of killed virus alone. This group of mice corresponds well with a group of animals immunized with a living but "attenuated" vaccine. Spread of infection from immunized to normals occurred in three instances, in all three before any death had occurred in the control cages, in two out of three instances within a fortnight. The indication is clearly that spread may occur from mice immunized with living

vaccine to normal susceptibles, even when the immunized mice have not been subsequently infected with a potent virus, but spread under these conditions appears to be far less frequent. Three of the 50 immunized mice died with lesions suggestive of ectromelia. The same considerations apply here as in immunized and infected mice.

The infected non-immunes—the third double column of Table XLI—gave rise, as would be expected, to frequent spread. One or more of the 5 contact mice died in 7 of the 10 cages. It is, at first sight, somewhat surprising that spread was a little less frequent among this group than among the infected immunes, but an explanation is probably to be found in the very different length of contact exposure. It will be noted that all the 50 infected non-immunes died. In each cage the first death was on the 4th day, 46 of the 50 mice were dead by the 5th day, all were dead within 7 days.

The group of vaccinated survivors from a previous epidemic was too small to allow of any definite conclusion. The single death among the contact mice on the 79th day may, we think, reasonably be neglected, since by that date accidental spread of infection had certainly occurred among our cages. The suggestion—it is clearly nothing more—is that this group of mice were non-infective.

Experiment 2.—A second experiment of the same general type was started on 30.x.33. The various groups were made up as follows:—

(*a*) Fifty mice that had received two doses of formolized vaccine (ten-day and three-day), the second dose being given on 20.x.33, were distributed among 10 cages, 5 mice to a cage, and to each cage 5 normal mice were added.

(*b*) Fifty mice that received the same two doses of formolized virus and, one week later (20.x.33), a dose of potent phenolized virus, were distributed in the same way and with the same additions of normal mice.

(*c*) Fifty mice that had received the same two doses of formolized virus and, seven days later, the same dose of potent phenolized virus, the latter having been given on 23.vi.33, were distributed in the same way and with the same additions of normal mice. The potentially infective mice in this group had received the same treatment as those in group (*b*), but 129 days had elapsed since they received the infecting dose of virus.

(*d*) Thirty surviving mice from group (*b*) of the preceding experiment were distributed in 6 cages (5 mice to a cage) and the same additions of normal mice were made. The potentially infective mice of this group had received the same treatment as those in groups (*b*) and (*c*), but 115 days had elapsed since they received the infecting dose of virus, and they had been submitted to a varying risk of contact infection during the 84 days of Experiment 1.

(*e*) Fifty normal mice were placed in 10 cages, 5 mice to a cage, and these cages were distributed among the cages of groups (*a*) to (*d*) to act as controls.

This experiment was continued for 105 days. The results, which are summarized in Table XLII, were very similar to those obtained in Experiment 1. The control cages of group (*e*) showed that cage isolation had failed. Ectromelia deaths occurred in 5 of the 10 cages, the first death occurring on the 30th day, and the remaining 4 infected cages yielded their first deaths between the 43rd and 80th days. In this experiment several of the small groups of normal mice exposed to contact infection in groups (*a*) to (*d*) became infected relatively late in the experiment, 10 of them from the 70th day onwards. Since infection at this period had clearly little significance, these late deaths are not recorded in Table XLII. All ectromelia deaths in the control group (*e*) and in the infective mice of groups (*a*) to (*d*) are recorded in the same way as in Table XLI. Of the mice exposed to contact infection in groups (*a*) to (*d*), only those deaths are recorded that occurred before the 28th day, i.e. before any deaths had occurred in the control cages of group (*e*). Where late ectromelia deaths occurred among these exposed normal mice the fact is indicated by reference to the footnote.

TABLE XLII

Showing deaths from ectromelia among infecting mice (5) *and mice exposed to risk* (5) *in various groups and sub-groups. Figures in brackets refer to day on which the first death from ectromelia occurred in that sub-group.*

Sub-group No.*	*Immunized (living virus).*		*Immunized and infected.*		*Immunized and infected survivors (c).*		*Immunized and infected survivors (d).*		*Controls (e).*
	Infecting.	*Exposed.*	*Infecting.*	*Exposed.*	*Infecting.*	*Exposed.*	*Infecting.*	*Exposed.*	
1	1 (93)	0	0	2 (13)	1 (93)	0†	0	0†	0
2	1 (96)	0	0	3 (17)	3 (79)	0†	2 (94)	0†	0
3	0	0	4 (7)	3 (9)	0	0	0	0	0
4	0	0†	0	3 (13)	0	2 (7)	0	0†	1 (80)
5	0	0	0	0†	0	0†	0	5 (10)	1 (30)
6	0	0†	0	0†	0	0	1 (92)	4 (9)	1 (43)
7	0	0	0	3 (6)	0	0†	—	—	3 (70)
8	0	0	1 (88)	0	0	1 (26)	—	—	0
9	1 (40)	0	0	0	0	0†	—	—	0
10	0	0	0	0	0	0†	—	—	1 (65)

* See footnote to Table XLI.

† Deaths from ectromelia occurred in this sub-group after the 28th day.

It will be seen that the results confirm those of Experiment 1, granting the same assumptions. The immunized and infected mice that had received their infecting dose of virus ten days previously were clearly infective to their normal companions (*see* second double column of Table XLII). There is a definite indication that mice similarly treated may be infective more than 100 days after receiving an active dose of virus (*see* third and fourth double columns of

Table XLII). There is here no clear indication that the mice immunized with living formolized virus, but not subsequently injected with potent virus (first double column of Table XLII), were infective to their companions, but it may be noted that the two groups that became infected (Nos. 4 and 6) showed their first deaths relatively early, in each case on the 45th day.

Summary

If we relied upon the spread of infection within certain of our cages, and its absence in others, irrespective of the time at which this spread occurred, we should not be justified in drawing any conclusions from these two experiments, for we were not successful in excluding cage-to-cage infection throughout the entire course of either of them. If, realizing that the risk of accidental cross infection was present throughout, and that the event could at best be postponed under the conditions in which we were working, we are prepared to accept as valid a comparison based on the time at which infection occurred in the different cages, we think that the following conclusions are probably in accord with the evidence.

(1) Mice that have been immunized with two doses of formolized virus—the virus, in the second dose at least, being still living—are sometimes infective for some days or weeks after immunization, though in this series of experiments the exact period is impossible to determine, and the infectivity appears to be of a low order.

(2) Mice that have been immunized with two doses of formolized virus and subsequently infected with a fully active virus are highly infective to normal companions for at least two to three weeks after receiving the infecting dose, even though they themselves show no sign of disease.

(3) This infectivity may, in some instances, persist for several months; but it is probable that the average infectivity of immunized and infected mice decreases considerably with lapse of time.

(4) In so far as these experiments afford any guide to probable happenings under field conditions, it would seem that the immunization of stock animals with a living vaccine would not be free from risk, if the immunized animals were subsequently permitted to mix with non-immunized stock.

SECTION VIII

THE EFFECT OF DIET ON THE EPIDEMIC SPREAD OF INFECTION

Among the non-specific factors that might reasonably be expected to exert a significant effect on host resistance, and hence on the spread of infection within a herd, diet holds an important place.

The association between famine and pestilence is attested in many of our epidemiological records, and there is no reasonable doubt that extreme deprivation renders a human or animal herd more liable to attack by a variety of microbial parasites. It is not, however, with such wide departures from the normal that we are here concerned. Our problem is to determine, if we can, the effect produced by far less obvious deficiencies, deficiencies that might actually occur in human populations living well above the poverty line because of some change in the kind of food consumed, or, still more readily, among our live stock because of the frequency of artificial, and therefore restricted, feeding.

In the case of one such deficiency, that of vitamin A, it may be said that the problem has been solved, at all events in the qualitative sense. The studies of Drummond (1919), Hess *et al.* (1921), Cramer (1923, 1924, 1927), Werkman (1923 a, b), Gross (1924), Wolbach and Howe (1925), Goldblatt and Benischek (1927), Green and Mellanby (1928, 1930), Hotta (1928), Reiter (1929), Lassen (1930, 1931, 1932), Gudjónsson (1930), McClung and Winters (1932), Greene (1933) and others seem to have established the fact that a diet from which vitamin A is absent renders animals more liable to contract a variety of spontaneous infections, and more susceptible to experimental infection with various pathogenic bacteria. It would take us too far afield to discuss in any detail the available evidence in regard to the mechanism by which A-avitaminosis produces its effects, but we may note (Cramer, 1923, 1924; Wolbach and Howe, 1925) that it is associated with atrophic changes in the epithelium of various mucous surfaces, so that one of its effects may well be to reduce the efficiency of the first-line defence that hinders the access of bacteria to the tissues. This, however, appears not to be the whole story. Hotta (1928), Reiter (1929) and Lassen (1930, 1931, 1932) report that A-avitaminotic animals are more susceptible than normal controls to the subcutaneous, intraperitoneal or intravenous injection of pathogenic bacteria, indicating a defect in the defence mechanisms of the general tissues of the body.

A problem that more immediately concerns us is the quantitative as opposed to the qualitative relation between A-avitaminosis and infection. At what level of vitamin A deficiency does resistance to infection begin to fall? May a vitamin A intake that is sufficient to suppress the more obvious evidences of avitaminosis be insufficient to ensure maximal resistance? Would a liberal supply of vitamin A, substantially in excess of that generally supposed to be required for the maintenance of health, increase the average resistance of the recipients above the normal level? To these questions there is as

yet no answer. We may note that Lassen found it necessary to induce a pronounced degree of A-avitaminosis before he could demonstrate a significant break in resistance; but this observation taken alone cannot be given great weight, and the experimental data are quite insufficient to allow any just conclusion to be reached in regard to this important point. Such evidence as is available from observations carried out on man under natural conditions is, in our view, far too conflicting to aid in the solution of the problem on its quantitative side, which is clearly susceptible to attack along ordinary experimental lines. It would seem wise to suspend judgment until this attack has been made.

The experimental evidence with regard to the effect of other dietary deficiencies on resistance to infection is too slight or too confused to justify any general discussion; but it seems desirable to summarize briefly those experiments that have been carried out with the immediate object of determining the effect of dietetic factors on the spread of infection, if only to emphasize how chaotic is our present knowledge.

The earlier experiments of Webster and Pritchett (1924), although undertaken as part of a general study of experimental epidemiology, were concerned with the effect of dietetic factors on the resistance of individual mice, not directly with their influence on the spread of infection among a herd. The diets they compared were the ordinary mouse diet of the Rockefeller Institute, consisting of a daily ration of bread soaked in fresh pasteurized Grade B milk warmed to at least 60°–70° C., supplemented by two weekly feedings of an oatmeal and buckwheat mixture and one weekly feeding of dog biscuit, and a modified McCollum diet consisting of 67·5 per cent. whole wheat flour, 15 per cent. casein, 10 per cent. milk powder, 5 per cent. butter fat, 1 per cent. sodium chloride, and 1·5 per cent. calcium carbonate. Pregnant does were fed on each of these diets; and the young, after weaning, were kept on the same diets that their mothers had received until they attained a weight of 16–18 g. They were then tested by the injection through a stomach tube of 2–5,000,000 *Bact. aertrycke*. Three experiments of this type are recorded, the number of mice in each group in each experiment varying from 10 to 36. No tabulated protocols are given, and the only method of arriving at the actual figures of mortality is to apply the percentage figures, read from the small-scale curves, to the numbers in each group, which are given in the text. On this basis the numbers of deaths in the two groups in the three different experiments would seem to have been as follows:—

	McCollum diet.		*Institute diet.*	
	No. tested.	*No. died.*	*No. tested.*	*No. died.*
Experiment 1 ..	10	1	10	8
Experiment 2 ..	26	6	26	26
Experiment 3 ..	33	4	36	22
Total ..	69	11	72	56

These figures give a mortality of 15·9 per cent. among the mice fed on the McCollum diet as compared with 77·8 per cent. among the mice fed on the Institute diet, a very striking difference.

In two further experiments recorded in the same paper, mice fed on these two diets were compared in their resistance to mercuric chloride and to *botulinum* toxin, the former administered through a stomach tube, the latter by intraperitoneal injection. In each case the McCollum mice appeared rather more resistant than those fed on the Institute diet; but the difference was in time to death rather than in the number of survivors. There was no evidence that the McCollum diet conferred a resistance of the same order as that demonstrated in the experiments with *Bact. aertrycke.*

Pritchett (1927) carried out a more extensive series of experiments along somewhat similar lines, except that the various diets were fed to the mice for far shorter periods before infection—10 to 14 days. The purpose of this series of experiments was to determine which constituent of the McCollum diet was responsible for the increased resistance.

In one small experiment groups of 18–47 mice were fed on the Institute diet, on the Institute diet plus 10 per cent. whole milk, on the Institute diet plus 10 per cent. casein, on the Institute diet plus 10 per cent. whole wheat flour, and on the McCollum diet minus the butter fat. The results, so far as any significance can be attached to such small groups, suggest that no effect was produced by adding any of these constituents of the McCollum diet to the Institute diet, while the McCollum diet minus the butter fat increased the resistance of the mice, though not to the same extent as the full McCollum diet employed in the earlier experiments. The remainder of the numerous tests recorded in this paper are concerned with the influence of the fat constituents of the diet, especially in relation to the possible rôle of vitamin A. In a few cases it is necessary to estimate figures from chart readings, but in the majority of tests the results are given in tabular form, and can be read directly. They allow adequate comparisons to be made between the mortality rates of mice on the Institute diets and on each of the following test diets:—

(1) Institute diet plus 5 per cent. cod liver oil.
(2) „ „ „ 5 per cent. butter fat.
(3) „ „ „ 5 per cent. " Crisco " fat.
(4) McCollum diet minus fat.

Cod liver oil was selected as a fat with a consistently high vitamin A content; but butter fat was known to show seasonal variations in its vitamin content, and the tests were carried out at intervals over a twelve months' period; " Crisco " was a hydrogenated vegetable oil, from which vitamins were supposed to be absent.

Comparing the mortalities in the groups on these diets with the mortalities in the groups on the Institute diets included in the same tests, the results are as follows:—

	Diet.	No. tests.	No. mice tested.	Per cent. mortality.
A	Institute	11	434	69·4
	Institute + cod liver oil	11	435	53·3
B	Institute	5	139	61·2
	Institute + butter	5	138	49·3
C	Institute	5	139	61·2
	Institute + Crisco	5	137	53·3
D	Institute	3	119	81·5
	McCollum minus fat	3	93	47·3

These figures are not easy to interpret. In every comparison the mice on the Institute diet show the higher mortality. But it seems to make little difference what kind of fat is added to the diet, or whether the test diet is deficient in fat, as in the McCollum diet from which the 5 per cent. butter fat has been withdrawn. This diet indeed gives the most favourable comparison with the control, though the mortality is far higher than that recorded in the earlier experiments with the complete McCollum diet. This, combined with the fact that the mice on the Crisco diet show only slightly less benefit, as compared with the controls, than those on the cod liver oil or butter fat diets would seem to tell strongly against the view that the vitamin A content is the determining factor in the influence exerted by these diets on resistance to *Bact. aertrycke* infection.

In an experiment carried out at a later date, Webster (1930 d) studied the effect of a change from the Institute to the McCollum diet, or *vice versa*, in herds of mice infected with *Bact. enteritidis* and recruited by the addition of two normal mice a day. The record of the four herds concerned runs from May, 1927, to June, 1929. From the events in these herds Webster concludes that a change from the Institute to the McCollum diet led to a fall in the mortality rate, while a change from the McCollum to the Institute diet had a reverse effect. This would clearly accord with *a priori* expectations based on the earlier experiments with small groups of mice, and the record given in the text is compatible with this interpretation; but the issue is confused by the fact that, in two of the herds, a change in the type of immigrant mice, from a relatively resistant to a relatively susceptible breed, was made within a few months of the change from the McCollum to the Institute diet, so that the period during which the four herds were comparable in all respects other than diet is a relatively short one.

Our own experiments in this field (Topley, Greenwood and Wilson, 1931 a) may be very shortly summarized. The method that we employed in the first of these experiments was that of the closed epidemic, to which frequent reference is made in this report. The diets that we tested were as follows:—

Control diet: An abundant diet of whole oats and the provision in drinking vessels of a mixture of equal parts of water and pasteurized milk, the milk and water, in the drinking vessels, being steamed at 100° C. for ten minutes.

Diet A: Whole meal flour 60 parts, casein 20 parts, fresh butter 5 parts, sodium chloride 1 part, calcium lactate 2 parts.

Diet B: Whole meal flour 60 parts, casein 20 parts, vitamin A concentrate 5 parts, sodium chloride 1 part, calcium lactate 2 parts.

Diet C: Whole meal flour 20 parts, casein 20 parts, butter 40 parts, sodium chloride 1 part, calcium lactate 2 parts.

Diet D: Whole meal flour 20 parts, casein 20 parts, lard 33 parts, sodium chloride 1 part, calcium lactate 2 parts.

Diet E: Whole meal flour 60 parts, casein 20 parts, butter 5 parts.

The mice on diets A–E were given water to drink instead of the milk and water mixture. The vitamin A concentrate contained in diet B was a commercial liver concentrate stated to contain twenty times the vitamin A content of good cod-liver oil as judged by the antimony trichloride test.

Diets C and D were included for the following reasons. One of us (W.W.C.T.), in an unpublished series of experiments, had found that the inoculation into mice of killed suspensions of *Bact. aertrycke* was followed, under suitable conditions, by symptoms closely resembling insulin shock, and that the injection of non-fatal doses of insulin greatly accelerated the death of mice that had been injected with such suspensions. The recent studies of Delafield (1931, 1932, 1934) on the changes in blood sugar concentration that follow the intravenous injection of such suspensions, or of bacterial extracts, into rabbits have added further and far more precise evidence that the toxic action of this organism is associated with a derangement of carbohydrate metabolism. Observations by Bainbridge (1925) led him to the conclusion that an increase in the fat/carbohydrate ratio in the diet increases the resistance of mice to insulin. It seemed desirable to see whether such a modification would affect their resistance to *Bact. aertrycke.*

The experiments differed in no essential way from others in which the method of the closed epidemic has been employed. An adequate number of mice were maintained for 14 days on each of the experimental diets. At the end of that period 300 normal mice, all of which had been maintained on the standard control diet, were injected intraperitoneally with 1,000 living *Bact. aertrycke.* These inoculated mice were divided into 12 groups each of 25, and to each group were added 100 of the mice that had been maintained for the previous 14 days on one or other of the diets under test, each of the six diets being tested in duplicate. Each herd was then maintained for 60 days on the same diet as that on which the susceptibles had been fed before exposure to infection.

All mice dying were examined by the usual technique, and, on the 60th day, all survivors were killed.

The results are summarized in Table XLIII which gives the expectation of life, limited to 60 days, for the 100 exposed mice in each of the 12 herds.

TABLE XLIII

Herd.	*Diet.*	${}_{60}E_x$ *(each herd).*	*S.E.*	${}_{60}E_x$ *(pairs of herds).*	*S.E.*
Control ..	Oats and milk	56·8	1·01	56·4	0·74
,, ..	,, ,, ...	56·0	1·08		
A1.. ..	Basal	48·0	1·81	48·2	1·30
A2.. ..	,, ...	48·5	1·87		
B1.. ..	Basal + vitamin A ..	39·8	1·88	38·1	1·38
B2.. ..	,, ,, ,, ..	36·4	2·02		
C1 ..	Excess butter	37·8	1·93	37·6	1·37
C2 ..	,, ,, ...	37·5	1·96		
D1 ..	Excess lard	40·0	1·89	43·0	1·32
D2 ..	,, ,, ...	46·0	1·82		
E1.. ..	Basal without salt ..	48·0	1·89	49·0	1·27
E2.. ..	,, ,, ,, ..	50·0	1·69		

Taking the column giving the ${}_{60}E_x$ values for the duplicate pairs of tests, several points of interest may be noted. In the first place, as the figures for the two control groups show, the strain of *Bact. aertrycke* employed proved to be of an unexpectedly low epidemic potency (*see* pp. 151–152). It was of high virulence—284 of the 300 inoculated mice died—and it had considerable powers of spread —46 of 88 survivors from the first control herd were harbouring *Bact. aertrycke* in their spleens when they were killed and examined on the 60th day; but it tended, under conditions of contact spread, to give rise to non-fatal rather than to fatal infections. Next, it will be noted that each of the herds on the test diets showed a higher mortality than the two on the control diets; so far from any of these diets having raised the resistance of the mice they appear to have had the opposite effect. The basal diet which, apart from the absence of milk powder, did not differ greatly from the McCollum diet employed by Webster, was no exception; but this, and the same diet without the salt mixture, gave the lowest mortalities apart from those shown by the two control herds. The addition of vitamin A concentrate, of excess butter, or of excess lard, was associated with a definite increase in mortality.

These results differed so widely, and so unexpectedly, from those recorded by Webster and Pritchett that an attempt was made to test the same diets by a technique bearing a greater resemblance to that which they employed. Four groups, each of 50 mice, were taken and maintained for 14 days on the control diet, diets

A (basal), B (basal plus vitamin A), or C (excess butter). On the 14th day each mouse of each group was injected intraperitoneally with 1,000 *Bact. aertrycke*. Each mouse was, thereafter housed in a separate cage for 14 days, and was fed on the same diet as it had received before inoculation.

The results are summarized in Table XLIV. They differ, as will be seen, from those obtained in the closed epidemics. There is here no suggestion that the replacement of the control by any of the three test diets is associated with an increase in mortality; indeed, the mice on the test diets suffered a slightly lower mortality, and lived on the average slightly longer, than the control group; but the difference is in no case statistically significant.

TABLE XLIV

Diet.	*Per cent. mortality.*	*S.E.*	*Mean survival time limited to 14 days.*	*S.E.*
Control	82·0	5·43	6·9	0·70
Basal	74·0	6·20	7·9	0·70
Basal plus vitamin A ..	78·0	5·86	8·6	0·61
Excess butter	74·0	6·20	8·5	0·61

This clearly suggests that some factor, other than the effect of the diet in raising or lowering resistance, was operating in our epidemic cages, and this is an obvious possibility. It may well be that the various synthetic diets were more readily contaminated with excreta than the whole oats that were fed to the control groups, and that the rate of passage of bacteria from infected to healthy mice was thus increased. Moreover, the addition of the vitamin A concentrate to the diet, and still more the addition of an excess of butter, produced an oiliness of the coat that may have induced the mice to spend a disproportionate time on their toilet, and thus again have provided increased opportunities for infection.

The most that we should be prepared to conclude from this series of experiments is that, if any benefit at all was derived from the diets tested, it was so slight as to be more than counterbalanced by other influences that may have increased the facilities for contact infection.

It was undesirable to leave the problem in this entirely indecisive state, and an experience reported by Glenny and Allen (1921) seemed to offer an opportunity of investigating it along slightly different lines. These observers record that, owing to a temporary failure of supplies, a large stock of guinea-pigs were not given the usual supply of green food in addition to the basal diet of bran, oats and hay, but were given mangolds as a substitute. This period of deprivation lasted for some six weeks and was associated with a rapid rise in the death rate, due in part to infection with *Bact. enteritidis* (Gaertner), in part to a broncho-pneumonia

apparently caused by infection with *Bact. faecalis alkaligenes*. After the re-introduction of fresh green food to the diet no fresh cases of infection occurred, and the death rate reached the normal level of about 1 per cent. within a fortnight. Later they attempted to test the relative value of mangolds, swedes and green food as anti-infective dietary factors. Batches of guinea-pigs were fed on each diet and were cared for in the ordinary way, in an infected environment and without any attempt at isolation. It is stated that the results confirmed the view that an adequate supply of green food would suppress the epidemic, and showed that swedes had a greater value than mangolds in this respect.

We attempted to test these diets on mice, using the method of the closed epidemic. The same control diet of whole oats, milk and water was employed. The mice in three herds received this diet alone. The mice in three herds received in addition a daily supply of cabbage. The mice in three herds received a daily supply of mangolds. The mice in three herds received a daily supply of carrots. The effect of the addition of carrots was studied because of the relation demonstrated between carotene and vitamin A. All other details of technique were the same as in the experiments recorded above, except that, in the present series of tests, the strain of *Bact. aertrycke* was of greater epidemic potency. The results are summarized, in the usual way, in Table XLV.

TABLE XLV

Diet.	$_{60}E_x$ *(each herd).*	*S.E.*	$_{60}E_x$ *(in groups).*
Control (1)	37·7	2·20	39·6
,, (2)	41·3	1·72	
,, (3)	39·8	2·09	
Control plus cabbage (1)..	33·7	2·11	38·3
,, ,, ,, (2)..	39·3	1·77	
,, ,, ,, (3)..	41·8	1·86	
Control plus mangolds (1)	40·8	1·95	34·1
,, ,, ,, (2)	26·5	1·77	
,, ,, ,, (3)	34·9	1·86	
Control plus carrots (1) ..	28·3	1·78	30·0
,, ,, ,, (2) ..	25·1	1·70	
,, ,, ,, (3) ..	36·5	1·89	

Taking the third column of figures, those giving the mean $_{60}E_x$ values for each of the four groups, it is clear that neither cabbage, mangolds nor carrots have raised the resistance of the mice. There is a suggestion that the addition of carrots, and perhaps of mangolds, has resulted in an increased mortality, but this conclusion would, we think, be unsafe, for the differences in average duration of life between the groups are no greater than those observed within the groups.

Our findings, in any case, show no accord with those reported by Glenny and Allen. The most obvious suggestion would seem to be

that mice differ in some essential way from guinea-pigs in their nutritional requirements.

Discussion and Summary

We feel that apart from the generally-accepted view that a gross deficiency in vitamin A renders animals more liable to infection, it is impossible at the moment to draw any definite conclusions in regard to the influence of diet on resistance or susceptibility.

There is a clear suggestion in Webster and Pritchett's experiments that the McCollum diet that they employed increased the resistance of the mice to which it was fed, and in Pritchett's experiments that the addition of various kinds of fat to a diet consisting mainly of bread and milk has a similar beneficial effect. But none of these experiments gives any direct clue to the constituent of the McCollum diet that is responsible for this increase in resistance, nor do Pritchett's results seem compatible with the view that the determining factor is a high content of vitamin A. Her findings, apart from those with the McCollum diet minus fat, rather suggest that a relatively high fat content is a significant factor; but our own experience, so far as it goes, is hardly in accord with this view.

The most striking differences are those recorded by Webster and Pritchett when comparing the Institute diet of bread and milk with the complete McCollum diet; and the fact that the McCollum diet minus fat yielded better results, in Pritchett's series of tests, than any of the bread and milk diets to which fat had been added suggests that some other constituent, or some other balancing factor, was more important than the fat content; though it must be noted that the withdrawal of the fat had lessened the value of the McCollum diet, so far as we can fairly judge by comparing Pritchett's results with the earlier findings of Webster and Pritchett —unfortunately no direct comparison of the McCollum diet with and without fat appears to have been made.

For the almost consistently negative results of our own experiments we have no explanation to offer, other than the suggestions put forward above.

In the particular case of the mouse—and it is the mouse that concerns us here—we feel that too little is yet known in regard to its nutritional requirements to allow of any adequate study of the problem by the methods of experimental epidemiology. The crude experiments outlined above have given highly confusing answers. But the problem is of such obvious importance that it clearly deserves more detailed study along more systematic lines. Our colleague, Dr. Marion Watson, is at the moment engaged on such a study, investigating growth, fertility and "normal" mortality rates in the mouse on a variety of diets, as well as susceptibility to experimental infection with *Bact. aertrycke*. If, and when, these studies yield significant results, we hope to test her findings under conditions of epidemic spread.

SECTION IX

THE RÔLE OF BACTERIOPHAGE IN EPIDEMICS OF MOUSE TYPHOID

It is obvious that the bacteriophage, described independently by Twort in 1915 and by D'Herelle in 1917, might have an important influence on the epidemic spread of infection. That it does in fact exert such an influence has been claimed in no uncertain terms by D'Herelle (1926), and, in relation especially to cholera, similar claims have more recently been advanced by several observers (*see* Morrison, 1932).

If we accept the view, which the balance of evidence now appears to support, that the name " bacteriophage " must be regarded as a generic term for a multitude of filtrable viruses adapted to prey upon particular species of bacteria, there would seem some grounds to hope that, in a specific phage of adequate " virulence," we might find an almost ideal agent for the control of epidemic disease. A living bactericidal agent, acting specifically on the particular pathogenic organism concerned, and self-propagating so long as the bacterium itself was spreading among the hosts at risk, would, indeed, be almost too good to be true. So far as our experience is concerned, the dream remains a dream. Before considering possible reasons for its non-fulfilment it will be convenient to summarize the experimental evidence.

Experiment Phage I.

Our first attempt (Topley, Wilson and Lewis, 1925 b) was to determine whether the administration to mice of an active *Bact. aertrycke* phage would lessen the degree of intestinal infection as judged by the frequency of recovery of the organism from the intestine. In the first experiment a number of mice, housed in separate cages, were given repeated doses of a living culture of *Bact. aertrycke* by the mouth, each dose containing approximately 2×10^6 bacilli. The faeces of each mouse were examined several times a week by a method that allowed relatively small numbers of *Bact. aertrycke* to be detected with considerable accuracy (Topley and Ayrton, 1924). As soon as any mouse had excreted *Bact. aertrycke* on two occasions it was transferred to the experimental group. The first mouse so transferred was given an active phage filtrate by mouth. The second was given no phage, but maintained as a control; and so on until two groups, each of 10 mice, had been collected. The phage was at first administered to each mouse daily with a dropping pipette, but later in the experiment this practice was discontinued, and each mouse was supplied daily with 10 c.c. of a 1/10 dilution of phage in a drinking vessel of the ordinary inverted test tube type, from which it drank readily. From the

date of entering the experimental or control group the faeces of each mouse were examined at frequent intervals, similarly spaced in the case of each mouse, until death occurred, or until the 42nd day, when the survivors were killed.

It will be noted that, in this experiment, each mouse was known to be excreting *Bact. aertrycke* in its faeces before phage was administered.

The results may be very shortly summarized.

Mice receiving phage (10) :—

Number of specimens of faeces examined = 142
Percentage of specimens yielding *Bact. aertrycke* = 29·6 per cent.
Number of deaths from mouse typhoid = 5

Mice not receiving phage (10) :—

Number of specimens of faeces examined = 177
Percentage of specimens yielding *Bact. aertrycke* = 26·0 per cent.
Number of mice dying of mouse typhoid = 2

There was no evidence that the phage was lessening the frequency of *Bact. aertrycke* in the faeces, or influencing the infection in any way.

Experiment Phage II.

The second experiment differed from the first, in that the phage and the living bacteria were administered together, so that we were testing the effect of the former on the initiation of infection in the intestinal tract, as well as on an infection already initiated.

In this experiment 20 mice were taken, and fed on a mixture of a 24 hours' broth culture of *Bact. aertrycke* and an active phage filtrate; 20 control mice were fed on the culture alone. This feeding was repeated three times at weekly intervals. The faeces of each mouse in each group were 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 on any day refused to yield a specimen for examination, or died before the experiment was completed. On the 42nd day the experiment was brought to an end, and all survivors were killed.

The results of this experiment were as follows.

Mice receiving phage (20) :—

Number of specimens of faeces examined = 269
Percentage of specimens yielding *Bact. aertrycke* = 16·0 per cent.
Number of mice dying of mouse typhoid = 5

Mice not receiving phage (20) :—

Number of specimens of faeces examined = 256
Percentage of specimens yielding *Bact. aertrycke* = 23·5 per cent.
Number of mice dying of mouse typhoid = 5

Here again there is no evidence that the phage exerted any significant influence. The frequency of isolation of *Bact. aertrycke*

was slightly greater among the controls than among the phages receiving mice; but no close approximation could be expected with so variable a phenomenon, and the number of mice excreting was the same in the two groups, sixteen in each. Moreover, the numbers of deaths from mouse typhoid were identical.

Experiment Phage III.

Even with these results it did not seem impossible that, under natural conditions of spread, the presence of phage in large amounts among a herd at risk might lessen the frequency of contact infection. We therefore started an epidemic of mouse typhoid 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 in regard to the number of mice of any given cage age which it contained. To each group of 20 mice 80 normal mice were added, and each closed epidemic was allowed to run its course for 84 days. The mice in two of the cages were provided each day with a drinking vessel containing a 1/10 dilution of an active lytic filtrate, those in the other two cages were given a 1/10 dilution of peptone water to serve as controls.

The results of this experiment, though in no sense dramatic, seemed to offer a suggestion that the phage was not altogether without effect. Up to the 25th day there was no difference between the behaviour of the phage-receiving herds and the controls, the mortality in the four herds during this period varying from about 35 to 50 per cent., one phage-treated herd showing the lowest mortality, the other the highest. From this period onwards, however, the mortality in the phage-treated herds lessened, while that in the control herds did not. On the 84th day, when the experiment was terminated, the survivors in the two herds to which phage was administered numbered 39 and 34 respectively, the survivors in the two control herds numbered 23 and 16. Although the phage had clearly been ineffectual in inhibiting the onset of severe epidemics, it seemed possible that it had had some influence in bringing these epidemics to an earlier close. The results were, however, clearly indecisive, and the experiment was therefore repeated.

To allow for the possibility that the phage had undergone some adaptation during the experiment recorded above, and was for this reason more efficacious during the later epidemic period than during the earlier, we proceeded as follows. To the survivors of each of the four herds we added sufficient mice to make the total number up to 100. To one herd that had received phage during the previous epidemic phage was again administered; to the other it was not. To one herd that had received no phage during the previous epidemic it was now administered, to the other it was not. As before, each herd was observed for a period of 84 days.

The results were disappointing. A severe epidemic developed in each herd and there was no significant difference between one herd and another. The actual figures were as follows. The herd that received phage during both epidemics had 23 survivors; the

herd that received phage during the first epidemic but not during the second had 23 survivors; the herd that received no phage during the first epidemic but received it during the second had 13 survivors; the herd that received no phage during either epidemic had 13 survivors.

It may be noted, in regard to all the preceding experiments, that the comparison between the phage and no-phage groups is not an entirely clean one. Although we have never recovered an *aertrycke* phage from the faeces of normal mice, nor from an experimentally infected mouse during the early days of infection, it is by no means uncommon to recover a phage from the faeces of a mouse that has been infected with *Bact. aertrycke* for a long period, while the examination of specimens of faeces from large numbers of mice during a closed epidemic of the type described above, or during any epidemic in which the herd is continuously recruited by immigration, will usually reveal the presence of phages among some proportion of the mice at risk. Numerous specimens of faeces were in fact examined in this way during the experiments summarized above. It would be wearisome to record the results in detail, but it may be noted that, while a phage was recovered at one time or another from almost all the groups to which no phage was intentionally given, the frequency of phage recovery was very much greater among the mice to which the lytic filtrate was administered. There was, of course, no *a priori* reason for supposing that the type of phage recovered from the control groups was the same as that administered to the other groups; but this point was not specially examined. The normal appearance of an active phage, of one type or another, in any herd that is suffering from a prolonged epidemic prevalence must obviously be taken into account in considering the probable rôle of these viruses in the spread of infection, and we shall return to it in a subsequent paragraph.

Experiment Phage IV

At a later date (Topley and Wilson, 1925) a further series of experiments was undertaken by a somewhat different technique. D'Herelle (1926) records the abrupt cessation of epidemics of fowl typhoid following the subcutaneous injection of a phage filtrate into the birds at risk. We had ourselves, in earlier experiments, found that a filtrate from a phage-lysed culture was an effective immunizing agent when injected intraperitoneally 14 days before the injection, by the same route, of a dose of living and virulent *Bact. aertrycke*, though it had no protective effect when given one day before the living organisms. This, of course, is in no sense surprising, since the filtrate will contain a high concentration of antigenic material derived from the bacterial bodies. We thought, therefore, that it would be of interest to test the effect of injecting an active phage into the tissues of mice exposed to risk during a closed epidemic.

An epidemic of mouse typhoid was therefore started by feeding 80 mice on a strain of *Bact. aertrycke* known to be sensitive to the

phage that we proposed to employ. Three weeks later, when several deaths from mouse typhoid had occurred, 420 normal mice were added to the 60 survivors. During the following week there were 45 deaths from mouse typhoid among these 420 normal mice, showing that an epidemic of the disease was under way.

At this date 360 survivors from the 420 normal mice were divided into four groups of 90 each and were treated as follows :—

(1) One group was placed in a large cage, and each mouse was injected intraperitoneally with 0·25 c.c. of phage. This herd was labelled P1.

(2) A second group was placed in a similar cage but no phage was given. This herd was labelled C1.

(3) A third group was dispersed into 18 groups of 5 mice each, phage being given as in group (1). This group was labelled P2.

(4) A fourth group was dispersed as in (3), but no phage was given. This group was labelled C2.

The survivors from groups (3) and (4) were re-aggregated into a single herd on the 42nd day of the experiment. All groups were observed for 84 days.

The behaviour of groups (1) and (2) as compared with that of groups (3) and (4), i.e. the effect of segregation followed by re-aggregation, does not concern us here. We are interested only in comparing group (1) with group (2), and group (3) with group (4), i.e. in assessing the effect of the phage inoculations.

The relevant figures are as follows :—

(1) Herd P1—phage given—survivors on 84th day = 2
(2) Herd C1—no phage given—survivors on 84th day = 8
(3) Herd P2—phage given—survivors on 84th day = 16
(4) Herd C2—no phage given—survivors on 84th day = 16

All the epidemics were severe, and there is no evidence that the administration of the phage by the intraperitoneal route exerted any beneficial effect.

We have, in more recent years, carried out a few experiments with other phages, isolated from different sources, mainly to determine whether these would react differently from those studied in the earlier experiments. We have not, in these instances, tested our lytic filtrates in closed epidemics, but have examined only their protective power when injected into mice simultaneously with living cultures of *Bact. aertrycke*, or their immunizing power when used as a bacterial vaccine. Since these experiments have not been previously recorded, they may perhaps be very briefly summarized.

Experiments with phage P

This was a phage isolated from the intestinal contents of a pig. It was somewhat more active than those used in the earlier experiments.

Seventy-five mice were immunized by two intraperitoneal injections of this phage, and 75 mice by two intraperitoneal injections of a formolized culture of *Bact. aertrycke*. The dose of the bacterial

vaccine was 500 × 10^6 bacilli; the amount of bacterial substance in the lytic filtrate could not be estimated with any accuracy.

One week later 30 of the phage-vaccinated mice were injected intraperitoneally with 1,000 *Bact. aertrycke*, 30 with a mixture of 1,000 *Bact. aertrycke* and 0·25 c.c. of phage P filtrate; 30 of the mice immunized with the bacterial vaccine were injected with the same dose of *Bact. aertrycke*, 30 with *Bact. aertrycke* and phage. A group of 30 normal mice received *Bact. aertrycke*, another group of 30 *Bact. aertrycke* and phage. The number of bacilli injected and the total volume of the inoculum were in all cases the same. All mice were observed for 28 days. The results may be summarized as follows:—

Immunization with	*Test inoculum* Bact. aertrycke *plus :—*	*Deaths.*
Phage	Phage	23/30
Phage	No phage	22/30
Bacterial vaccine	Phage	18/30
Bacterial vaccine	No phage	19/30
Nil (controls)	Phage	30/30
Nil (controls)	No phage	30/30

The phage produced some active immunity when used as a bacterial vaccine, perhaps slightly less than that produced by a formolized bacterial suspension; but it had no protective action when mixed with the test dose of living bacilli and inoculated with it.

Comparison of the protective action of P phage with that of a phage C, derived from sewage

These tests were made on groups of 30 mice. The suspension of living *Bact. aertrycke* (500 × 10^6) was mixed with 0·25 c.c. of phage filtrate and injected intraperitoneally. All mice were observed for 28 days. The results were as follows:—

Phage tested.	*Deaths.*
P	28/30
C	28/30
P + C	28/30
None (controls)	29/30

Neither phage appeared to have any protective effect.

Tests of the protective action of P phage and of phages derived from it by isolation from surviving mice, P6, P29 and PQ

These tests were carried out in groups of 20 mice by exactly the same method as in the previous series of tests. The results were as follows:

Phage tested.	*Deaths.*
P	20/20
P6	20/20
P29	19/20
PQ	19/20
None (controls)	19/20

Clearly none of these phages had any trace of protective action, behaving in this respect like the phages we had tested in the earlier series of experiments.

Discussion and Summary

As a preliminary to a discussion of the significance of these results it will be well to subject them to very careful criticism. One point, not touched on in the description of the experiments themselves, may be dealt with very summarily. The failure to obtain any amelioration of the disease in individual mice, or in an experimental herd, was not due to the appearance of modified strains of *Bact. aertrycke*, resistant to the particular phage that we happened to be employing. There was, as one would expect, no difficulty in developing such resistant strains by the ordinary in-vitro methods, but we could obtain no evidence that such adaptation was occurring *in vivo*. Many hundreds of strains of *Bact. aertrycke*, isolated from the faeces of infected mice during life, or from the spleen or heart's blood after death, were examined for sensitivity to the phage under test. In only a single instance in one experiment was a resistant strain encountered. This was isolated from the heart of a mouse dying during one of the epidemics in which phage was being administered. The strain from the spleen of the same mouse was fully sensitive. There is, indeed, no doubt at all that active phage and sensitive bacteria frequently co-exist in the tissues in the course of fatal infections. It has been a common experience to obtain very marked phage action on primary plates from broth cultures taken from the heart or spleen of mice dying from mouse typhoid after they have received phage by mouth. The filtrate from such a primary broth culture is often highly active. Webster (1930, *d*) records analogous observations in an epidemic of *Bact. enteritidis* infection among mice, in the course of which an active bacteriophage made its appearance.

Much more cogent criticism could, we think, be brought against our findings on the ground that the phages we were employing were not of maximal activity. This was, in fact, the case. Most of them produced lysis in dilutions of 10^{-5}, some in dilutions of 10^{-6}, but none of them produced the complete and rapid clearing of young broth cultures that is associated with phages of the most active type.

Our own feeling is that these findings are adequate as a confrontation of the extreme position adopted by D'Herelle, who, in his discussion of the use of the phage in controlling epidemics of fowl typhoid, states that a weak or moderate activity of the phage is sufficient to render the animal resistant to infection, the bacteria being destroyed in the intestine before they can multiply. It is suggestive, also, that almost all those who have studied the action of the phage in experimental animal infections have reported results as disappointing as our own—Levy (1925), Wollman (1925), Richet and Hauduroy (1925), Bronfenbrenner and Korb (1925–26), Ebert

and Peretz (1929) in the case of mouse typhoid, Pyle (1926) in salmonella infections of fowls, Compton (1928, 1930) and Doorenbos (1929) in experimental plague in rats, Clark and Clark (1927) in a haemolytic streptococcal infection in the rabbit, Colvin (1932) in a streptococcal infection in the guinea-pig, and Cowles and Hale (1931) in anthrax in the mouse. It is, however, true that these workers were concerned with the therapeutic action of the phage, not with its possible effect in limiting the spread of the disease, and these problems are not identical. Their results, like ours, are not in accord with some of the claims that have been made for the phage as a therapeutic agent; but that is not the point at issue.

Limiting ourselves to the epidemiological viewpoint there is clearly no support for D'Herelle's view that the rise and fall of an epidemic merely registers the fluctuations between two agents, the pathogenic bacterium and the phage; but such a view hardly needs serious discussion. We have shown that an active phage may be distributed at large among a herd at risk during the rise and peak of an epidemic wave, and that its presence appears to make no significant difference to the epidemic process, and our findings are in accord with a mass of reports from the field which shows quite clearly that phages of various types are frequently associated with the presence of serious infection, in an individual or in a herd.

Our findings do not, and cannot, prove that there do not exist phages of a different type that, by their greater activity, and perhaps by an ability to exert their action in the intestinal tract, or in the tissues, as well as in the test tube, might be able to prevent the initiation of an epidemic, or, once initiated, to bring it to an end. Argument along these lines can, of course, be carried to infinity, since each new failure may be met by the objection that the wrong kind of phage has been used; but there are, we think, sound reasons for adopting the view that the hunt should not be abandoned yet. A significant finding, in this regard, is afforded by results recorded by Andrewes and Elford (1932). Working with a coli-phage of high activity they found that it not only lysed the sensitive bacteria, but killed them almost immediately, even under conditions in which lysis did not occur. The addition of sodium citrate prevented the lysis but not the killing. The main interest of their results, from our present point of view, lies in the curious quantitative relations that these experiments brought to light. It was found that no detectable killing occurred in mixtures containing less than 10^6 phage particles per c.c., while almost complete killing occurred as soon as this limit was exceeded. Moreover, although there was an upper limit of *Bact. coli* concentration for any given concentration of phage, beyond which no killing effect was demonstrable, a variation of the *Bact. coli* concentration over a wide range below this limit made very little difference to the proportion of the organisms killed. Within this range, 10^3–10^7 *Bact. coli* per c.c. for a mixture containing 10^6 phage particles per c.c.,

some 95 per cent. of the bacilli were killed irrespective of the number present, so that the effect appeared to depend on the absolute concentration of phage particles in the mixture rather than on the relative concentrations of bacteria and phage. It is, of course, unwise to argue from conditions in the test tube to conditions in an animal's intestine; but results such as these suggest the possibility that there may be some limiting concentration of phage, or level of phage activity, below which no effect whatever may be produced while, if this level were exceeded, a dramatic effect might be obtained. The necessity for employing highly "virulent" phages has, of course, been urged often enough by those who support the claims of phage therapy or phage prophylaxis. The interest of such an experiment as that of Andrewes and Elford lies in the demonstration of a lack of simple proportionality in a phage effect; so that one cannot argue, from the complete failure to demonstrate any protective action with a variety of phages, which certainly produce lysis in concentrations less than those actually employed, that an increase in phage activity would be unlikely to yield more successful results.

It is probably in connection with the prevention and treatment of cholera that the pros and cons of the field use of phage are to-day most actively debated. Reference has already been made to Morrison's observations; and Asheshov's studies on cholera phages (Asheshov *et al.*, 1930) provide an excellent example of the arguments that may be adduced on the basis of virulence and polyvalency. There is, however, in the case of this particular disease, another line of argument that must not be overlooked. Cholera is the classical example of a pure intestinal infection. It is possible that phage action might be effective in such a disease, even if quite ineffective in diseases in which extensive tissue invasion is an essential factor in the production of the clinical syndrome. The adoption of this assumption would, of course, jettison most of the claims that have been made for phage by its more enthusiastic supporters, and would confine its sphere of usefulness within narrow limits.

Such a view is certainly tenable; in fact, the very numerous observations on the inhibitory effect of colloids on phage lysis, and the results of many studies on the fate of phage particles after injection into the animal body, lend it considerable support. It may, however, be doubted whether these findings are final and decisive. If a disease of experimental animals were known that was clearly analogous to cholera in man the obvious next step would be to examine the effect on it of a highly virulent phage administered under controlled conditions. Unfortunately we know of no such disease. Is it waste of time to examine the problem further in relation to those diseases, whether included in the "intestinal" or "respiratory" category, in which early tissue invasion occurs? We do not think that it is; though the outlook is certainly not hopeful. It would, in reality, be going too far to

suggest that in cholera itself tissue invasion plays no part; and it seems a little doubtful whether a bactericidal agent the action of which was confined entirely to the intestinal lumen would exert the dramatic effects that have been claimed for phage in the treatment of this disease. Moreover, even if we adopted the assumption that phage is without effect once tissue invasion has occurred, it does not follow that it will have no influence on the colonization on a mucous surface that probably precedes entry to the deeper tissues. It is, in any event, so difficult to obtain reliable data from the ward or from the field, that it seems desirable that experimental studies should be continued.

SECTION X

THE EFFECT OF DISPERSAL OF AN INFECTED HERD

We have so far made no mention in this report of the effect of altering the condition of aggregation of our herds. This is not because we believe this factor to be unimportant—we suspect that its importance may be decisive—but because the few observations that we have as yet made on this point are too fragmentary to serve as the basis of any general discussion. It has been our desire, as we have already emphasized, to obtain as complete and detailed a picture as possible of the behaviour of our herds under conditions of maximal aggregation, before attempting to study the effect of varying that particular factor. It is our hope that in the near future we shall be able to turn our attention to this aspect of our general problem.

In the meantime we may summarize very briefly the few scattered observations that have been recorded.

In the first experiment of this kind (Topley, 1922) 305 normal mice were aggregated in a large cage, and to them were added 24 mice infected with *Bact. aertrycke.* During the following 7 days 15 mice died, and one that died on the 7th day showed all the post-mortem appearances of mouse typhoid. On the 8th day the surviving 314 mice were dispersed as follows. The 20 mice that remained from the 24 infected animals were removed from the herd. Of the 294 normal mice 100 (Group A) were placed in a single large cage; 94 (Group B) were dispersed into four herds, three of 25 and one of 19, each herd being housed in a separate cage; 100 (Group C) were dispersed in ten cages, 10 mice to a cage. These three groups were observed for 68 days. The survivors of Groups B and C were then re-aggregated into single herds, and observation was continued for another 93 days. The total and specific mortalities within these groups during each of the two periods are set out in Table XLVI. In this case the mice that died but were wholly or partially eaten by their companions are listed as non-specific deaths. Many of the small groups of 10 mice showed no evidence of infection with mouse typhoid during their period of isolation, and it would be entirely misleading to list the deaths of unexamined mice in these cages as due to infection with *Bact. aertrycke.* In this experiment, therefore, the term "specific death" is confined to those mice from which *Bact. aertrycke* was isolated at necropsy. We shall be underestimating a little the specific death rate during periods when mouse typhoid was actually spreading in a herd, just as we are overestimating it a little when we follow the convention of including unexamined mice among the specific deaths. The percentage mortalities during the period of re-aggregation of Groups B and C are calculated on the total numbers in those groups at the beginning of the experiment.

TABLE XLVI

Showing the mortality among groups of mice during periods of dispersal and re-aggregation

Group.	Dispersal.		Re-aggregation.		Total period.	
	Total mortality per cent.	*Specific mortality per cent.*	*Total mortality per cent.*	*Specific mortality per cent.*	*Total mortality per cent.*	*Specific mortality per cent.*
A Undispersed (100 mice).	95·0	65·0	3·0	2·0	98·0	67·0
B Four large groups (94 mice in all).	90·4	74·5	7·5	4·2	97·9	78·7
C Ten groups of 10	45·0	6·0	31·0	10·0	76·0	16·0

Clearly the dispersal into four large groups has made little difference in the behaviour of Group B. The dispersal, at this stage, into ten small groups of 10, has, however, produced a considerable effect. The total mortality in C during the period of dispersal is half that in A or B; the specific mortality less than 1/10th. Re-aggregation, however, had the effect of inducing a fresh epidemic spread, and during the succeeding 93 days this herd suffered a considerable mortality, at least 1/3rd of which was specific. In the final result this herd showed many more survivors than herds A or B (24 per cent. as against 2·0 and 2·1 per cent.), and the specific death rate for the whole period was much lower (less than 1/4); but the occurrence of so many "non-specific" deaths makes it impossible to assess with any accuracy the advantage that this herd derived from the preliminary period of dispersal. The suggestion is clearly that, *when dispersal is carried out at the beginning of an epidemic period*, the division of a herd into many small isolated units greatly decreases the mortality during the succeeding ten weeks or so, and that, although the re-aggregation of such a herd is followed by a fresh spread of the disease, the final mortality is lower than in a similar herd that has not been dispersed during the earlier stages.

In a much later experiment (Topley and Wilson, 1925) an opportunity was taken, in connection with an experiment on the influence exerted by a bacteriophage on epidemic spread (*see* pp. 182–183) to test this point again.

An epidemic of mouse typhoid was started by feeding 80 mice on a culture of *Bact. aertrycke*. Three weeks later, when several deaths from mouse typhoid had occurred, 420 normal mice were added to the 60 survivors. During the following week 45 of these 420 susceptibles died of mouse typhoid, showing that an epidemic

was well under way. At the end of this period 360 survivors from the 420 susceptibles were treated as follows :—

Group A : 90 mice were placed in a single cage and treated with phage.

Group B : 90 mice were placed in a single cage but not treated with phage.

Group C : 90 mice were dispersed into 18 sub-groups of five mice apiece and were treated with phage.

Group D : 90 mice were dispersed into 18 groups of five mice apiece, but were not treated with phage.

The survivors from groups C and D were re-aggregated into single herds 42 days later. All groups were observed for 84 days.

We are not here concerned with the effect of the phage (which was discussed on pp. 182–183), and the only comparison that concerns us is that between the behaviour of groups A and B on the one hand and C and D on the other. The final mortalities were as follows :—

Group C :	dispersed and re-aggregated ..	82·2
Group D :	„ „ „ ..	83·3
Group A :	never dispersed	97·8
Group B :	„ „	91·1

The difference is not large, but it is consistent. Dispersal followed by re-aggregation appears to have had some effect. In this case, in which dispersal was carried out at a later stage of epidemic spread than in the experiment recorded above, the effect of the dispersal was strikingly different. There was no *immediate* difference in mortality. For the first 20 days or so the mortality was almost equally severe in each of the four herds ; but about the 25th day the death rate in each of the dispersed herds showed a definite slowing, while that in each of the undispersed herds continued unabated for about another ten days. It may be noted that, in this experiment, the re-aggregation of the dispersed herds was followed by no detectable increase in mortality, suggesting that each of the dispersed units had in this case reached a state of equilibrium before re-aggregation occurred.

We may, in this connection, refer to another small series of experiments carried out at an early stage of this work (Topley and Wilson, 1923). We have noted that, under the conditions obtaining in our long-continued epidemics, infection never dies out. In the small series of experiments referred to we proceeded in another way. Small groups of mice infected with mouse typhoid or with pasteurellosis were placed in contact with equally small groups of normal mice. After a varying number of days the contacts were removed from the infected and were isolated. After a period of isolation they were placed in contact with another small group of normal mice. After a period of close contact in the same cage these two groups were separated, and the third group, after a period of isolation, was placed in contact with a fourth, and so on. By this method of group-to-group infection, periods of contact alternating with

periods of isolation, we were never able to induce infection to spread beyond three successive groups. These results seem suggestive, but the experiments were carried out on far too small a scale to be conclusive.

Taking these scattered experiences as a whole, they are at least in conformity with the view that the conditions of contact exert an exceedingly important effect on epidemic spread; but the real nature of that effect can only be determined by *ad hoc* experiments, carried out on an adequate scale.

SECTION XI

SUMMARY AND CONCLUSIONS

At the end of each section of this report we have set out the main conclusions that we should draw from the observations there recorded. In this final section it may be useful to bring these conclusions together, present them as a whole, and discuss very briefly the general significance of the picture they present :—

(I) *In regard to the general character of the epidemic process, as it is revealed in herds of mice living in close and continuous contact and subject to the continuous or intermittent migration of susceptibles.*

We think it has been proved that :—

(*a*) *In relation to secular time.*

(1) The disease will never normally die out. Since in experimental practice a herd may be reduced to a very small number of individuals, it *might* happen that no individual left in it was either clinically sick or a carrier ; it might happen that the disease became extinct. But we think our experience is wide enough to demonstrate that such an event would be a mere accident of small numbers, and that in large herds maintained under the conditions of our study the disease will be perennial.

(2) The form of the mortality curve—the occurrence of a succession of well-differentiated waves, or their replacement by a fluctuating mortality without periods of quiescence, or the almost complete smoothing out of such fluctuations into an approximately steady death rate—depends in the main on the rate of immigration. With very low rates of immigration there will be well-separated waves and quiet intervals ; with high rates of immigration there will be minor fluctuations, or an almost steady death rate.

(3) The average death rate over any long period of time is not highly correlated with the immigration rate, at least over a wide range of the latter variable. The ultimate size of the herd is thus mainly determined by the rate of immigration ; and the tendency is towards an invariant total population with an invariant death rate.

(4) This condition of equilibrium, though it may be continued for long periods, is fundamentally unstable ; and when it is seriously disturbed, by some extrinsic or intrinsic factor, the system tends to pass through a period of violent fluctuations before equilibrium is established again at the same, or at some other, level.

(*b*) *In relation to life-table time, i.e. to time as measured for each mouse from the date of entrance to the herd.*

(1) In epidemics initiated by virulent and infective strains of *Bact. aertrycke, Past. muriseptica* or the virus of ectromelia, in which the corresponding diseases are displayed in their characteristic and fatal form, the rate of mortality during the early days of herd life is very high. Approximately half the entrants are dead after 25 days, three-quarters to four-fifths after 50 days. The actual numbers living at each cage age, the l_x values, vary over a moderate range from one severe epidemic to another; but the early mortality is always of this high order. At later cage ages the level of mortality decreases—as measured by the ${}_5q_x$ of our life tables, the probability of dying within 5 days. This decrease usually sets in about the 25th–30th day of cage age and becomes more marked during the succeeding weeks. Somewhere between the 40th and 60th days of cage age it usually attains a low level that, in spite of minor fluctuations which we think are due to sampling errors, tends towards a constant value, the surviving mice thereafter dying out logarithmically.

(2) The expectation of life of the surviving mice—most conveniently expressed as the limited ${}_{60}E_x$ (i.e. the average length of life following cage age x, the maximum value being limited to 60 days)—rises continuously after the 20th–30th day of cage life towards a level which, while greatly in excess of that of new entrants, never reaches the limited expectation of life of normal mice living in the same environment, but not exposed to contact with an infective disease. The approach to this "normal" level is closer in the case of ectromelia than in either of the bacterial infections. If we choose to equate expectation of life to "resistance," we may say that the average resistance of surviving mice increases with survival in herd, but never becomes absolute. In the long run the great majority eventually succumb to the reigning disease.

(II) *In regard to the mechanisms that determine these events.*

(*a*) We think it certain that both selection, by death of the more susceptible, and natural immunization play a part in the increased average resistance displayed by surviving mice. We think there is little doubt that the part played by natural immunization is exceedingly important, and that it is probably more important than the part played by selection.

(*b*) As a working hypothesis, we think there is considerable support for the view that the resistance attained by any

one mouse after prolonged survival in an infected herd is not maintained at a steady level, but is subject to rather wide fluctuations, and that ultimate death from the reigning disease is to be attributed, in part at least, to fluctuations of this kind.

(*c*) We think it has been proved in the case of mouse typhoid, and rendered highly probable in the case of the other diseases that we have studied, that, under the conditions of our experiments, infection occurs very early in herd life, and that few mice escape it for more than 14 days. We should, therefore, regard an infected herd as a highly complex system, consisting of mice suffering from a fatal infection, others in a state of infection-equilibrium that ends in death or recovery at some later period, others undergoing natural immunization by an infection of slighter degree, and a small minority not yet infected. There are, we think, reasons for suspecting that a small proportion of mice, possessed of unusually effective innate mechanisms of the kind that hinder the access of bacteria or viruses to the tissues, may, in spite of the reception of repeated doses of the parasite, remain uninfected, that is unchanged in their immunological state, for unusually long periods of time. Their ability to escape infection is, however, never absolute.

(*d*) We think that the differences we have observed in the form in which epidemics may be propagated in secular time—the occurrence or non-occurrence of differentiated waves of mortality—are due to the state of equilibrium established in this complex system, which may be shifting or temporarily stabilized. Fundamentally it is always unstable, and subject to sudden disturbances. We do not think that the observed fluctuations in mortality—the epidemic waves—are due to any fixed cycle of bacterial or virus development, to seasonal or similar disturbances, or indeed to any single determining cause. Various causes may set the system swinging; but they do not, we think, in our cages, determine the period of the swings.

(III) *In regard to the influence of particular factors, other than those discussed above.*

We think it has been shown that the level of mortality in an infected herd, the proportion of immunizing to fatal infections, and the degree to which infection occurs, are largely determined by the characters of the bacterial strain with which the epidemic is initiated; that virulence and infectivity vary independently; that a highly potent "epidemic" strain possesses both these characters; and that a strain of low epidemicity may give rise to a variant of high epidemicity during a long-continued epidemic.

We should, therefore, regard differences in the virulence or infectivity of different bacterial strains of the same species as of the first importance in determining the general characters of an epidemic; and possible variations in these characters as a potential cause of changes in the prevailing type of any epidemic infection.

We do not, on the other hand, think that our observations afford any evidence that changes of this kind play a part in the sequence of epidemic waves during any given epidemic prevalence.

(IV) *In regard to possible modes of interference with the normal course of events in our infected herds, apart from changes in the conditions of contact.*

(*a*) We think it has been proved that ARTIFICIAL IMMUNIZATION by the most effective methods at present available, will increase the resistance of entering mice to a level at which they have, on entrance, an expectation of life not greatly inferior to that of those survivors that have experienced the full effects of selection and natural immunization. In conformity with our findings that natural immunization is more effective in the virus disease, ectromelia, than in the bacterial diseases, mouse typhoid and pasteurellosis, we have found that artificial immunization against ectromelia appears to be more effective than artificial immunization against mouse typhoid. In no case, however, is the immunity attained complete. The immunized mice die, in the long run, of the prevailing disease; nor, in mouse typhoid, at least, does the immunization of all entrants to a herd show any tendency to bring an epidemic to a close.

With regard to the effect of artificial immunization on the infection rate, as opposed to the death rate, our experience indicates that the infection of immunized animals is exceedingly common, and that, at least in ectromelia and most probably in the bacterial diseases, many of the immunized and infected survivors are infective for normal mice; so that, even if it were possible to devise a method of immunization more effective in lowering mortality than those we have hitherto employed, it is doubtful whether we should, by this method, be able to eliminate infection from our herds, and so render safe the admission of susceptible immigrants.

We would emphasize that the relatively unsatisfactory results we have obtained, especially in mouse typhoid, in the control of mortality by this method, must be assessed in relation to the extremely severe conditions of exposure to which our mice have been subjected. It is quite possible that under conditions of more restricted contact

the results with our present reagents would be more favourable. We also think it possible that methods of immunization might be devised which, at least in relation to their effects on mortality, might prove successful even under the severe conditions of trial existing in our herds. Both these possibilities we hope to explore.

(*b*) Although we have not ourselves been able to obtain any evidence that any change in our standard DIET exerts a favourable influence on the course of mortality, we think that the observations of other workers suggest that such changes might exert a significant effect, though we should be surprised if this effect were very great. In view of the proved effect of A-avitaminosis in lowering the resistance of animals to various infections, this possibility clearly merits further exploration.

(*c*) All our experiments on the influence of BACTERIOPHAGE on mouse typhoid have yielded entirely negative results; and in this they accord with the findings of many other workers who have studied the effect of this agent on experimental infections in laboratory animals. The possibilities that would be opened by a successful exploitation of bacteriophage action in the control of epidemic disease are, however, so attractive that we do not think that the investigation of this problem should be abandoned at the present stage. Although all the present indications are definitely against the efficacy of this agent *in vivo*, except perhaps in cholera, and although the argument of inadequate virulence or insufficient polyvalency of the phage employed cannot be accepted as a satisfactory explanation of an unlimited number of negative results, we do not think that this field has yet been explored to the point at which further search becomes useless. It is, at least, far more likely that clear and definite answers, favourable or unfavourable, will be obtained by controlled animal experiment than by any relatively uncontrolled trial in the field.

(V) *In regard to possible interference in the course of an epidemic by modifying the conditions of contact.*

The few observations that we have so far made on this problem allow of no conclusion beyond a suggestion that dispersal, to be effective, must be carried out in the very early stages of the epidemic process. That modifications of our technique resulting in a lessened intimacy or frequency of contact within a herd would profoundly alter the rate of spread of infection, and with it the nature of the equilibrium attained, we can be reasonably sure without troubling to do any experiments on the point.

> What we cannot tell in any other way than by direct experiment is what these changes will amount to in quantitative terms. We think that we have now obtained sufficient information with regard to the behaviour of our herds under conditions of close and continuous contact, to have some hope of measuring accurately the effect of changing these conditions in various ways.

Finally, it may not be altogether amiss to consider the significance of these findings in relation to events in the natural world outside our cages—though it is, of course, impossible, in such a report as this, to attempt even the briefest review of the mass of epidemiological and bacteriological literature from which obvious analogies might be drawn.

Taking first the secular trend of events, our observations, and the interpretations that we have placed upon them, are in general accord with the view expressed by Hamer (1906) that the periodicity of such an epidemic disease as measles is probably due to periodic changes in the constitution of the population exposed to risk, leading, after each epidemic wave, to a gradual re-accumulation of susceptibles. These natural epidemic waves are not, of course, the minor fluctuations of our mortality curves. They correspond rather with the widely spaced waves observed with very slow rates of immigration, or with the effect produced by adding susceptibles to the population surviving from an epidemic prevalence. In the natural world, taken as a whole, the re-accumulation of susceptibles is by births rather than by immigration; but there are specialized herds, such as schools in general and boarding schools in particular, in which, as Dudley's observations have so clearly shown, the immigration of non-immunes term by term is probably a decisive factor in determining the course of events.

In any of those common endemic-epidemic diseases from which many or most of our people suffer at one time or another during their lives, but which occur in epidemic form only at more or less widely-spaced intervals, we should regard this ever-varying state of the immunological constitution of the herd as the main factor determining the intervals at which the epidemic waves occur. It follows that we should postulate, as a major component of that mysterious entity, the epidemic constitution, the existing immunological constitution of the herd in relation to all those pathogenic parasites to which it is, for the time being, exposed.

In regard to the mechanism by which this immunological constitution is determined, our findings clearly support the *Durchseuchung* hypothesis; and we should regard sub-lethal or latent infection as the essential factor involved in the immunization of any human or animal herd. In relation to our discussion of the relative importance of innate as compared with acquired immunity, it seems relevant to point out that well over a thousand years' exposure to measles, a disease that exerts its main killing power before puberty and should hence exert a maximal selective effect,

has not sufficed to lower its incidence in the slightest degree; while the immunizing effect of a wave of disease is too obvious to need comment, and Stocks (1928) has recorded observations suggesting that this effect is not confined to those who pass through a clinically diagnosable infection.

In recording our failure to demonstrate any seasonal influence on the mortality in our infected herds, we were careful to emphasize that this finding could have no significance in the world at large. The seasonal incidence of particular epidemic diseases is one of the commonplaces of epidemiology. The only comment that we would offer on this point is that "seasonal" should not be interpreted in a purely meteorological sense. That cold or heat, humidity or dryness, have, in themselves, an influence on the human and animal body, and hence on its resistance to disease, we do not doubt. That they exert an indirect effect, in determining the ease and frequency of the spread of infection—as, for instance, through their influence on the numbers of insect vectors—is well established. But it must be remembered that social, as well as meteorological, events show a seasonal rhythm. Some of these are themselves meteorologically determined, as, for instance, the balance of indoor and outdoor life. Others, such as the breaking-up and reassembly of schools, are independent of weather conditions. Many of these social happenings will have a direct effect on the immunological constitution of the various herds at risk, and will hence impose a "seasonal" rhythm on the working of the mechanisms that we have considered above.

The observations that we have outlined in section VI will clearly incline us to adhere to the views of these epidemiologists who attach a major importance to the evolution or importation of "epidemic strains" of particular bacteria or viruses. The temporary prevalence of a strain, or strains, of a particular epidemic character would, on this view, play a large part in determining the reigning epidemic constitution. The numerous studies of Griffith (1926, 1927, 1928, 1933), Griffith and Gunn (1928) and of Glover and Griffith (1930, 1931) on infections due to haemolytic streptococci afford one recent instance from the domain of human medicine; and the recording by Dudley and Brennan (1934) of their experiences at Chatham, where the occurrence during a period of fifteen months of eleven cases of cerebro-spinal fever with only one death was followed by a non-contact meningococcal carrier rate fluctuating for some seventeen months about an average value of 54 per cent., without the occurrence of a single case of the disease, affords obvious analogies with the experiments described on pp. 148–160. Among the particularities of epidemic behaviour, determined by the biological characters of the prevailing strain of parasite, it seems likely that the ratio between immunizing and disease-producing or killing infections holds an important place.

In this connection we would note again that there are many reasons for suspecting that the association of one bacterium with

another, or of one virus with another, or, more especially perhaps, of a bacterium with a virus, may play a part in determining the character of an epidemic no less important than that played by variations in the biological characters of a single parasite. So far as experimental epidemiology is concerned, this is a problem that awaits attack.

On the problem of the epidemiological effect of variations in the closeness and continuity of contact, or in the opportunities for the spread of infection in other ways, the experimentalist has as yet no adequate data that he can compare with the mass of evidence collected by the field observer. The few and inadequate experiments recorded in this report afford only one analogy of interest, and that of doubtful significance. If we compare the effect of dispersing an infected herd of mice with the probable effect of school closure during an epidemic prevalence, and confine our attention to the effect on the boys or girls exposed to risk, the suggestion—it is at the moment nothing more—is that the nature of this effect will depend in the main on the stage of the epidemic process at which the school is closed. If it is closed early, when only a small proportion of those at risk are infected, the advantage reaped *by the scholars* will probably be large. If closure is delayed until infection is widespread, the scholars' advantage will be relatively slight, though not insignificant. In terms of practical politics and ignoring again the possible effect on home contacts, this means that the possible benefits of closure following the emergencies of clinically diagnosable cases of any given disease can only be assessed when we know the probable nature of the actual equilibrium of which these cases are a manifestation. If the ratio of " carriers " to " cases " is low, prompt closure will be effective ; if this ratio is high, closure might not greatly lessen the scholars' risk. There can, in fact, be no general rule. Unless we know something of the immunological constitution of our herd we cannot assess the probable effect of dispersing it.

We may, before leaving this particular problem, make another general note. We should expect herd structure, in terms of opportunity for the spread of infection, to have an important influence, not only in determining the frequency of disease or death, but in determining the average or ultimate immunological constitution of the herd. We should, therefore, allot to this factor an important place among the determinants of an epidemic constitution, postulating, of course, that if we wish to define an epidemic constitution as a system operating over a large geographical area for a considerable period of time—a definition that, though perhaps in accord with classical epidemiology, does not seem to us obligatory —we must limit our variations in herd structure to those having a similar distribution.

This, perhaps, would be the most appropriate place to consider the hypothesis of the importance of the " velocity of infection " to which Dudley (1923, 1926) has been led by his studies on

diphtheria and other epidemic infections in man. Our experimental data do not, however, as yet provide us with any material that can usefully be compared with these field investigations. For the moment we would only note that we should be inclined to view this problem rather in terms of the relative velocities of immunization and disease production within a herd, than in terms of the relative velocities of reception and elimination of the parasite by an individual. These aspects are, of course, not independent; our observations suggest that, so far as the individual is concerned, the probability of immunization or death may be largely determined by the size and spacing of the doses of the parasite received. On problems of this nature we hope shortly to have more experimental evidence to offer.

Turning finally to possible methods of interference with the natural course of events, we would merely note that, of those we have attempted, artificial immunization has afforded the only significant results; though it has not, under the conditions of our experiments, approached the successes recorded from the field. Within their range of effectiveness, our findings accord with field observations in attaching a greater efficiency to antiviral than to antibacterial immunization. Antitoxic immunization, of the type involved in diphtheria or scarlet fever prophylaxis, we have had no opportunity to study. We hope in the future to be in a position to assess the value of active immunization under conditions more nearly approaching those existing in nature. There is, we think, one other suggestion of practical importance in the observations recorded in this report. So far as our present methods of immunization are concerned there is no indication that a lowering of mortality is associated with an equivalent lowering of infection. Many immunized survivors are apparently infected and infective. If this applies to field immunization in an equal degree, there seems little hope of the complete elimination of any particular infective disease along these lines. The immunological constitution of a herd may, perhaps, be maintained in a state in which overt cases of the disease no longer occur; but it seems likely that any relaxation of artificial immunization would result in a re-accumulation of susceptibles and a re-appearance of disease. If it were possible to induce an active immunity that prevented infection as well as disease the immediate position would be more hopeful, though even then the danger of re-importation might well prove very formidable if immunization were relaxed.

Apart from general hygienic methods designed to lessen the opportunities for infection—and there are many important diseases in which such methods are not applicable—the present indications would suggest that active immunization is likely to remain the only procedure which, acting alone, will exert an important effect on the prevalence of an infective disease; but the acceptance of such a conclusion does not, in our view, afford any reason for neglecting the study of other factors, such for instance as dietary

variations, which, even if undramatic in their isolated effects, may well be of great cumulative importance. The records of descriptive and statistical epidemiology make it abundantly clear that the amelioration, or disappearance, of an endemic or epidemic infection is more often the result of a summation of effects, many of them unidentifiable, than of any single known factor. The identification of such minor or accessory factors, and the assessment of their relative importance, will clearly involve comparisons between large groups of animals under controlled conditions. In studies of this kind, the methods of experimental epidemiology offer obvious advantages.

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No. 168. Alcohol and Inheritance: An Experimental Study. By F. M. Durham and H. M. Woods. [1932.] 1*s.* 3*d.* (1*s.* 4½*d.*).

(Book). Alcohol: its Action on the Human Organism. Second Edition. [1924.] 1*s.* paper covers, 1*s.* 6*d.* cloth bound.

Anaerobic Bacteria: *see* WOUND INFECTIONS.

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No. 121. Borna Disease and Enzootic Encephalo-Myelitis of Sheep and Cattle. By S. Nicolau and L. A. Galloway. [1928.] 5*s.* (5*s.* 2½*d.*).

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Bacteriology (MISCELLANEOUS):

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No. 51. The Laboratory Diagnosis of Acute Intestinal Infections, including the Principles and Practice of the Agglutination Tests. By the Committee upon Pathological Methods. [1920.] 4*s.* 6*d.* (4*s.* 8*d.*).

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See also NUTRITION; RICKETS.

Dental Disease:

No. 70. The Structure of Teeth in relation to Dental Disease. By J. Howard Mummery. [1922.] 2*s.* (2*s.* 1*d.*).

No. 97. The Incidence of Dental Disease in Children. By the Committee for the Investigation of Dental Disease. [1925.] 1*s.* 6*d.* (1*s.* 7½*d.*).

No. 140. Diet and the Teeth: An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. [1929.] 17*s.* 6*d.* (18*s.*).

No. 153. Diet and the Teeth: An Experimental Study. Part II. A.—Diet and Dental Disease. B.—Diet and Dental Structure in Mammals other than the Dog. By May Mellanby. [1930.] 2*s.* 6*d.* (2*s.* 8½*d.*).

No. 159. The Influence of Diet on Caries in Children's Teeth (Interim Report). By the Dental Committee. [1931.] 6*d.* (7*d.*).

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No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] 2*s.* (2*s.* 1½*d.*).

(Book). Diphtheria: its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1923.] 12*s.* 6*d.* (13*s.* 3*d.*).

See also EPIDEMIOLOGY (Nos. 75, 137 and 195).

Dust: *see* VENTILATION, ETC.

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Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean:—

No. 6. III.—Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16. By Paul Fildes and others. IV.—Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] 4*s.* 6*d.* (4*s.* 7½*d.*).

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Reports of the Committee upon the Physiology of Hearing.

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No. 201. The Determination of Iodine in Biological Substances. By C. O. Harvey. [1935.] 1*s.* (1*s.* 1½*d.*).

See also CHILD LIFE; RICKETS; DENTAL DISEASE; STANDARDS (No. 202); BACTERIOLOGY (No. 210).

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No. 79. Bacteriological and Clinical Observations on Pneumonia and Empyemata, with special reference to the Pneumococcus and to Serum Treatment. By E. E. Glynn and Lettice Digby. [1923.] 5*s.* (5*s.* 3*d.*).

Pneumothorax, Artificial: *see* TUBERCULOSIS.

Print, Legibility of: *see* VISION.

Protozoan Infections:

No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] 2*s.* (2*s.* 1½*d.*).

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No. 170. Studies in the Psychology of Delinquency. By G. W. Pailthorpe. [1932.] 2*s.* (2*s.* 2*d.*).

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No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1*s.* (1*s.* 1*d.*).

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No. 62. Medical Uses of Radium: Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5*s.* (5*s.* 3*d.*).

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Respiration:

No. 198. Test for Respiratory Efficiency. By A. Moncrieff. [1934.] 1*s.* (1*s.* 1½*d.*).

Rheumatism: *see* CHILD LIFE (No. 114).

Rickets:

No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4*s.* (4*s.* 2*d.*).

No. 68. Rickets: the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2*s.* 6*d.* (2*s.* 7½*d.*).

No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4*s.* (4*s.* 3*d.*).

No. 77. Studies of Rickets in Vienna, 1919–22. [1923.] 7*s.* 6*d.* (7*s.* 10½*d.*).

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Salvarsan: *see* VENEREAL DISEASES; STREPTOCOCCAL INFECTIONS; STANDARDS, BIOLOGICAL (No. 128).

Scarlet Fever: *see* EPIDEMIOLOGY (Nos. 137, 180).

Scurvy: *see* NUTRITION (No. 146).

Shock, Surgical:

Reports of the Committee on Surgical Shock and Allied Conditions:—

No. 25. Wound-Shock and Haemorrhage. [1919.] 4*s.* (4*s.* 5½*d.*).

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No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] 3*s.* 6*d.* (3*s.* 8½*d.*).

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Wassermann Test : *see* VENEREAL DISEASES.

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