TRANSIENT FLUCTUATIONS IN THE RESISTANCE OF MICE TO INFECTION WITH *B. AERTRYCKE.*

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(With 6 Text-figures.)

During the past 3 years a given strain of *B. aertrycke* has been inoculated into mice at fairly frequent but irregular intervals, for the purpose of serving as a control in certain experiments which have not yet been published. Since analysis shows that the results are of some interest, it is considered advisable to report them separately.

Technique.

The strain of *B. aertrycke* used was obtained from an experimental epidemic about the year 1924. For 2 years or so after its isolation it was kept in narrow stab agar tubes and subcultured from time to time; for the last 3 years it has been subcultured monthly, the stab agar culture being incubated for 18 hours at 37° C., and then stored in a dark cupboard at room temperature. When a virulence test was to be made, the strain was seeded into 5 c.c. of casein broth, contained in a $\frac{5}{8}$ inch test-tube; after 24 hours at 37° C. the culture was diluted with sterile Ringer's solution till there were approximately 100 viable bacilli in 0·5 c.c. of suspension; this quantity was then inoculated intra-peritoneally into 20 mice. Counts of the viable bacilli were made on the actual suspension used for inoculation. During the first year the diluent used was sterile tap water, but for the last 2 years Ringer's solution has been employed. All mice that died during the next fortnight were examined post-mortem, cultures being taken from the heart's blood and spleen; mice that survived a fortnight were killed, and spleen cultures taken. No mouse was considered to have been specifically infected unless *B. aertrycke* was isolated from the tissues, and identified by agglutination.

The mice used for inoculation were taken from the laboratory stock, which was furnished by four or five different breeders. Parti-coloured mice only were used, generally of between 17 and 23 grm. in weight; on some occasions it was impossible to obtain all the mice of this weight, and the deficit was supplied usually by younger, rarely by older animals. Before the start of this enquiry, Lockhart (1926) investigated the possibility of variations between mice from different breeders in their susceptibility to infection with *B. aertrycke*, and failed to detect any significant differences in this respect; it does not therefore seem probable that the results to be recorded in this paper are likely to be due to the use of different strains of mice. The mice were fed on oats, supplemented

5 days in the week by a ration of autoclaved milk diluted with an equal quantity of water; over the week-end the milk was replaced by water.

The virulence tests were commenced towards the end of October 1926 and are recorded up to October 1929; for convenience October 18th, 1926, has been taken as the starting and October 17th, 1929, as the finishing point. The results are shown in Table I and Fig. 1.

Table I. *Results of successive inoculations of batches of* 20 *mice with a single strain of* B. aertrycke *over a period of* 3 *years.*

Stock culture.

Date	Dose*	Died	Specific deaths	Killed	Specifically infected	Total infected	Average expectation of life†
Oct. 18th, 1926–Oct. 17th, 1927.							
28. x. 26 ...	91	7	**7**	13	11	18	11·75
4. xi. 26 ...	50	16	**15**	4	4	19	9·0
18. xi. 26 ...	55	13	**13**	7	6	19	9·8
30. xi. 26 ...	30	13	**13**	7	5	18	9·25
16. xii. 26 ...	44	12	**12**	8	6	18	10·1
21. xii. 26 ...	31	12	**12**	8	5	17	9·6
28. xii. 26 ...	52	12	**12**	8	7	19	9·6
3. iii. 27 ...	72	18	**17**	2	2	19	7·3
18. iii. 27 ...	83	11	**11**	9	8	19	10·3
5. iv. 27 ...	45	19	**19**	1	1	20	6·4
10. v. 27 ...	163	17	**17**	3	3	20	8·0
3. vi. 27 ...	206	11	**11**	9	8	19	10·85
23. vi. 27 ...	90	11	**10**	9	7	17	9·55
8. vii. 27 ...	80	7	**7**	13	12	19	11·6
14. vii. 27 ...	80	13	**13**	7	7	20	8·65
21. vii. 27 ...	35	14	**14**	6	4	18	8·4
10. viii. 27 ...	44	6	**6**	14	9	15	12·05
13. x. 27 ...	32	5	**5**	15	9	14	12·45
Sum	1283	217	**214**	143	114	328	174·65
Arithmetic mean...	71·27	12·06	**11·89**	7·94	6·33	18·23	9·70
Standard deviation	—	—	**3·77**	—	—	—	—
Oct. 18th, 1927–Oct. 17th, 1928.							
20. x. 27 ...	110	14	**14**	6	6	20	8·9
4. xi. 27 ...	95	8	**8**	12	11	19	10·75
16. xi. 27 ...	58	13	**13**	7	7	20	8·5
7. xii. 27 ...	178	8	**8**	12	11	19	11·35
21. xii. 27 ...	107	14	**14**	6	5	19	8·35
4. i. 28 ...	160	16	**16**	4	4	20	9·4
19. i. 28 ...	128	13	**13**	7	7	20	10·2
27. i. 28 ...	112	15	**15**	5	5	20	9·2
9. ii. 28 ...	71	12	**12**	8	8	20	11·2
24. ii. 28 ...	83	13	**13**	7	5	18	9·15
8. iii. 28 ...	90	15	**15**	5	5	20	7·5
23. iii. 28 ...	91	18	**16**	2	2	18	8·95
12. iv. 28 ...	34	13	**13**	7	7	20	9·7
27. iv. 28 ...	71	11	**11**	9	9	20	10·45
15. v. 28 ...	92	12	**12**	8	5	17	9·65
24. v. 28 ...	36	9	**9**	11	9	18	10·7
13. vi. 28 ...	109	11	**11**	9	8	19	9·6
4. vii. 28 ...	114	15	**15**	5	4	19	7·0
19. vii. 28 ...	88	16	**16**	4	4	20	6·55
1. viii. 28 ...	109	12	**12**	8	7	19	9·15
15. viii. 28 ...	118	15	**15**	5	4	19	8·0
2. x. 28 ...	157	13	**13**	7	7	20	10·1
16. x. 28 ...	85	17	**17**	3	3	20	7·1
Sum	2296	303	**301**	157	143	444	211·45
Arithmetic mean...	99·84	13·17	**13·09**	6·83	6·22	19·31	9·19
Standard deviation	—	—	**2·45**	—	—	—	—

* Dose of actual living organisms injected. † In days.

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Table I—*continued.*

Oct. 18th, 1928–Oct. 17th, 1929.

Date	Dose*	Died	Specific deaths	Killed	Specifically infected	Total infected	Average expectation of life†
30. x. 28 ...	108	13	**12**	7	6	18	9·0
20. xii. 28 ...	118	18	**18**	2	2	20	7·85
22. i. 29 ...	147	14	**14**	6	6	20	8·65
26. ii. 29 ...	97	6	**6**	14	13	19	12·4
26. iii. 29 ...	163	14	**14**	6	6	20	8·65
16. iv. 29 ...	136	8	**8**	12	11	19	11·45
30. iv. 29 ...	90	9	**9**	11	8	17	11·2
8. v. 29 ...	92	15	**15**	5	4	19	7·95
28. v. 29 ...	60	14	**14**	6	5	19	9·2
4. vii. 29 ...	121	15	**15**	5	4	19	7·75
13. viii. 29 ...	89	20	**20**	0	0	20	5·4
26. ix. 29 ...	48	9	**9**	11	9	18	9·85
17. x. 29 ...	97	17	**17**	3	3	20	8·8
Sum	1366	172	**171**	88	77	248	118·15
Arithmetic mean...	105·1	13·23	**13·15**	6·77	5·92	19·08	9·09
Standard deviation	—	—	**3·99**	—	—	—	—

Summary for 3 years Oct. 18th, 1926–Oct. 17th, 1929.

Date	No. of experiments	Mice injec.	Dose*	Died	Specific deaths	Killed	Specifically infected	Total infected	Average expectation of life†
1926–7	18	360	1283	217	**214**	143	114	328	174·65
1927–8	23	460	2296	303	**301**	157	143	444	211·45
1928–9	13	260	1366	172	**171**	88	77	248	118·15
Sum	54	1080	4945	692	**686**	388	334	1020	504·25
Arithmetic mean	—	20	91·6	12·81	**12·71**	7·19	6·18	18·89	9·34
Standard deviation	—	—	—	—	**3·39**	—	—	—	—

It will be noted that though the average number of specific deaths was 12·7, there were numerous and often very marked deviations, reaching from 5 deaths on the one hand to 20 on the other. Such an extreme deviation as this is 4·9 times the standard error for deaths in the two samples, and is therefore very unlikely to be determined by errors of random sampling. It seems possible that these fluctuations might be caused by variations in dosage, variation in the virulence of the culture, or variation in the resistance of the mice.

Variations in dosage.

It was stated that the mice were injected with approximately 100 viable bacilli; the actual number injected varied, however, considerably, chiefly owing to the greater or less degree of growth in different batches of broth. In Table II the doses given are classified under four divisions, and the specific deaths associated with them are recorded. Examination of these figures shows

Table II. *Effect of dosage on virulence.*

Dose*	No. of exps.	Average no. of specific deaths
30–59	14	11·79 ± 3·41
60–89	11	13·73 ± 3·44
90–119	19	12·68 ± 3·34
120 and over	10	12·90 ± 2·91

that there is no very evident relationship between the size of the dose and the number of specific deaths that follow it. The same conclusion emerges from

* Dose of actual living organisms injected. † In days.

examination of Table I, in which it is seen that a low dose is frequently accompanied by a high number, and a high dose by a low number of specific deaths.

These results confirm those already obtained by Topley (1927) and by Lockhart (1926), both of whom have considered in some detail the effect of

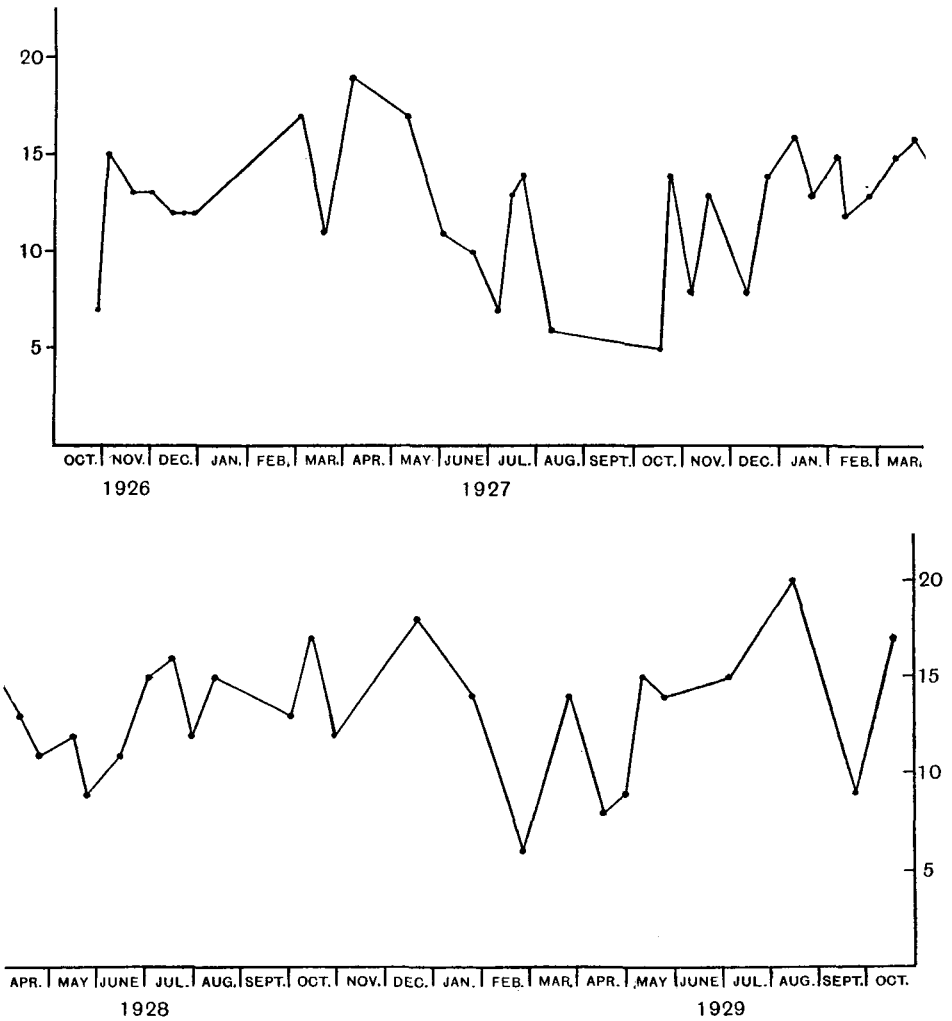

Fig. 1. The number of specific deaths in successive batches of 20 mice inoculated intra-peritoneally with *B. aertrycke*.

variations in dosage of *B. aertrycke*. Lockhart, for example, found that the average percentage mortality following the intra-peritoneal inoculation of mice with 10 million organisms was 90·75, whereas after a dose of only 10 organisms it was 30·75. That is to say a millionfold decrease in dosage sufficed to lower the percentage mortality only from about 90 to about 30 per cent.

Dealing with doses in the neighbourhood of those reported in this paper, evidence has already been brought (Wilson, 1930) to show that, with strains

of relatively high virulence, an increase in dosage from 100 to 1000 leads to not more than about two extra deaths in each batch of 20 mice. It would appear, therefore, that the variations in dosage obtaining in these experiments are not a sufficient explanation for the marked fluctuations which occurred in the number of specific deaths.

Variations in virulence of the strain.

In the absence of an animal of standard resistance, it is clearly impossible to decide from simple inoculation experiments whether fluctuations in the mean number of deaths are due to a change in virulence of the culture or to variations in susceptibility of the mice. The problem has to be approached in other ways. We propose to bring forward two pieces of evidence, both of which appear to indicate that the fluctuations in the number of deaths observed were not due, in any marked degree, to changes in virulence of the infecting strain. One of these we shall give now; the other will be more conveniently considered in a later section.

It was shown in a previous paper (Wilson, 1930) that four main types of *B. aertrycke* could be distinguished on the basis of morphology, colonial appearance, virulence to mice, and to a lesser extent of agglutinability with salts, acids and specific sera. The *A* or smooth virulent type, in a dose of 100, killed on an average about 14 mice out of 20; the *B* or smooth, weakly virulent type killed 1 or 2 mice; the *C* or rough, ichthyotic, weakly virulent type killed 2 or 3 mice; while the *D* or true rough type, usually failed to kill any mice at all. Admixture of *B*, *C* or *D* types with the *A* type had little effect on the virulence of this type, provided that more than a certain small number of organisms of this type were present in the inoculum. It was further shown that if a given strain of *B. aertrycke* was submitted to daily sub-cultivation under conditions that were known to lead to a loss of virulence, the virulent *A* type, of which the strain at first consisted, was gradually replaced by one or more of the less virulent types; and that not until the *A* type had been completely or almost completely replaced, did the virulence of the whole culture exhibit a marked fall.

In order to decide whether, in the particular experiments under consideration, the fluctuations in the number of deaths were determined by changes in virulence of the strain, we submitted the culture at each virulence test to an examination similar to that previously described (Wilson, 1930). The strain was examined on 24 occasions between April 1928 and October 1929, and on 20 of these it consisted purely of the *A* type; on the other four occasions it was mixed with relatively small numbers of the *D* type. The average number of specific deaths following inoculation of the 20 pure *A* strains was $13{\cdot}1 \pm 3{\cdot}48$; the average number of specific deaths following inoculation of the four $A + D$ strains was $13{\cdot}75 \pm 2{\cdot}95$. These figures do not suggest that the constitution of the strain underwent any serious alteration during the last 18 months of the experiment; and since the fluctuations in the number of deaths were as marked

during this period as during the preceding 18 months, there is no reason for attributing these fluctuations to changes in the virulence of the strain.

VARIATIONS IN SUSCEPTIBILITY OF THE MICE.

Several workers have recorded observations on seasonal fluctuations in the susceptibility of different animals to toxic products and to infection. Südmersen and Glenny (1909) give records of the minimal lethal dose of diphtheria toxin for guinea-pigs between January 1908 and July 1909. They found that during the summer a larger dose of toxin was required than during the winter. The maximum susceptibility of the animals was in January, the minimum in September. Hunt (1910) brought evidence to show that the resistance of mice to acetonitrile could be greatly modified by changes in the dietary. In general such substances as dextrose, oatmeal, liver or kidney greatly increased their resistance, while eggs, milk, cheese and various fats had the opposite effect. But even with mice fed on a standard diet there was a definite variation in resistance at different times of the year. In contrast to Südmersen and Glenny's experiments with diphtheria toxin and guinea-pigs, Hunt found that the maximum susceptibility of mice to acetonitrile was in August, the minimum in January. Pearce, Brown and van Allen (1924) found that the results obtained by the transplantation of a spontaneous malignant tumour of rabbits were subject to considerable variation; and they brought evidence to suggest that the season and weather at the time at which the experiments were carried out probably played an important part in determining the results. The figures obtained during the second year of the experiment were not altogether in consonance with those obtained during the first year, and the authors carefully refrain from expressing more than a tentative opinion on the seasonal nature of the fluctuations in the animals' resistance. Pritchett (1925, 1926) examined the resistance of four strains of mice from different breeders to intra-gastric inoculation of the mouse typhoid bacillus. The tests, which were performed monthly, were commenced in September 1923 and continued till September 1925. Marked fluctuations were observed in the death-rate at different times of the year; and though certain strains of mice showed greater variation than others, the general correspondence between the different strains was fairly close. Pritchett concluded that the fluctuations were of a definitely seasonal nature. The reason for this conclusion is not altogether clear. In Fig. 2 her curves expressing the average mortality of the four different breeds of mice during two consecutive years are superimposed. It will be seen that during the first year the mortality was low in the autumn, rose throughout the winter to attain a maximum in May, fell during June and July, and rose again to a peak in September. On the other hand, during the second year the mortality was high in the autumn, fell during the winter to a minimum in February, and rose again to a maximum in April, after which it remained fairly steady. Simple inspection of the curves is sufficient to show that there is little or no seasonal correspondence between the mortality in the two successive years. The results

are, however, of very great interest in demonstrating the fluctuations in death-rate that occur after inoculation with a given strain of different batches of mice kept under standard conditions.

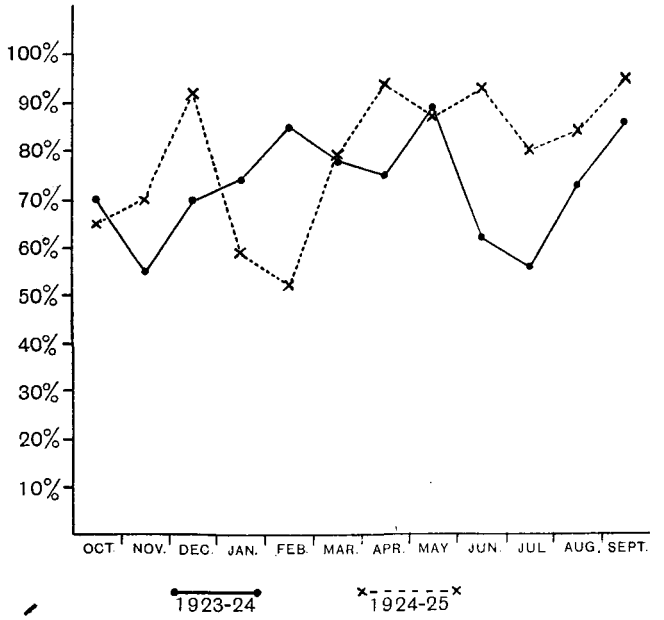

Fig. 2. Percentage of deaths following intra-gastric inoculation of mice with the mouse typhoid bacillus—Pritchett's experiments.

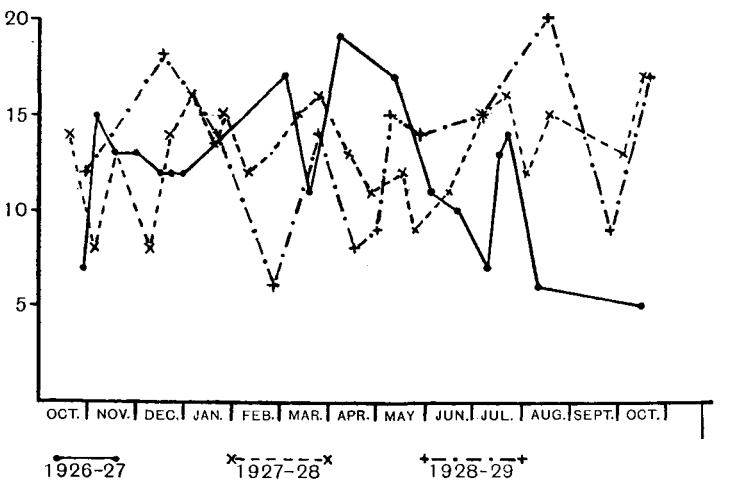

Fig. 3. The number of specific deaths in successive batches of 20 mice inoculated intra-peritoneally with *B. aertrycke*. Same figures as in Fig. 1, but curves plotted for separate years.

Our own results, concerning the intra-peritoneal inoculation of mice with *B. aertrycke* during three consecutive years, are plotted in Fig. 3, the curves for the different years being superimposed. Owing to the irregularity of the

intervals at which the tests were made, the fluctuations appear very marked. The general trend of each curve is better appreciated from Fig. 4, in which the average number of specific deaths over 3-monthly periods have been plotted. The results are very striking. During 1926–7 there was a high mortality in the early spring and a low mortality in the early autumn. (For the purpose of description we regard March to May as the spring months, June to August as the summer months, September to November as the autumn months, and December to February as the winter months.) During 1928–9 the conditions were reversed, the maximum mortality being in the autumn, the minimum in the spring. The results of the year 1927–8 were more or less intermediate, there being two maxima, in spring and autumn, and two minima, in winter and summer.

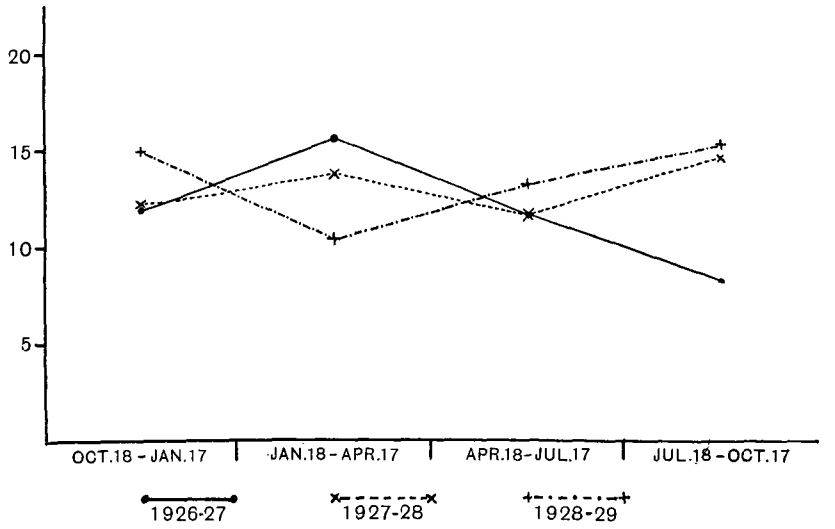

Fig. 4. The average number of specific deaths over successive 3-monthly periods in batches of 20 mice inoculated intra-peritoneally with *B. aertrycke.*

These curves demonstrate the injudiciousness of applying the term "seasonal" to fluctuations observed in the death-rate of animals submitted from time to time to given experimental procedures. The use of this term should be reserved for fluctuations which, even though quantitatively dissimilar, are yet alike in occurring with regular sequence in the same direction at corresponding periods of successive years. From this definition it follows that no conclusions can be drawn from results obtained in an experiment lasting over a single year. However apparent "seasonal" fluctuations may be in such an experiment, there is no justification for assuming that similar fluctuations would have occurred at corresponding periods of the following year or years. This point is very well exemplified by results recorded by Blake and Okell (1929) on the varying susceptibility of mice to intravenous injection of the toxin of *B. shigae.* The curve, showing the mortality of their mice during the year 1926–7, is plotted in Fig. 5, and superimposed on it is the curve obtained

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for the corresponding period in our own experiments. Since no estimation of the virulence of our culture was made from December 1926 to March 1927, we have interrupted our curve for this period by a dotted line. Considering that Blake and Okell were testing the toxicity of a dead product, while we were testing the virulence of a living bacterium; that the estimations were not made on corresponding days of each month; and that the number of estimations in any given month varied considerably, it is surprising that the two curves show such general correspondence. From their results Blake and Okell conclude tentatively that the fluctuations are probably of a seasonal type; but since their experiments were not repeated over another year, it seems clear that the use of the term "seasonal" is rather premature. It is entirely possible

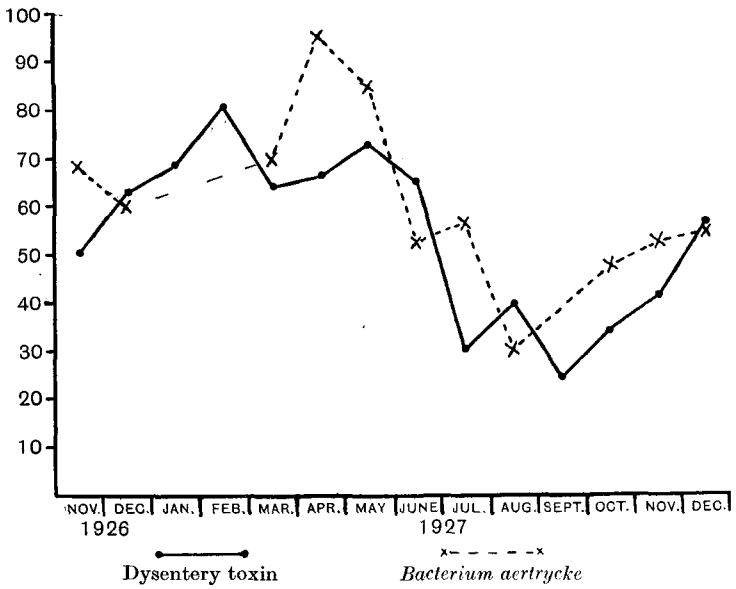


Fig. 5. Continuous line shows average percentage of deaths in each month amongst mice inoculated intravenously with dysentery toxin—Blake and Okell's experiments. Interrupted line gives similar figures for mice inoculated intra-peritoneally with *B. aertrycke*—author's experiments.

that if their experiments had been continued, they would have observed much the same lack of correspondence between seasonal fluctuations from year to year as Pritchett observed in her series, and we observed in ours. The same criticism applies to most of the other work that has been recorded on the so-called "seasonal" variation in susceptibility of animals to different experimental procedures. For this reason we prefer, for the moment, to refer to such variations as "transient fluctuations." It may be that if the diet, the age, the weight, and other variable factors in the animals under observation were more perfectly standardised, these fluctuations might really prove to be of a seasonal nature, but until this can be definitely shown, it seems wiser not to commit ourselves to a conclusion which is not supported by the evidence at present available.

It has already been pointed out that variations from time to time in the number of deaths following the inoculation of a given species of animal with a living bacterium may be ascribed to alterations in the virulence of the micro-organism or in the susceptibility of the animals; unless other evidence, necessarily of a circumstantial nature, can be adduced, it is impossible to decide which of these two factors is responsible. In dealing, however, with a poisonous chemical compound, such as acetonitrile, or a standard toxin such as that formed by the diphtheria or the Shiga dysentery bacillus, we are on surer ground, and it is fair to conclude that significant deviations from the mean number of deaths are attributable to an altered susceptibility of the animals under test. We shall now bring further evidence to suggest that the transient fluctuations in the death-rate in our experiments were likewise due to variation in the susceptibility of the mice.

FURTHER EVIDENCE IN FAVOUR OF VARIATION IN THE SUSCEPTIBILITY OF MICE.

During the progress of another experiment it became important to ascertain whether daily sub-cultivation under anaerobic conditions had any effect on the virulence of *B. aertrycke*. Accordingly an experiment was commenced, using the following technique: On June 13th, 1928, the stock strain of *B. aertrycke* was tested for virulence in the usual way, and was seeded into 7 c.c. of casein broth contained in a $\frac{3}{4}$ inch test-tube. This culture was then incubated anaerobically by a modification of the platinum black method originally described by Laidlaw (1915). Each day, except on Sundays, a fresh subculture was made. In case it should become contaminated, or be accidentally destroyed, the anaerobic culture was carried over in duplicate. Except for the fact that occasionally one tube leaked while the other did not, these two cultures were maintained under as nearly as possible identical conditions. Virulence tests were carried out at irregular intervals, the stock strain being tested simultaneously with the two anaerobic strains. The results up to September 26th, 1929, when the 400th subcultures were tested, are recorded in Table III and Fig. 6. Though frequent and marked fluctuations occurred in the mortality, there is no evidence to indicate that anaerobic cultivation under the conditions described had any permanent effect on the virulence of the strain. The most striking point, however, is that the fluctuations caused in the number of deaths at any one test occurred in the same direction with all three cultures. There is only one exception to this observation, namely on March 26th, 1929, when the deaths caused by the control culture and by *V* 42 went up, while those caused by *V* 43 came down. When it is considered that the stock culture was maintained under entirely different conditions from the anaerobic cultures, that it was kept in a stab agar tube, and was subcultured only once a month, and that when required for a virulence test it was grown aerobically for 24 hours in a $\frac{5}{8}$ inch test-tube containing 5 c.c. casein broth made from a different batch from that used for the anaerobic cultures, it is

remarkable that the results should show such a degree of parallelism. The odds against such a result being attributable to chance are impossibly high. It is difficult to conceive how cultures kept under such different conditions could have varied simultaneously in virulence; and one is forced to the conclusion

Table III. *Comparison of stock and anaerobic cultures.*

Date	*V* 41 stock		*V* 42 anaerobic		*V* 43 anaerobic	
	Dose	Specific deaths	Dose	Specific deaths	Dose	Specific deaths
13. vi. 28 ...	109	**11**	—	—	—	—
19. vii. 28 ...	88	**16**	32	**18**	65	**17**
1. viii. 28 ...	109	**12**	20	**11**	178	**10**
15. viii. 28 ...	118	**15**	185	**19**	177	**19**
2. x. 28 ...	157	**13**	375	**11**	405	**11**
16. x. 28 ...	85	**17**	87	**13**	126	**14**
30. x. 28 ...	108	**12**	128	**3**	120	**5**
20. xii. 28 ...	118	**18**	223	**16**	37	**17**
22. i. 29 ...	147	**14**	173	**12**	183	**15**
26. ii. 29 ...	97	**6**	123	**8**	157	**13**
26. iii. 29 ...	163	**14**	197	**17**	176	**10**
16. iv. 29 ...	136	**8**	97	**8**	111	**8**
28. v. 29 ...	60	**14**	184	**15**	316	**15**
13. viii. 29 ...	89	**20**	102	**18**	54	**19**
26. ix. 29 ...	48	**9**	245	**17**	390	**15**
Sum	—	**199**	—	**186**	—	**188**
Arithmetic mean...	—	**13·27**	—	**13·29**	—	**13·43**
Standard deviation	—	3·66	—	4·54	—	4·02

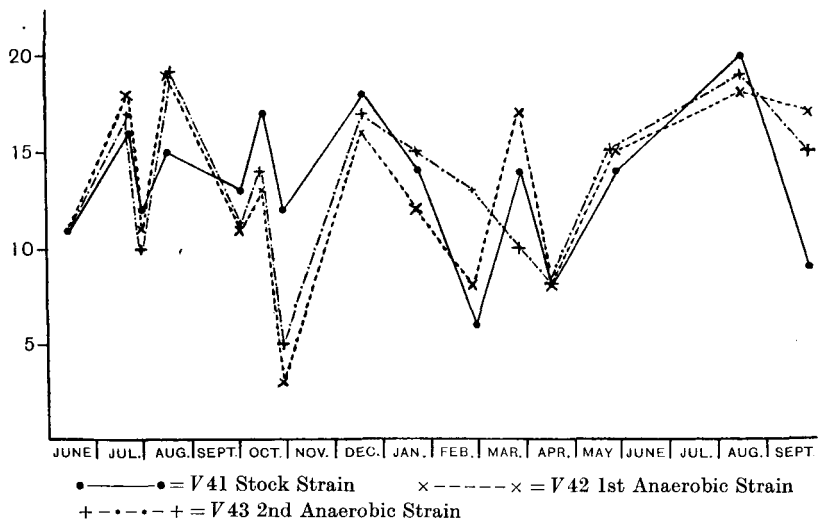


Fig. 6. The number of specific deaths in successive batches of mice inoculated intra-peritoneally with the stock strain of *B. aertrycke*, and with two separate anaerobic cultures of this organism.

that the fluctuations were due in great part to a varying susceptibility of the mice at successive tests.

The one factor that the stock and the anaerobic cultures had in common was that the dilutions for the virulence tests were all made in Ringer's solution. Though it is known that this solution is satisfactory for maintaining the

viability of the organisms for some hours (Wilson, 1922), it seemed possible that its composition might vary from time to time depending on the amount of salt precipitated during sterilisation, and that this variation might be associated with a deleterious effect on the organisms which were suspended in it. From the data available in these experiments there is no satisfactory method of testing the plausibility of this suggestion. It might be argued that if the Ringer had a nocuous effect at some times and not at others, the number of viable organisms in the suspensions used for inoculation would be fewer on the former and greater on the latter occasions. There should, in fact, be a parallelism between the doses of the three strains similar to that between the specific deaths. A graphic reproduction of the doses shows however very little parallelism between the stock and the anaerobic cultures. It might likewise be argued that if the Ringer was acting as a disinfectant, and was causing a decrease in the viable organisms, the deaths following injection of the lower doses would be less than those following injection of the higher doses. The results of examining the figures from this point of view are recorded in Table IV. Though the figures for some of the groups are very few, there is no indication that a low dosage is associated with a low death-rate.

Table IV. *Relation between the dosage and the number of specific deaths following inoculation of the stock strain V* 41, *and the two anaerobic strains V* 42 *and V* 43.

	Dose 20–59	Specific deaths	Dose 60–89	Specific deaths	Dose 90–119	Specific deaths	Dose 120 and over	Specific deaths
V 41	1	**9**	4	**67**	5	**63**	4	49
V 42	2	**29**	1	**13**	2	**26**	9	118
V 43	2	**36**	1	**17**	1	**8**	10	127
Sum	5	**74**	6	**97**	8	**97**	23	**294**
Arithmetic mean	—	**14·8**	—	**16·2**	—	**12·1**	—	**12·8**
Standard deviation	—	4·02	—	2·27	—	4·31	—	4·02

It is not easy therefore to explain the parallelism in the deaths caused by the three cultures under consideration by assuming that the Ringer used for preparing the inoculation suspensions varied from time to time in constitution. The possibility remains that, though the Ringer may not have caused a decrease in the number of viable organisms, it may yet have affected their virulence. This supposition is of such a nature as to render its proof or disproof a matter of the greatest technical difficulty, and it must for the present be regarded as a possible, though perhaps rather improbable, hypothesis.

It seems fair to conclude from this comparative experiment that the more or less parallel fluctuations which occurred in the deaths caused by the three strains under test were determined mainly by variations in the susceptibility of the mice.

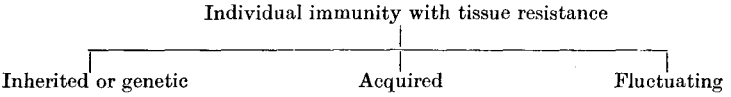
DISCUSSION.

Evidence has been brought forward to suggest that in an experiment lasting over 3 years the fluctuations in the number of deaths amongst samples of 20 mice, following the injection of a given strain of *B. aertrycke*, may probably

be attributed to a variation from time to time in the resistance of the mice rather than to changes in the virulence of the organism. Before enlarging on the significance of this concept, it would be as well to define the term "resistance."

This term is generally regarded as the converse of "susceptibility," but the exact connotation of either term is almost as vague as that of the term "virulence." We are not concerned here with considering that type of resistance to disease which is manifested by certain animals under natural conditions, but which does not depend on a true tissue immunity. As an example of this type we would quote the rabbit, which, though highly susceptible experimentally to the bovine type of tubercle bacillus, rarely, if ever, contracts tuberculosis under field conditions. Nor do we wish to consider the resistance possessed by an animal in virtue of its being a member of a herd. For a discussion of these types of immunity the reader is referred to a recent publication by Topley and Wilson (1929).

The type of resistance that we wish to examine is essentially that dependent on the tissues and relating to the individual animal. Under this heading it seems possible to recognise three subdivisions which we may represent in diagrammatic form as follows:



We would designate as inherited or genetic immunity that type of resistance which depends on the inherited characteristics, whether anatomical or physiological, of the species, race, or individual in question. Instances of this type of immunity are the resistance of Algerian sheep to anthrax (Chauveau, 1880), and of all animals so far tested to gonorrhoea.

Acquired immunity may be either active or passive, and each of these may develop naturally or as the result of an artificial stimulus. Thus active immunity may be acquired naturally by contact with infected animals, or it may follow some artificial procedure such as vaccination. Passive immunity may develop naturally, as by the ingestion of colostrum or milk, containing antibodies (Dalling, 1928), or it may result from the injection of immune serum.

The term "fluctuating immunity" is intended to refer to that type of resistance which, as the result of transient variations in the functional activity of various tissues or organs, resulting from the action of environmental factors, is subject to fluctuations from time to time. The greater resistance to mouse typhoid of mice fed on a full MacCollum diet, as compared with that of mice fed on a simpler diet of bread and pasteurised milk supplemented by an oatmeal and buckwheat mixture and dog biscuit (Webster and Pritchett, 1924), may be cited as an instance of the type of case which is here *envisaged.* Numerous other examples might be quoted such as the diminished resistance of diabetics to suppurative infections, the susceptibility to intestinal disturbances so common in visitors to hot countries and to hilly districts, the fall

in their natural resistance to anthrax of hens partly immersed in cold water (Pasteur, 1878), the effect of over-exercise in converting a latent infection of rats with *B. enteritidis* into an active one (Boycott and Price-Jones, 1926), and so on.

So far as our immediate problem is concerned it is necessary to assume that the fluctuating characters on which susceptibility depends are so responsive to certain undetermined environmental changes that the average resistance of various samples of mice is synchronously increased or lowered to a significant degree within relatively short periods of time, and under conditions of housing, feeding, and the like, which are not obviously subject to wide variations.

We would regard this fluctuating immunity as superimposed on the basal genetic, or the more incidental acquired immunity. The three types of immunity are necessarily bound up with each other, and the actual resistance possessed by any given animal at any given moment is the summation of these three types. Leaving aside the subject of acquired immunity, the point we wish to make is that a distinction must be drawn between the genetic resistance, which is common to all animals of the race or species, and the type of resistance which is subject to fluctuation from time to time with changing environmental conditions. On *a priori* grounds it is probable that variations in genetic immunity do occur between different animals of the same race or species, though what the extent of these variations may be, it is impossible to say. There seems to be no practicable method by which these variations may be determined. If, for example, we submit a batch of 20 mice to some experimental inoculation, and we find that 14 mice die and 6 survive, we are not justified in concluding that the 6 survivors owe their life to a greater degree of inherited immunity. If the same test could be repeated 3 months later on the same mice, it is quite possible that some of the previous survivors would die and that some of the mice which succumbed before would now survive. But a repetition of the test is impossible, because as soon as the mice have been injected they cease to be normal animals. Whatever the extent of variation in genetic immunity may be amongst animals of a single race or species, it is quite evident that it will not account for such instances as have been quoted in which the resistance of the animals has been raised by an improved diet or lowered by over-exercise. There is evidence that such fluctuations in immunity are relatively transient, and it therefore seems justifiable to suppose that they are determined by some temporary alteration in the physiological activity of the animal.

It seems not improbable that variations in this fluctuating type of immunity are concerned, in some small measure at least, with the epidemic prevalence of endemic diseases.

Of the mechanisms that determine this particular type of resistance we are mostly ignorant, but it is permissible to surmise that it depends on such factors as the state of the gastro-intestinal mucosa, the efficiency of the reticulo-endothelial system, the activity of the ductless glands, and the local blood supply to the tissues.

One other point must be raised in this connection. The term resistance or susceptibility is commonly used in relation to infection. Certain workers, however, have employed these terms in reference to toxins and drugs. There seems to be no objection to this extension of meaning provided the process or product against which the resistance is manifested is clearly stated. But caution is needed in this regard. So far as we understand Webster (1924) he appears to consider that the resistance of an animal such as the mouse to infection with *B. aertrycke* is determined by the same factors as are responsible for the resistance of the same animal to inoculation of *Cl. botulinum* toxin or of a general protoplasmic poison such as mercuric chloride. This view is based on the fact that when a group of mice were inoculated with *botulinum* toxin or with $HgCl_2$, and the survivors were subsequently submitted to infection with the mouse typhoid bacillus, these survivors were found to be more highly resistant than a control group of normal mice. It would appear that the preliminary treatment with toxin or with $HgCl_2$ acted like a comb, serving to weed out the less generally resistant members of the group; and in so far as this is true, it is justifiable to regard the resistance of the animals as non-specific. But over and above this there is a specific immunity which renders, for example, the rabbit susceptible to the bovine but not to the human type of tubercle bacillus. It is impossible to regard this specificity as being determined by the same factors as are responsible for protecting the animal against a general protoplasmic poison, such as $HgCl_2$.

In the particular experiments recorded in this paper the fluctuations from time to time in the number of deaths caused by the stock culture (Fig. 1) are greater than can be explained by chance. We have brought evidence to show that in all probability they were not caused in any large degree by variation in dosage or in the virulence of the culture. We are therefore led to conclude that the main cause of these fluctuations is a variation in the susceptibility of the animals under test. This conclusion is supported by the fact that in a comparative experiment, in which the virulence of three different cultures was tested simultaneously on several occasions, the fluctuations in the number of deaths almost invariably occurred in the same direction. From the way in which the mice were selected at random from the general stock, it is extremely difficult to believe that these fluctuations were due to a variation in the innate or genetic resistance of the animals. We would therefore attribute them to alterations in the "fluctuating immunity" of the mice, determined by changes in the environmental factors to which the animals were subjected.

Apart from the interest that these experiments have in suggesting that the propagation of certain epidemics may be in part determined by fluctuations in the resistance of the population at risk, they seem to have an important bearing on the interpretation of the results of virulence experiments in general. It is clear that if we wish to measure the absolute virulence of any given strain at different times, it is necessary to standardise the dosage, and to use animals of the same degree of resistance. The standardisation of the dosage within

reasonable limits presents little difficulty, but the selection of animals of uniform resistance presents a problem that at the moment appears frankly insoluble. If it is true that fluctuations in resistance occur from time to time even in closely in-bred stocks of mice, as appears probable from Pritchett's results, it is clearly impossible to select mice of standard susceptibility for testing. The outcome therefore of any virulence test will depend on the interplay of two factors, one or other, or both of which may be actively varying. The most we can do is to obtain some idea of the relative virulence of two or more cultures tested simultaneously. A similar difficulty has been already encountered in what is really a much simpler problem, namely the standardisation of insulin. Trevan and Boock (1926) found that the proportion of mice developing convulsions after the subcutaneous injection of a given batch of insulin varied from time to time; thus, on one date 0·0015 mg. of insulin per 20 grm. body weight of mouse convulsed 80 per cent., on another date only about 35 per cent. of mice. "For this reason," they conclude, "the mouse-convulsive dose can only be used for the comparison of the relative activities of two samples of insulin." In practice, they calculate the relative activity of two samples of insulin from the respective convulsion rates for the two samples when injected at the same time.

SUMMARY AND CONCLUSIONS.

1. The virulence of a given strain of *B. aertrycke* was tested on 54 occasions during the 3-year period, October 1926–October 1929. Each test was made by inoculating intra-peritoneally about 100 viable organisms into 20 parti-coloured mice, usually of 17–23 grm. in weight, drawn at random from the general laboratory stock. The average number of specific deaths caused by this procedure was $12{\cdot}71 \pm 3{\cdot}39$, but very marked deviations were noticeable, varying from a minimum of 5 to a maximum of 20. These deviations occurred without any observable regularity, and appeared to have no definite seasonal relationship. Evidence is brought to suggest that they were chiefly determined not by variations in the dosage or in the virulence of the strain, but by fluctuations in the resistance of the mice.

2. The meaning of the term "resistance" is discussed, and it is suggested that the term "fluctuating immunity" should be used to denote those variations in resistance which occur from time to time, and which are due to alterations in the physiological behaviour of the animal dependent on changing environmental conditions. This fluctuating immunity should be distinguished from innate, inherited, or genetic immunity on the one hand and from acquired immunity on the other.

3. It is pointed out that the existence of fluctuations from time to time in the susceptibility of animals to infection renders fruitless any attempt to obtain an absolute measure of virulence. The most that can be done is to compare the relative virulence of two or more strains injected simultaneously.

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