THE EPIDEMIC POTENCY OF STRAINS OF *BACT. AERTRYCKE* OF VARYING VIRULENCE.

A REPORT TO THE MEDICAL RESEARCH COUNCIL.

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(With 5 Charts.)

One of the questions, to which the experimental study of epidemics has as yet given no decisive answer, is the possible significance of variations in bacterial virulence. The experiments here recorded relate to this problem, in the particular case of mouse-typhoid spreading within a closed community.

Webster and his co-workers (Webster, 1923, *a*, *b*, *c*, *d*; 1924, *a*, *b*; Webster and Pritchett, 1924) would regard the virulence of any single strain of *Bact. aertrycke*, the causal organism concerned, as but little variable. While admitting (Webster and Burn, 1928, *a*, *b*, *c*) that variations in virulence may occur in bacteria belonging to this group under the influence of a bacteriophage, they consider that any single strain maintains a constant level of virulence for an indefinite period of time, under any of the usual methods of cultivation, and that variations in virulence are difficult, if not impossible, to induce by any of the methods usually adopted. In particular, they believe that such variations play no significant part in the epidemic spread of disease.

Our own experiments, and those of our colleagues (Lockhart, 1926), many of which have not yet been recorded, have convinced us that *Bact. aertrycke*, when cultivated in the laboratory under various conditions, varies in virulence to a greater degree, and more frequently, than Webster and his colleagues suppose; although our own experience is in accord with theirs, in so far as we have found that a single strain, maintained by massive subculture in a solid medium, and at infrequent intervals, usually maintains its virulence unaltered over months or years. This aspect of the problem will, however, be dealt with more fully in subsequent reports.

The present series of experiments is concerned with the epidemic behaviour of strains of appreciably different virulence. With regard to the origin of these strains, we would merely note that they are all derived from strains isolated during the course of the experimental epidemics which we have studied during the past 10 years, and are, so far as we can tell, descendants of the original strain with which we started. Their after-history, as regards the time during which they have been maintained in subculture, and the

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environmental conditions to which they have been submitted, varies widely. We may add that none of them has been intentionally submitted to the influence of a bacteriophage, or has shown any evidence of bacteriophage-action. None of them has displayed the cultural characteristics typical of a "rough," as opposed to a "smooth" strain.

EXPERIMENTAL METHOD.

The method adopted has been the same in the case of each of the five strains with which we are here concerned. At the commencement of each experiment 125 mice have been injected intraperitoneally, each with 1000 bacilli contained in 0·25 c.c. of Ringer's solution. These mice have then been divided into five batches, each of 25, and to each batch have been added 100 normal mice. Each of the five test-populations, containing 25 infected mice and 100 susceptibles exposed to risk, has been housed in an experimental cage of the usual type, and observed for 60 days, or for a longer period. All dead and uneaten mice have been submitted to the usual post-mortem examination, including the isolation and identification of the infecting organism. At the end of each experiment all survivors have been killed, and cultures have been prepared from a fragment of the spleen. In addition, many strains isolated from mice dying during the course of the epidemic, or from survivors killed at its close, have been tested for virulence by the intraperitoneal injection of 30 mice with 1000 bacilli from a 24-hour agar culture, grown at 37° C.

The reliability of the numerical results obtained in experiments of this kind, and the best method of comparison between one set of figures and another, have been discussed in an earlier paper (Topley, 1927), and need not be referred to here. The estimated sampling error involved in a single experiment does not, indeed, arise; since each experiment has been repeated in a five-fold series, carried out under identical conditions.

We would, therefore, merely note that we have based our comparisons between the fates of the different populations exposed to risk, or between the inoculated mice and their susceptible companions, on the mean expectation of life, limited to 60 days; and that, in calculating the probable error of the mean expectation of life of the mice exposed to risk in each complete five-fold experiment, we have taken the standard deviation of the five means, and not the standard deviation of the survival time of the whole 500 mice, thus making the larger allowance for errors of random sampling. A further comment upon this point will be found below.

The results obtained in the separate experiments will be briefly recorded, and then compared with one another.

Exp. 1. This experiment was carried out with a strain of *Bact. aertrycke* of moderately high virulence. The exact virulence of the strain was not tested on the day on which the experiment was commenced, nor were any of the strains isolated during the five epidemics tested in this way. The course

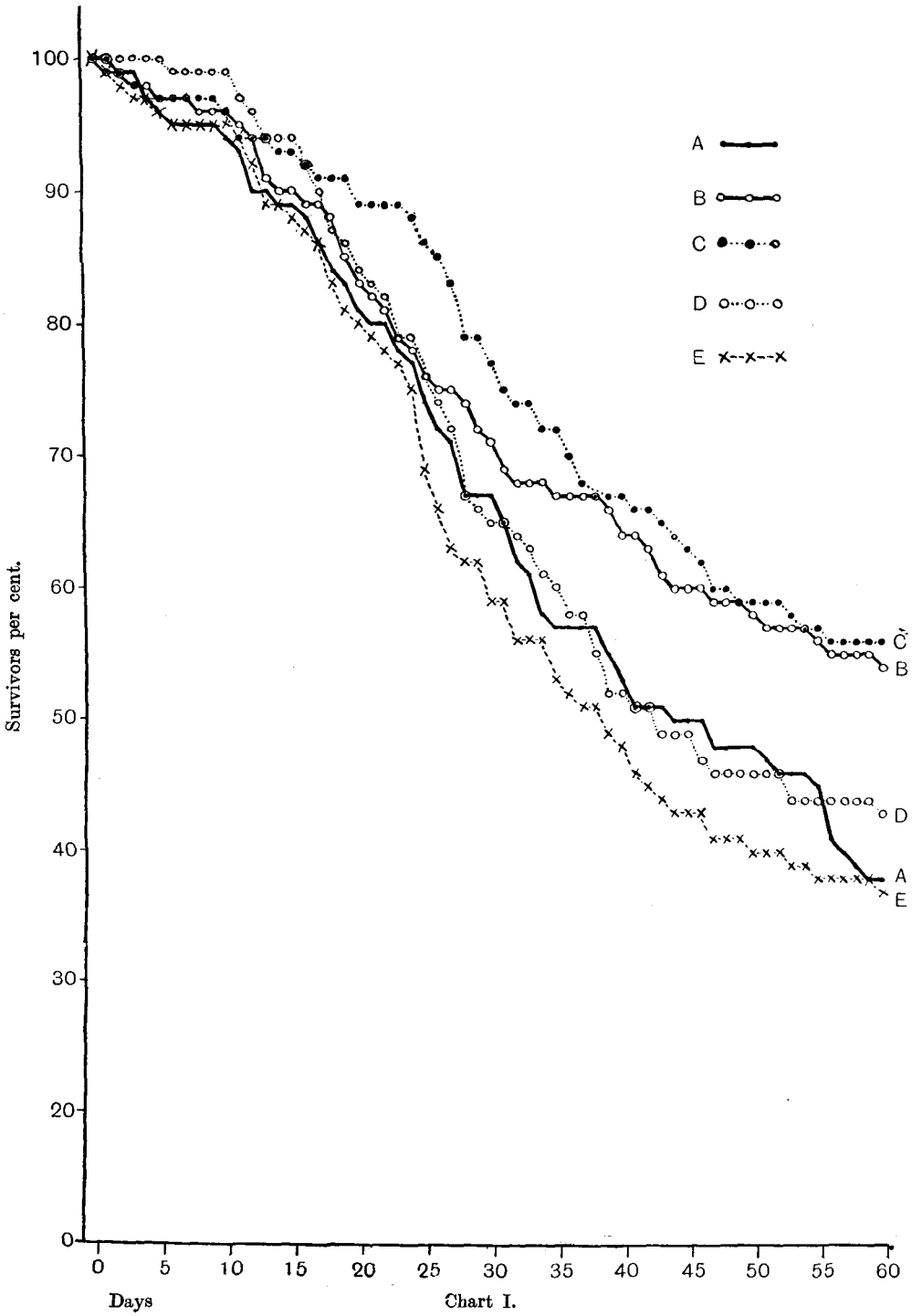

Chart I.

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of the epidemic during the first 60 days is recorded in Chart I, and the numerical results are set out in Table I, so far as they concern the fate of each batch of 100 mice exposed to risk.

Table I. *Showing the mean expectation of life, limited to* 60 *days, of the* 100 *mice exposed to risk in each of the five test-populations of Exp.* 1, *and for the five populations taken together.*

Epidemic		Mean expectation of life
1/A	=	40·74 ± 1·31 days
1/B	=	44·62 ± 1·31 „
1/C	=	46·41 ± 1·20 „
1/D	=	41·63 ± 1·23 „
1/E	=	38·64 ± 1·31 „
1/A–E	=	42·41 ± 0·84 „

The survivors from this experiment were killed on the 84th day, and submitted to the usual post-mortem examination. The results of the spleen-cultures are recorded in Table II.

Table II.

Epidemic	No. of survivors on 84th day	No. with positive spleen-cultures	% with positive spleen-cultures
1/A	27	16	59·26
1/B	47	35	74·47
1/C	51	36	70·59
1/D	40	30	75·00
1/E	32	21	65·63
1/A–E	197	138	70·05

These five epidemics were of about the same order of severity, as judged by the mortality-curves, and by the mean expectation of life. The mean expectation of life for the whole series, 42·41 days, may be taken as a measure of the epidemic potency of the strain of *Bact. aertrycke* employed. In this, as in previous experiments of this kind, the surviving mice had not, for the most part, escaped infection, but had resisted it: 70·05 per cent. of the survivors were harbouring *Bact. aertrycke* in their spleens.

Exp. 2. The strain of *Bact. aertrycke* employed in this epidemic was tested for virulence on the day on which the experiment was commenced by intra-peritoneal injection of 1000 bacilli into each of 25 mice. These mice were observed for 14 days, during which time all died of *Bact. aertrycke* infection.

The results, as regards the mice exposed to risk, are recorded in Chart II and Table III.

Table III. *Showing the mean expectation of life, limited to* 60 *days, of the* 100 *mice exposed to risk in each of the five test-populations of Exp.* 2, *and for the five populations taken together.*

Epidemic		Mean expectation of life
2/A	=	30·07 ± 1·16 days
2/B	=	34·83 ± 1·22 „
2/C	=	29·63 ± 1·11 „
2/D	=	28·39 ± 1·17 „
2/E	=	33·42 ± 1·34 „
2/A–E	=	31·27 ± 0·73 „

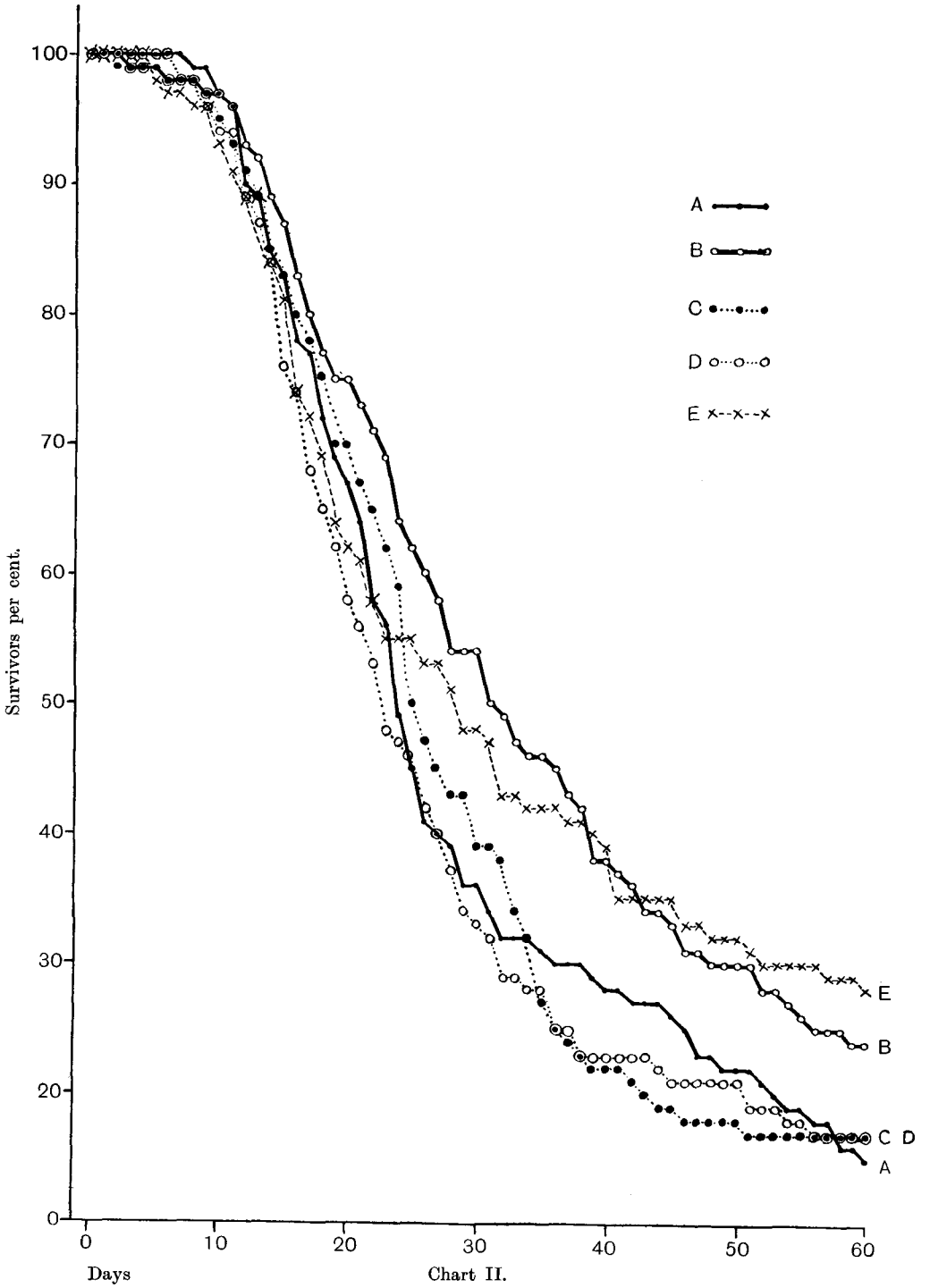

Chart II.

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The survivors from this experiment were killed on the 60th day. The results of the spleen-cultures are recorded in Table IV.

Table IV.

Epidemic	No. of survivors on 60th day	No. with positive spleen-cultures	% with positive spleen-cultures
2/A	15	12	80·00
2/B	24	13	54·17
2/C	17	15	88·24
2/D	17	14	82·35
2/E	28	26	92·85
2/A–E	101	80	79·21

Three of the strains isolated from the spleens of the mice killed on the 60th day of this experiment were tested for virulence by the intraperitoneal injection of 1000 bacilli into each of 30 mice. The results are shown in Table V.

Table V.

Strain	No. of mice inoculated	No. dying within 14 days
2/A 94	30	30
2/B 77	30	30
2/C 85	30	30

It will be noted that this series of epidemics, initiated by a highly virulent strain of *Bact. aertrycke*, were of greater severity than those of Exp. 1, the mean expectation of life being 31·27, as compared with 42·41 days. The percentage of survivors harbouring *Bact. aertrycke* in their spleens was also higher, 79·21 as compared with 70·05.

The virulence tests carried out at the commencement and conclusion of the experiment showed that the bacteria present in the spleens of the surviving, and apparently healthy mice, had lost none of their initial virulence.

Exp. 3. This experiment was carried out with a strain of *Bact. aertrycke* known to be of relatively low virulence. The exact virulence of the culture employed was tested by the inoculation of 25 mice, with the usual dose, on the day on which the experiment was commenced. Of the 25 mice, 6 died of typical *Bact. aertrycke* infection, during the 14 days of observation. The results of this experiment are recorded in Chart III and Table VI.

Table VI. *Showing the mean expectation of life, limited to* 60 *days, of the* 100 *mice exposed to risk in each of the five test-populations of Exp.* 3, *and for the five populations taken together.*

Epidemic		Mean expectation of life
3/A	=	36·67 ± 1·13 days
3/B	=	40·01 ± 1·12 ,,
3/C	=	39·52 ± 1·09 ,,
3/D	=	31·75 ± 1·06 ,,
3/E	=	38·20 ± 1·07 ,,
3/A–E	=	37·23 ± 0·90 ,,

The survivors from this experiment were killed on the 60th day. The results of the spleen-cultures are recorded in Table VII.

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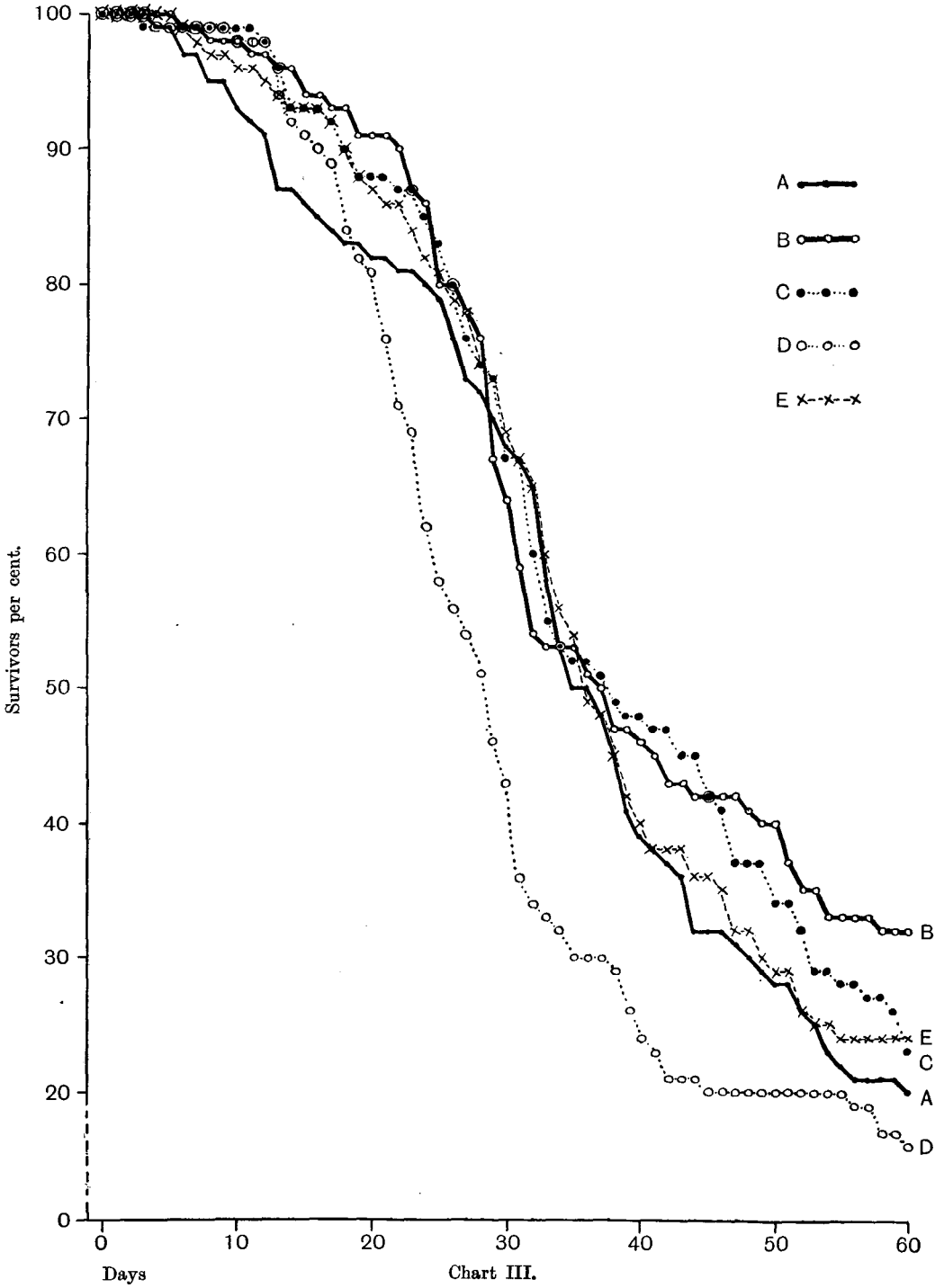

Chart III.

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Table VII.

Epidemic	No. of survivors on 60th day	No. with positive spleen-cultures	% with positive spleen-cultures
3/A	20	18	90·00
3/B	31	23	74·26
3/C	23	17	78·91
3/D	16	14	87·50
3/E	24	19	79·17
3/A–E	114	91	79·82

During the course of these epidemics, the strains of *Bact. aertrycke* isolated from the spleens of certain of the mice dying during the epidemic period, and from those of survivors killed on the 60th day, were tested for virulence in the usual way. Some of these strains were isolated from the spleens of the mice which were inoculated at the commencement of the experiment. These strains are marked with an obelisk (†) in Table VIII, in which the results of these tests are recorded.

Table VIII.

Strain	Day of exp. on which isolated	Fate of mouse from which strain was obtained*	No. of mice inoculated	No. of mice dying within 14 days
Orig.	—	—	25	6
3/B 20	25	*D*	30	30
3/C 14	24	*D*	30	28
3/D 34	24	*D*	30	30
3/A 90	60	*K*	30	30
3/C 94	60	*K*	30	29
3/E 82	60	*K*	30	28
3/A. In. 11†	60	*K*	30	29
3/B. In. 13†	60	*K*	30	5
3/D. In. 15†	60	*K*	30	5

* *D* = died on day stated. *K* = killed on 60th day.

† Mice inoculated at commencement of experiment.

The results obtained in this experiment are of considerable interest. As judged by the mean expectation of life these five epidemics were of considerable severity; but the strain with which they were initiated was of low virulence. The virulence tests, carried out with the strains recovered from mice dying during the course of the epidemics, showed that the strains then prevalent in the cage were of a high order of virulence. The strains isolated from certain of the surviving mice, among those exposed to risk, were fully virulent. Three strains were tested, which had been isolated from the spleens of mice previously inoculated with the relatively avirulent strain at the commencement of the experiment, the mice having survived throughout the epidemic period. One of these was fully virulent, the other two were of the same low order of virulence as the original strain.

It is clear that two strains of *Bact. aertrycke*, of very unequal virulence, were present in each of the five experimental cages during the progress of this series of epidemics. It is difficult to avoid the conclusion that the virulent strain was derived from the relatively avirulent strain, with which the epidemics were initiated.

Exp. 4. The strain employed in initiating this series of epidemics appeared to be quite avirulent when tested in the usual way. Of 30 mice, each inoculated intraperitoneally with 1000 bacilli, none died within 14 days. It may be noted that *Bact. aertrycke* was recovered from the spleens of 25 of these 30 mice, when they were killed on the 14th day.

The results of this experiment are recorded in Chart IV and Table IX.

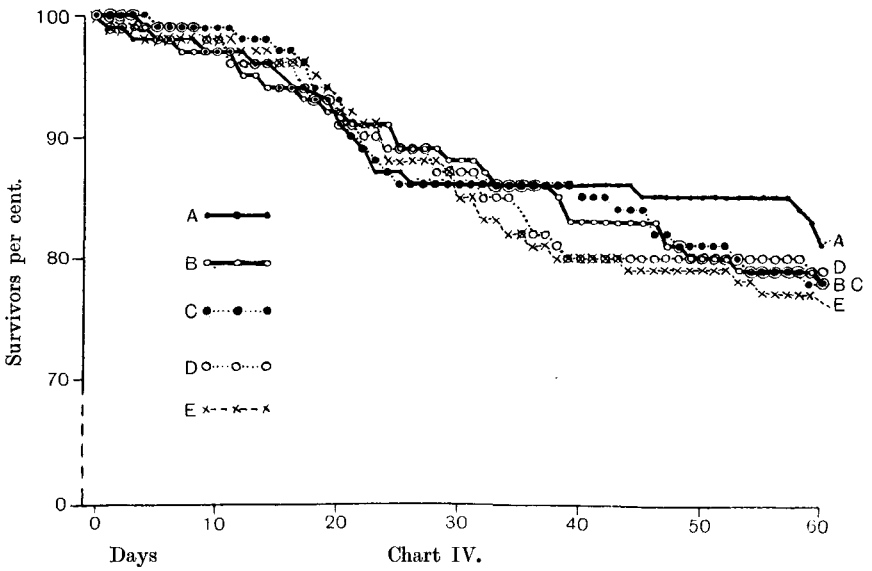

Chart IV.

Table IX. *Showing the mean expectation of life, limited to* 60 *days, of the* 100 *mice exposed to risk in each of the five test-populations of Exp.* 4, *and for the five populations taken together.*

Epidemic		Mean expectation of life
4/A	=	53·52 ± 1·06 days
4/B	=	52·85 ± 1·04 ,,
4/C	=	53·09 ± 1·00 ,,
4/D	=	51·50 ± 1·06 ,,
4/E	=	52·12 ± 1·06 ,,
4/A–E	=	52·62 ± 0·22 ,,

The survivors were killed on the 60th day. The results of the spleen-cultures are recorded in Table X.

Table X.

Epidemic	No. of survivors on 60th day	No. with positive spleen-cultures	% with positive spleen-cultures
4/A	81	3	3·70
4/B	78	8	10·26
4/C	78	8	10·26
4/D	79	7	8·86
4/E	74	12	16·22
4/A–E	390	38	9·74

It will be observed that the percentage of infected mice, at the end of the experiment, was far lower than in Exps. 1–3. The figures in this, and in other

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similar tables, refer only to the survivors from among the 100 mice exposed to risk in each epidemic; but it may be noted that, of 54 inoculated mice, which survived from the commencement of these five epidemics until the close of the experiment, 42 were found to be harbouring *Bact. aertrycke* in their spleens, when killed on the 60th day.

During the course of this experiment the strains isolated from several mice dying during the epidemics, or from survivors killed at their close, were tested for virulence in the usual way. The results are set out in Table XI.

Table XI.

Strain	Day of exp. on which isolated	Fate of mouse from which strain was obtained*	No. of mice inoculated	No. of mice dying within 14 days
Orig.	—	—	30	0
4/B 3	7	*D*	30	1
4/B 9	21	*D*	30	3
4/B 11	25	*D*	30	1
4/B 19	47	*D*	30	0
4/B. In. 6†	14	*D*	30	3
4/D 18	35	*D*	30	5
4/D 20	39	*D*	30	0
4/D. In. 1†	9	*D*	30	2
4/B 46	60	*K*	30	1
4/B 50	60	*K*	30	1
4/B 66	60	*K*	30	7‡
4/B 75	60	*K*	30	5
4/B 85	60	*K*	30	5‡
4/B 86	60	*K*	30	3

* *D* = died on day stated. *K* = killed on 60th day.
† Mice inoculated at commencement of experiment.
‡ One mouse gave negative post-mortem findings.

These results need little comment. The strain employed in initiating these epidemics was of very low virulence; nor was there any evidence of any increase in virulence during the course of the experiment. There is, perhaps, a suggestion that three of the six strains isolated from survivors possessed a slightly higher virulence than the original strain, or than those isolated during the earlier stages of the epidemic; but the differences are clearly not significant.

It will be noted that this strain, in spite of its lack of virulence, did spread to some extent among the population at risk, causing some latent and lasting infections, and some deaths. It may be added that in such experiments as this, in which the death-rate is very low, it is not easy to be certain what proportion of the total deaths are, in reality, due to mouse-typhoid. The regrettable cannibalism, so prevalent among mice, is very evident when the majority of the herd are in full enjoyment of health, and the intact corpses available for examination tend to be few in number. That many of the deaths in such slowly smouldering epidemics are due to specific infection there is no doubt, but it should be noted that a considerable proportion of the dead mice, which are in a condition to allow an autopsy to be made, give negative findings. Thus in Exp. 4/B, 22 mice died. Of these 10 were eaten by their companions, 7 yielded cultures of *Bact. aertrycke*, and 5 gave negative results. It has,

moreover, been our consistent experience, that the mice dying during epidemics of this kind present few characteristic lesions of mouse-typhoid. The post-mortem results, taken as a whole, resemble very strongly those observed during the pre-epidemic phase of the spread of infection among herds which are being recruited by continuous immigration.

Exp. 5. For the fifth experiment, another relatively avirulent strain was employed. In the preliminary virulence test 7 of 30 mice died within 14 days, but 2 of these showed no evidence of *Bact. aertrycke* infection. The results of this series of epidemics are recorded in Chart V and Table XII.

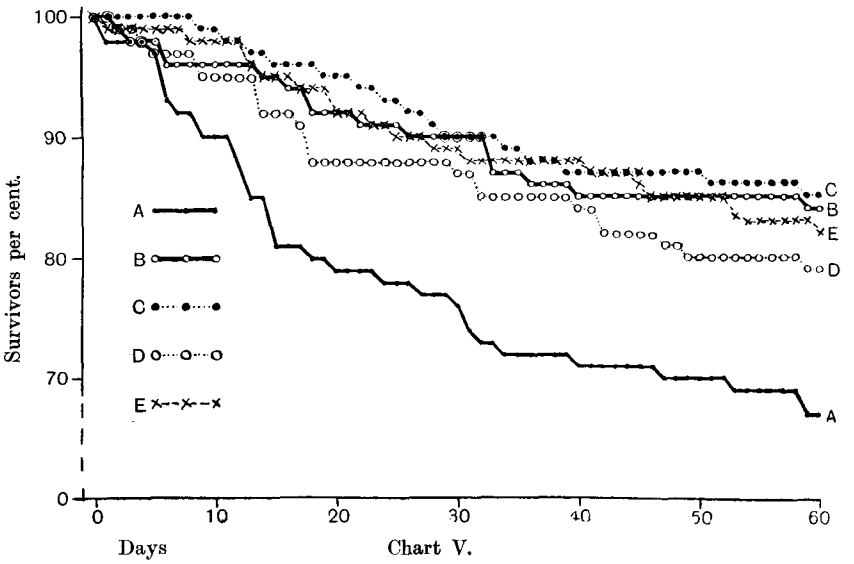

Chart V.

Table XII. *Showing the mean expectation of life, limited to* 60 *days, of the* 100 *mice exposed to risk in each of the five test-populations of Exp.* 5, *and for the five populations taken together.*

Epidemic		Mean expectation of life
5/A	=	45·72 ± 1·42 days
5/B	=	53·87 ± 1·03 ,,
5/C	=	55·01 ± 1·11 ,,
5/D	=	52·33 ± 1·13 ,,
5/E	=	54·14 ± 0·97 ,,
5/A–E	=	52·21 ± 1·01 ,,

This experiment was not brought to an end on the 60th day. The populations were maintained undisturbed until the 84th day, to give more time for any possible variation in virulence to occur. There was, however, no evidence of any such change. On the 84th day 50 normal mice were added to each cage, and the course of events was observed for a further 84 days. Again, no epidemic wave developed in any of the cages. Occasionally deaths occurred, and from the tissues of some of the dead mice *Bact. aertrycke* was isolated. These cases of infection included several of the more recently added

mice, as well as some of the survivors from the earlier period. The majority of such mice as died were, however, eaten by their companions, and a considerable proportion of the corpses not so consumed gave entirely negative findings at autopsy. The general character of the epidemics differed in no way from those observed in Exp. 4. From the 168th day the mice in 3 of the 5 cages were fed, on 5 days a week, with a bacteriophage-filtrate active against *Bact. aertrycke*, since it seemed possible that the well-known tendency of this agent to act as a stimulus to bacterial variation might lead to the appearance of more virulent variants. The course of events was observed for a further 46 days. Nothing of any interest occurred.

On the 214th day the survivors were killed and submitted to the usual post-mortem examination. The results are recorded in Table XIII.

Table XIII.

Epidemic	No. of survivors on 214th day	No. with positive spleen-cultures	% with positive spleen-cultures
5/A	26	4	15·38
5/B	39	1	2·56
5/C	68	1	1·57
5/D	56	4	7·14
5/E	55	12	21·82
5/A–E	244	22	9·02

As in the previous tables, the above figures refer only to the 100 mice, in each population, exposed to risk for the whole period of the experiment. Of the 250 mice added on the 84th day of the experiment, 172 survived until the 214th day. Of these, five only were found to be harbouring *Bact. aertrycke* in their spleens. It is of some interest to note that a considerable proportion of the mice, which were inoculated at the commencement of the experiment and survived until its close, were still infected. Of 31 of these mice, 10 yielded positive spleen-cultures.

The absence of any variation of the bacterial parasite in the direction of increased virulence, inferred from the absence of any wave of mortality during the prolonged period of observation, was confirmed by the results of virulence tests carried out on strains isolated from mice dying during the course of the epidemic. The results of these tests are recorded in Table XIV.

Table XIV.

Strain	Day of exp. on which isolated	Fate of mouse from which isolated*	No. of mice inoculated	No. of mice dying within 14 days
Orig.	—	—	30	7†
5/A 20	17	*D*	30	5†
5/A 33	58	*D*	30	1
5/A 35	60	*D*	30	3
5/A 54	104	*D*	30	0
5/A 56	113	*D*	30	2
5/B 16	58	*D*	30	4
5/D 11	17	*D*	30	3‡
5/E 26	101	*D*	30	2‡
5/E 27	102	*D*	30	0

* Died on day stated. † 2 mice gave negative post-mortem findings.
‡ 1 mouse gave negative post-mortem findings.

Discussion.

We may now pass to a comparison of certain of the results obtained in the various experiments which have been briefly recorded above.

The effect of differences in virulence of the infecting strains of *Bact. aertrycke* on the character of epidemics proceeding within a closed community is most readily judged by comparing the mean expectation of life of the mice exposed to risk in each of the five experiments.

In Table XV are set out the differences in the expectation of life for all possible pairs of experiments, the probable errors of these differences, and the ratio of each difference to its probable error.

Table XV.

Experiments compared	Difference in main expectation of life (days)	P.E. of difference	Difference/P.E.
1 and 2	11·14	1·113	10·01
1 „ 3	5·18	1·226	4·23
1 „ 4	10·21	0·865	11·81
1 „ 5	9·80	1·314	7·46
2 „ 3	5·96	1·158	5·15
2 „ 4	21·35	0·764	27·95
2 „ 5	20·94	1·250	16·75
3 „ 4	15·39	0·922	16·69
3 „ 5	14·98	1·352	11·08
4 „ 5	0·41	1·036	0·40

There is no appreciable difference between the mean expectation of life in Exps. 4 and 5. With each other pair the ratio of the difference to its probable error is greater than 4. The smaller differences, those between Exps. 1 and 3, and between 2 and 3, may be regarded as suggestive. In the case of each of the remaining pairs the ratios are high, sometimes very high, and the differences are clearly significant, in the statistical sense.

It happens that, in the case of Exp. 3, we have clear evidence that the strain employed to initiate the five epidemics was replaced, in the rôle of a killing agent, by a strain of greater virulence. From our present point of view this experiment may therefore be rejected, and with it disappear the differences of doubtful significance.

It seems safe to conclude that the epidemics of Exp. 2, initiated with a strain of high virulence, were significantly more severe than those initiated with the moderately virulent strain of Exp. 1, and that these in turn were significantly more severe than the epidemics of Exps. 4 and 5, initiated with strains of markedly low virulence.

There is a possible objection to our reading of the experimental facts which we must notice. It may be objected that our criterion of the range of "errors of sampling" of the partial expectations of life is imperfect. Suppose, for the sake of argument, that virulence—in the sense implied in this paper—were absolutely constant, then, owing to variations in the reactions of the hosts and in a great variety of environmental factors, there would of course be variations of the severity of the epidemic and consequently variations of the

mean lengths of life of the exposed to risk. It might well be that, in the progress of an epidemic, phases of high and low mortality would succeed one another—indeed we know that this happens—and that in one phase the scatter about the mean and of course the mean itself would differ much from the corresponding constants in another phase. Naturally the statistical tests we have applied in this paper would reveal significant difference between the constants. But, by hypothesis, these differences would not be due to variations of virulence. Is it not possible that our five experiments are merely different stages of a single observation?

The answer to that objection is we think provided by Tables V, VIII, XI and XIV. Were the suggested explanation true, there should be no correlation between the mortality experienced by the experimental populations and by the isolated test animals; these latter were not subjected to the influences which, by hypothesis, determined the phases of high or low mortality. The hypothesis of varying virulence, on the other hand, completely explains this correlation. It may be added—but we do not attach much importance to the observation—that the changes in severity of such a disease as Scarlet Fever, changes which can in no plausible way be related to environmental or host variations, seem to be congruent with the interpretation which best covers such experimental facts as here described.

We would emphasise that, in every case, there was a spread of infection from the inoculated mice to those exposed to risk. The strains of low virulence were not entirely without effect.

A point of some interest is brought out by a comparison between the mean expectation of life, in each experiment, and the proportion of the surviving mice which were found to be harbouring *Bact. aertrycke* when the experiment terminated. The relevant figures are set out in Table XVI. For convenience of comparison the experiments are arranged in ascending order of the mean expectation of life, that is in descending order of severity.

Table XVI.

Experiment	Mean expectation of life	Percentage of survivors harbouring *Bact. aertrycke*
2	31·27 ±0·733	79·21
3	37·23 ±0·896	79·82
1	42·41 ±0·837	70·05
5	52·21 ±1·013	9·02
4	52·62 ±0·271	9·74

It is clear that there is a high positive correlation between the severity of an epidemic and the proportion of infected survivors left after its subsidence. The point of particular interest is the low proportion of such infected individuals at the end of a mild, and slowly smouldering, epidemic. In the earliest paper dealing with the experimental study of epidemics (Topley, 1919), one of us suggested, on purely *a priori* grounds, that strains of bacteria possessing a low degree of virulence might spread widely among a population at risk during certain phases of an epidemic. The present series of experiments affords

no support for such a suggestion, so far as the spread of mouse-typhoid among a closed community is concerned. There is, at all events, no evidence that strains of low virulence produce numerous cases of non-fatal infection, associated with the presence of organisms in the spleen.

Finally, it is of some interest to compare, in each experiment, the mean expectations of life of the inoculated mice, and of those exposed to risk. The relevant figures are set out in Table XVII.

Table XVII. *Showing the mean expectations of life, limited to* 60 *days, of the inoculated and exposed mice in each experiment, the difference between these means, the probable error of this difference, and the ratio of the difference to its probable error.*

Experiment	Mean expectation of life (inoculated)	Mean expectation of life (exposed)	Difference	P.E. of difference	Difference/P.E.
1	19·82 ±1·054	42·41 ±0·837	−22·59	1·346	16·78
2	6·33 ±0·178	31·27 ±0·733	−24·94	1·304	19·13
3	44·34 ±1·123	37·23 ±0·896	+ 7·11	1·437	4·95
4	38·09 ±0·818	52·62 ±0·217	−14·53	0·846	17·17
5	38·94 ±1·046	52·21 ±1·013	−13·27	1·456	9·11

With the exception of Exp. 3, the mean expectation of life of the inoculated mice is significantly less than that of those exposed to risk, as, indeed, one would expect. The more severe the epidemic, the greater is this difference. In the case of Exp. 3, in which the mice were inoculated with a strain of low virulence, while a strain of high virulence appeared in each of the five cages during the course of the epidemic, the position is reversed. The inoculated mice, on the average, lived longer than their exposed companions. The difference is doubtfully significant, but it offers the suggestion that the inoculation of a relatively avirulent strain may have afforded some degree of protection against the later advent of a more virulent strain.

Summary.

Summarising the results obtained in this series of experiments, it would appear that, in the case of *Bact. aertrycke* infection spreading among a closed community of mice:

(1) The character and severity of the epidemic are mainly determined by the virulence of the infecting bacterial strain.

(2) Strains of high virulence may give rise to non-fatal persistent infections, maintaining their full virulence over long periods of time within the spleens of the infected mice.

(3) Strains of low virulence may persist for long periods of time within such closed populations, spreading to some extent among the mice at risk, causing occasional deaths, and leading to an appreciable number of latent infections.

(4) It would appear that virulence and power to spread are highly correlated.

(5) There is some evidence (Exp. 3) that an initially avirulent strain may, while spreading within a closed community, give rise to virulent variants, and so initiate a severe, explosive epidemic. The results obtained in Exps. 4 and 5 suggest that such a succession of events is by no means the invariable result of the introduction of a strain of low virulence among a susceptible population.

(6) There is a suggestion that infection with an avirulent strain may afford some degree of protection against subsequent infection with a highly virulent strain.

Such results as these do not, of course, prove that variations in virulence are an important factor in determining the course of naturally occurring epidemics, or in the long-continued experimental epidemics, maintained by a constant stream of susceptible immigrants, the character of which we have recorded in earlier reports. They do, perhaps, increase the *a priori* probability of this hypothesis. Its proof, or disproof, must await the results of an adequate study of the virulence of large numbers of strains, withdrawn at different times from a population suffering from a continued, and fluctuating, epidemic prevalence.

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