MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE. II.

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Experimental studies[1] indicate that strains of paratyphoid-enteritidis bacilli differ among themselves in virulence but that for each strain, virulence is relatively a fixed property. It seemed desirable, therefore, to test this point by a series of *per os* passages which would at the same time afford an opportunity to observe infection under more usual conditions and to analyze more accurately the relations existing between the host and the microbic invader.

EXPERIMENTAL.

Experiment 1.—The method adopted has been described elsewhere.[2] Mice weighing 16 to 18 gm. each, taken from the stock breeding room, were segregated two per jar. The six cultures used were obtained from the last intraperitoneal passage reported in the previous paper.[1] Twenty mice were used for each passage of every strain. The dose, 0.5 cc. *per os* of a 16 hour broth culture diluted 1:100, contained approximately 3,000,000 bacteria. The dead mice were autopsied and the cultures obtained from them were identified. From the first mouse to die of each series a blood culture from the heart was made, identified, and after 24 hours incubation, injected into another series of twenty mice. This procedure was repeated with each strain for a varying number of passages and was followed by a control passage with the original, unpassed stock culture. Then it was considered desirable to return to the cultures used for the first *per os* passages and conduct a duplicate series of passages, terminated as before, by a control passage with the original stock culture. Blood cultures were made on the last control series. The technique of this procedure has been described in a previous paper.[2]

Tables I to IV, which illustrate the methods of recording data in each series, show the duration of life and results of blood cultures

[1] Webster, L. T., *J. Exp. Med.*, 1923, xxxviii, 33.

[2] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 231.

following injection with *Bacillus enteritidis* and *Bacillus paratyphosus* B respectively. In Text-figs. 1 to 5 are plotted typical curves from each series and in Text-fig. 6 the curves for each strain are compared with one another and with the control Strain M. T. II.

TABLE I.

Per Os Passage. Strain B. enteritidis.

Passage No.	No. of bacteria per mouse.	No. of mice.	Duration of life after injection.	No. of survivors after 60 days.
			days	
1	4,000,000	20	5, 7, 8, 8, 9, 9, 9, 11, 11, 11, 11, 11, 11, 14, 16, 20*, 21, 39*	2
2	3,100,000	20	6, 8, 8, 9, 9, 9, 10, 12, 12, 12, 14, 15, 15, 18, 21, 22, 56*	3
3	3,400,000	20	5, 8, 8, 9, 11, 12, 13, 14, 19, 20, 21, 25, 26, 38, 40, 53, 55	3
4	3,000,000	18	5, 6, 7, 7, 8, 8, 8, 8, 9, 9, 9, 9, 9, 9, 10, 12, 12, 18	0
Control.	3,000,000	20	6, 6, 8, 8, 10, 10, 10, 10, 10, 11, 11, 12, 12, 13, 14, 15, 26	3
1 A	3,500,000	20	6, 8, 8, 8, 8, 8, 9, 9, 9, 9, 9, 10, 10, 10, 12, 15, 16, 28	2
2 A	4,000,000,	20	6, 6, 7, 7, 7, 8, 8, 8, 9, 11, 11, 11, 11, 11, 14, 26, 36	3
Control 2.	5,000,000	20	7, 8, 8, 8, 8, 8, 8, 8, 9, 10, 10, 10, 10, 11, 11, 11, 12, 12, 16, 29	0

* Autopsy cultures sterile.

TABLE II.

Per Os Passage. Strain B. paratyphosus B.

Passage No.	No. of bacteria per mouse.	No. of mice.	Duration of life after injection.	No. of survivors after 60 days.
			days	
1	4,000,000	19	18*, 19, 20*, 21†	15
1 A	5,000,000	20	13, 14†, 14†, 20, 22, 29†, 30	13
2 A	5,000,000	18	8, 14†, 16, 28	16
3 A	3,000,000	20	13, 21	18
Control.	3,000,000	20	6, 18, 19†, 25*	16

* Autopsy cultures sterile.

† Autopsy cultures not identified.

TABLE III.

Blood Cultures from Control 2 Series Mice Inoculated per Os with B. enteritidis on January 31, 1923.

Cage and mouse.	Cultured Feb. 1.	Cultured Feb. 3.	Cultured Feb. 5.	Cultured Feb. 7.	Cultured Feb. 9.	Cultured Feb. 14.	Cultured Feb. 21.	
1 A	−	1 col.	++	+++	Feb. 7. D.			
B	−	−	−	+	1 col.	++	Feb. 15. D.	
2 A	−	−	+++	Feb. 7. D.				
B	−	−	+++	" 7. "				
3 A	−	−	−	+	+++	Feb. 10. D.		
B	−	−	+++	++	Feb. 9. D.			
4 A	−	−	++	++	" 9. "			
B	−	−	+	+++	" 8. "			
5 A	−	−	++	Feb. 7. D.				
B	−	−	+	+	+++	Feb. 10. D.		
6 A	−	−	+++	Feb. 7. D.				
B	−	++	+++	" 6. "				
7 A	−	−	+	−	Feb. 9. D.			
B	−	−	+	−	++	Feb. 11. D.		
8 A	−	−	+++	Feb. 7. D.				
B	−	−	++	++	Feb. 9. D.			
9 A	−	−	+	+	1 col.	−	++	Feb. 28. D.
B	−	−	+++	Feb. 7. D.				
10 A	−	−	+	++	+++	Feb. 10. D.		
B	−	−	+	++	+++	" 11. "		

+ indicates 1 to 10 colonies; ++, 10 to 50 colonies; and +++, more than 50 colonies.

The tables and curves indicate that repeated *per os* passage does not alter the virulence of these strains. Strains M. T. I and *Bacillus enteritidis* are shown to have been most virulent and to act similarly.

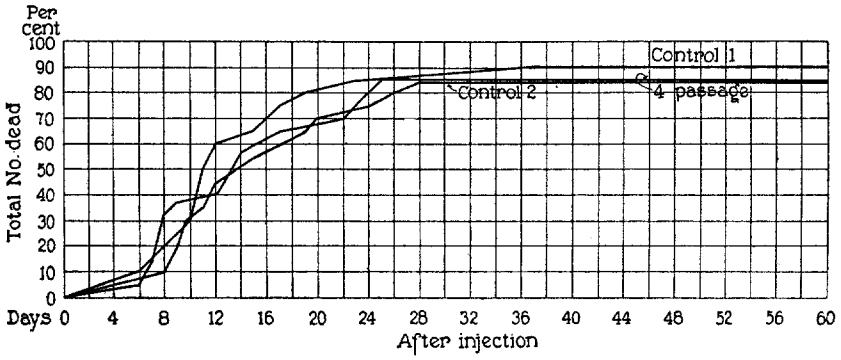
TABLE IV.

Blood Cultures from Control 1 Series Mice Inoculated per Os with B. paratyphosus B on February 8, 1923.

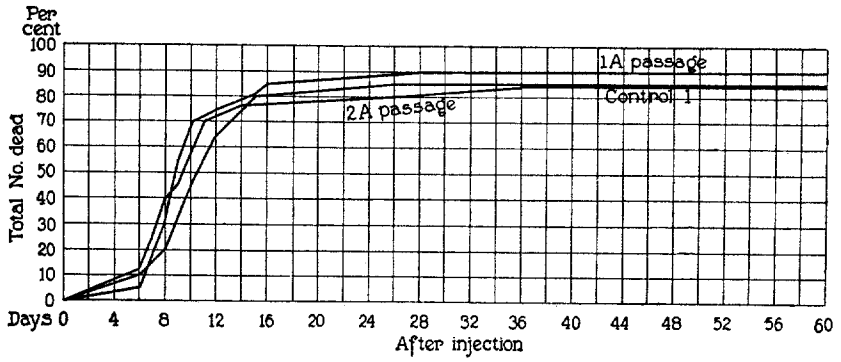
Cage and mouse.	Cultured Feb. 9.	Cultured Feb. 14.	Cultured Feb. 16.	Cultured Feb. 21.	Cultured Feb. 27.	Cultured Mar. 1.	Cultured Mar. 7.	Cultured Mar. 14.	Cultured Mar. 21.
1 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
2 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
3 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
4 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
5 A	—	—	—	—	Feb. 26. D.				
B	—	—	—	—	—	—	—	—	—
6 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
								1 col. M. T. I.	
7 A	—	—	—	—	—	—	Mar. 5. D.		
B	—	Feb. 14. D.							
8 A	—	+++	—	—	Feb. 27. Killed by accident.				
B	++	+++	—	1 col.	—	—	—	—	—
9 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
10 A	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—

+ indicates 1 to 10 colonies; ++, 10 to 50 colonies; and +++, more than 50 colonies.

After *per os* injection positive blood cultures developed promptly and 80 to 90 per cent of the mice died within 3 weeks. Strains M. T. II and *Bacillus aertrycke* (mutton) proved to be less virulent and behaved quite alike. About the same percentage of mice survived, some having shown persistently negative blood cultures, and a few having shown positive cultures and agglutinins. Strains *Bacillus*



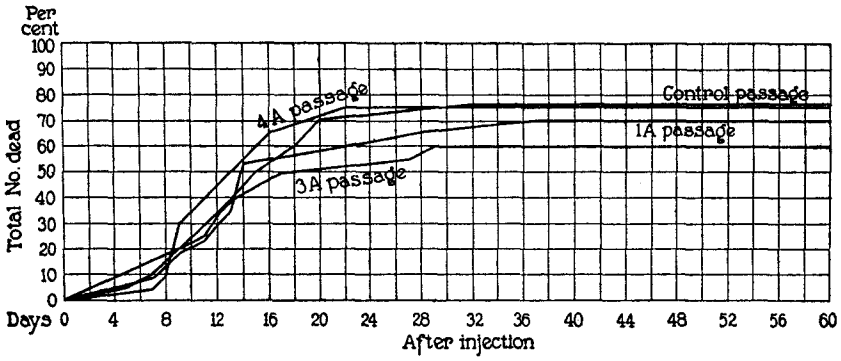
TEXT-FIG. 1. *Per os* passage of Strain M. T. I.



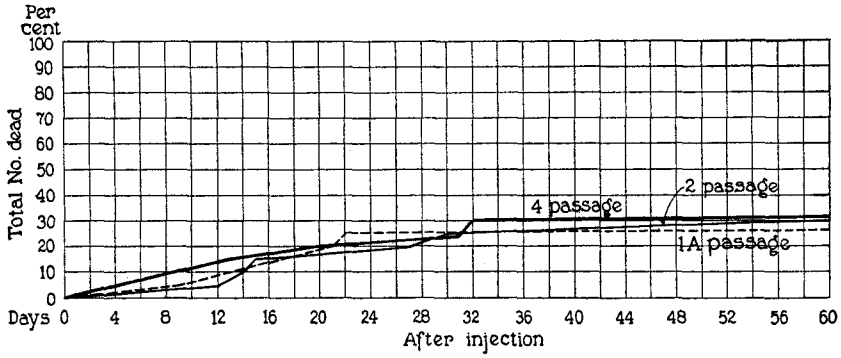
TEXT-FIG. 2. *Per os* passage of Strain *B. enteritidis.*

pestis caviæ and *Bacillus paratyphosus* B affected very few mice in any passage and usually did not invade the blood stream.

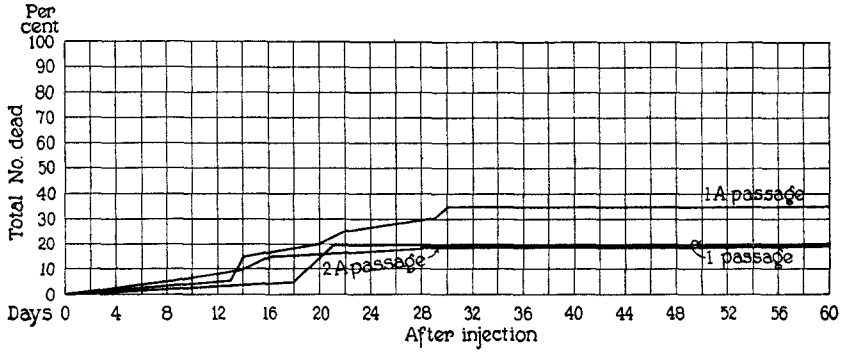
The fixed individual differences in susceptibility to the several pathogenic strains observed among the mice indicated that the reaction was of non-specific nature. In order to test this possibility a simple poisonous chemical compound was administered to a group of mice.



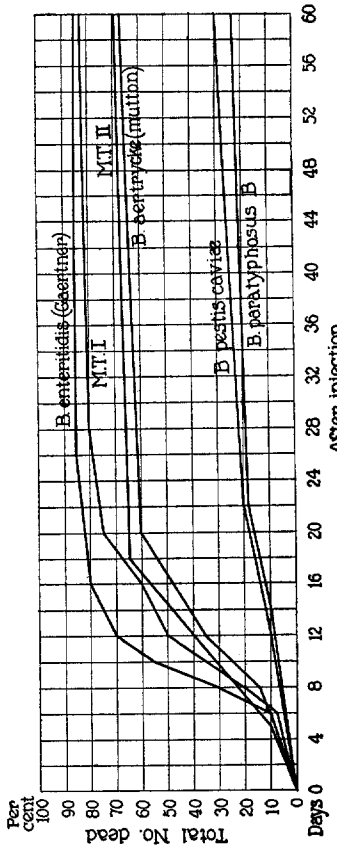
TEXT-FIG. 3. *Per os* passage of Strain *B. aertrycke* (mutton).



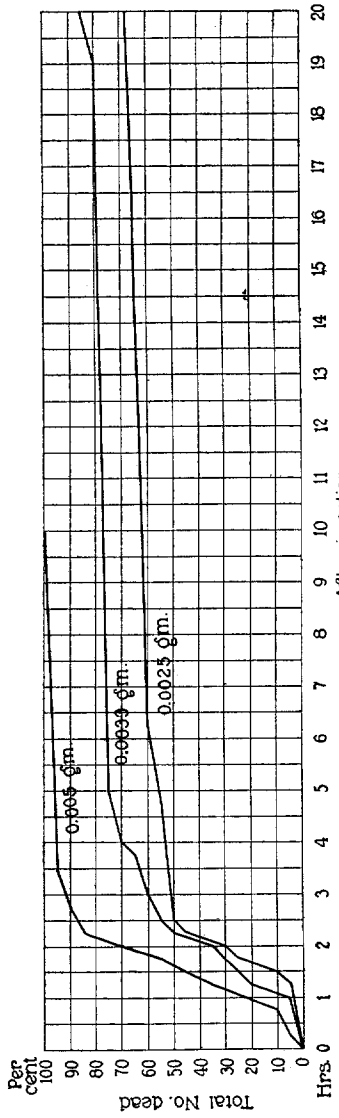
TEXT-FIG. 4. *Per os* passage of Strain *B. pestis caviæ.*



TEXT-FIG. 5. *Per os* passage of Strain *B. paratyphosus* B.



TEXT-FIG. 6. Standard mortality curve of six strains of the paratyphoid-enteritidis group.



TEXT-FIG. 7. Mortality curve following *per os* injections of mercury bichloride.

Experiment 2.—From a flask containing 1 gm. of mercury bichloride dissolved in 100 cc. of hot distilled water, subsequent dilutions were made in the usual bacteriological manner more to parallel biological technique than to attain chemical accuracy. The various doses in 0.5 cc. volume were administered *per os* by stomach tube to mice weighing about 16 gm. each.

Test 1: Two mice received 0.05 gm. each, two 0.005 gm., two 0.0005 gm., and two 0.00005 gm. The first two mice were dead in 5 minutes; one receiving 0.005 gm. died in 60 minutes, the other in 18 hours. The remaining mice survived 30 days.

Test 2: Two mice received 0.005 gm. each, two 0.0025 gm., two 0.00125 gm., two 0.0008 gm., and two 0.00061 gm. The 0.005 gm. mice died in 2 hours; the 0.0025 gm. mice were dead in 36 hours; the 0.00125 gm. mice were dead in 72 hours; the remainder survived 30 days.

Test 3: Twenty mice received 0.005 gm. each, twenty 0.0033 gm., and twenty 0.0025 gm. Text-fig. 7 shows the duration of life of these three series.

The mortality curve in Text-fig. 7, following 0.0033 gm. *per os* injection, is similar in form to that resulting from *per os* injection of Strain M. T. I or *Bacillus enteritidis* (Text-figs. 1 and 2), and the curve following the 0.0025 gm. injection resembles the M. T. II and *Bacillus aertrycke* (mutton) curves (Text-fig. 6). The comparison in the latter instance is of special interest since it has been shown previously[3] that the M. T. II curve (Text-fig. 6) may be superimposed upon the Amoss experimental epidemic curves.

Hence it appears that by an adjustment of the dose of mercury bichloride it is possible to secure mortality curves agreeing in form with those resulting from the *per os* injection of living bacteria.

DISCUSSION.

We wish now to correlate our findings with the results of earlier investigations along similar lines. Loeffler[4] and Danysz[6] made the first systematic studies on this group of bacilli in mice, rats, and guinea pigs. While it is true that their main purpose may be regarded as the perfecting of a method for the extermination of rodents, yet they and their successors incidentally or otherwise dealt with such

[3] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 269.

[4] Loeffler, F., *Centr. Bakt.*, 1892, xi, 129; xii, 1.

[5] Mereshkowsky, S. S., *Centr. Bakt., Ite Abt.*, 1895, xvii, 742.

[6] Danysz, J., *Ann. Inst. Pasteur*, 1900, xiv, 193.

questions as the natural spread of the infection in nature and the concurrent fluctuations of virulence among the bacilli.[4–20]

Perhaps it was Danysz who first emphasized the variations of the virulence in order to explain the rise and fall in mortality among the rodents exposed. He was followed by a number of investigators more or less upholding his views on this question, and lately Topley has also adopted this hypothesis as a partial explanation for the epidemic curves obtained in his studies of mouse typhoid.[21] There is, however, no real agreement in the opinions expressed, which range from statements that the virulence fluctuates[6,10,14,15,20] to statements that it remains constant,[13] or that the experimental results are irregular and not interpretable.[8,12,16,17]

We have been impressed with the lack of accuracy in respect to the constancy of dosage, and the small number of animals employed for the experimental tests. Hence our plan included using larger numbers of mice amounting in the end to about 1,400, and controlling the dosage of the bacilli. From the results, which in our case have been generally consistent, we believe that the following may be postulated:

First, the virulence of each of several strains of paratyphoid-enteritidis bacilli is apparently of fixed quantity, for mice at least. It would seem that since the strains used in the present experiments were originally isolated from man and several other animal species

[7] Klein, E., and Williams, H., *Lancet*, 1901, ii, 440.
[8] Kister, J., and Köttgen, P., *Deutsch. med. Woch.*, 1901, xxvii, 275.
[9] Krausz, A., *Deutsch. med. Woch.*, 1901, xxvii, 351.
[10] Bronstein, J., *Deutsch. med. Woch.*, 1901, xxvii, 577.
[11] Abel, R., *Deutsch. med. Woch.*, 1901, xxvii, 869.
[12] Rosenau, M. J., *Bull. Hyg. Lab., U.S.P.H., No. 5*, 1901.
[13] Issatschenko, B., *Centr. Bakt., 1te Abt., Orig.*, 1902, xxxi, 26.
[14] Markl, G., *Centr. Bakt., 1te Abt., Orig.*, 1902, xxxi, 202.
[15] Bahr, L., *Centr. Bakt., 1te Abt., Orig.*, 1905, xxxix, 263.
[16] Trautmann, H., *Z. Hyg. u. Infectionskrankh.*, 1906, liv, 104.
[17] Xylander, *Arb. k. Gsndhtsamte*, 1908, xxviii, 145.
[18] Bainbridge, F. A., *J. Path. and Bact.*, 1909, xiii, 443.
[19] Schern, K., *Arb. k. Gsndhtsamte*, 1909, xxx, 575.
[20] Steffenhagen, K., *Arb. k. Gsndhtsamte*, 1911, xxxvi, 198.
[21] Topley, W. W. C., *Lancet*, 1919, ii, 1; *J. Hyg.*, 1920–21, xix, 350; 1921, xx, 103. Topley, W. W. C., Weir, H. B., and Wilson, G. S., *J. Hyg.*, 1921, xx, 227. Topley, W. W. C., *J. Hyg.*, 1922, xxi, 10, 20.

and cultivated artificially for longer or shorter periods of time, there is good reason for supposing that the virulence of each for mammals in general is relatively a constant. It therefore seems permissible to exclude the factor of fluctuating virulence of the bacilli as explaining the epidemic mortality curve of mouse typhoid.

Next, it is clear from our studies that the different strains of paratyphoid-enteritidis bacilli vary among themselves in virulence for mice. Doubtless similar variations occur for other animals, including man. This inherent pathogenicity becomes, then, an important factor which may determine a severe outbreak of infection or the reverse among a susceptible group of animals. As the strains of high and low virulence may be antigenically identical, the transformation of one into the other cannot be predicated on the basis of bacteriological findings in sick or dead animals. In other words, to determine such transition, it is necessary to employ only accurately planned experiments in which the danger of substitution has been eliminated.

Finally, the experiments have brought out the fact, interesting and perhaps fundamental, that the degree of individual host susceptibility as exhibited by the mortality curve is a general property not restricted to the bacterial infections which we have been studying but inclusive of a chemical intoxication as, for example, with mercury bichloride. It is of importance to know that even when as few as twenty individual mice of a given age and weight, reared alike, are assembled, so small a group will contain individuals highly subject to infection or intoxication, as the case may be, individuals very resistant to the same injurious agencies, and individuals standing between these extremes.

CONCLUSIONS.

1. Six strains of paratyphoid-enteritidis bacilli, isolated from man and animals, differed markedly in virulence for mice.

2. The inherent virulence of each strain for mice was not affected by repeated intraperitoneal and *per os* mouse passage.

3. Individual differences in the susceptibility of the mice to each strain and to graded doses of mercury bichloride were demonstrable. Such variations appear to be of a non-specific nature.

We wish to thank Miss Allen Johnson for assisting with the technical work involved in the experiments reported in this and the preceding paper of the series.[1]