MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN MOUSE TYPHOID INFECTION.

By LESLIE T. WEBSTER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, August 25, 1922.)

An experimental epizootic of mouse typhoid caused by a member of the paratyphoid-enteritidis group, *Bacillus pestis caviæ*, has been studied by Flexner and Amoss at The Rockefeller Institute.[1,2,3] At the same time a number of tests were devised with various factors controlled or kept constant to analyze some of the observed phenomena.[4] It was shown that partial or complete protection of a general nature followed the administration of killed or living cultures intrapleurally, intraperitoneally, or *per os*. Also it appeared that after pleural or peritoneal injection, duration of life and type of disease were accurately related to dosage but that if the cultures were administered *per os*, duration of life was not so characteristically a function of dosage. Regardless of the number of organisms ingested, some animals proved refractory to infection by the gastrointestinal route, the normal portal of entry.

This latter observation would indicate that mice vary in their susceptibility to infection by *Bacillus pestis caviæ*; it throws light on the fact that throughout the course of mouse typhoid epizootics a certain percentage of animals survives, and it offers an admirable starting point for the study of disease as it occurs under natural conditions.

Sporadic infection as well as epidemic infection is concerned with three general variables, the distribution of the microbe, the virulence of the microbe, and the susceptibility of the host. With the first

[1] Flexner, S., *J. Exp. Med.*, 1922, xxxvi, 9.
[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25.
[3] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.
[4] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 71.

factor, the distribution and spread of the etiological agent, we shall not be concerned at present. But after the pathogenic organism has reached in proper quantity the usual portal of entry of its normal host, sporadic and epidemic infection will depend upon the balance between the second and third factors, microbic virulence and host susceptibility.

The following experiments, then, are an attempt to analyze the problems of infection by studying this equilibrium between microbic and host potentialities.

General Procedure.

Series of mice were injected *per os* by stomach tube with fixed doses of *Bacillus pestis caviæ*. At short intervals thereafter, the condition of the animals was noted, stools and blood were examined for the presence of the ingested microbe, the general character of the fecal flora was noted, and blood was tested for homologous agglutinins.

Technique.

White mice of uniform age and weight, obtained from the stock breeding room, were brought to the laboratory and maintained on the usual diet of bread and milk. To eliminate the contact factor and a possible reingestion of bacilli each animal was placed in a separate jar, strict cleanliness was observed in feeding and handling, and the jars were sterilized every week. Preliminary examination of blood and feces for bacteria of the paratyphoid-enteritidis group was always negative; in no cases were blood agglutinins demonstrable.

The strain of *Bacillus pestis caviæ* associated with the experimental epizootics[1,2,3] and employed in our earlier work[4] was used throughout these tests.

Stool cultures were examined in the following manner. The specimen of feces removed with care about 2 hours after feeding was emulsified with 5.0 cc. of salt solution in a sterile mortar. 0.2 cc. of this suspension was spread over a 14 cm. Petri dish containing plain agar plus 1 per cent dextrose. After 24 hours incubation the general character of the flora was noted and suspicious colonies were inoculated into plain broth. 24 hours later, agglutination tests were set up with

a formalinized broth suspension and immune rabbit serum with a titer of 1:20,000. Flocculation in a dilution of 1:10,000 was considered a sufficient criterion for the identity of the strain.[5]

It must be remembered that while a positive stool culture is reliable, a single negative test has little significance; at least three negative examinations are requisite for any definite conclusion.

Blood cultures were treated as follows: 0.02 cc. of blood from the tail vessels was spread over a dextrose agar Petri dish. After 24 hours incubation the plates were found either to be sterile or to contain the ingested microbe, usually in pure culture. Nevertheless, frequent agglutination tests were run as controls. The solid medium was found to be slightly more sensitive than fluid medium. Then, too, it allowed opportunity for rough quantitative estimates.

The amount of blood removed, 0.02 cc., corresponds to about $\frac{1}{100}$ of the total volume. This method is, therefore, about twice as accurate as that usually employed in human typhoid cases, and negative results indicate that very few if any organisms are circulating in the blood stream at that time.

Agglutination tests were performed about as described by Amoss.[3] A formalinized antigen was used; dilutions began at 1:20 and were doubled as far as 1:20,480.

Preliminary Experiments.

Experiment 1.—14 mice weighing 18 gm. each were placed in separate jars. On Mar. 13, 17, and 21, 1922, stool cultures showed no organisms of the paratyphoid-enteritidis group. On Mar. 22, agglutination tests were negative in dilutions of 1:20 and 1:40.

Mar. 22. The mice were given by stomach tube an 18 hour culture of *B. pestis caviæ* in a dilution of 1:100. This strain had been used frequently in other tests and was kept in stock on a plain agar stab.

At short intervals feces and blood were examined for the ingested organism, the general character of the flora was noted, the blood was tested for homologous agglutinins, and the condition of the animal was noted.

Individual protocols show the course of events (Table I).

It appeared immediately that the animals might be divided into at least three general groups, as follows: (1) Those which

[5] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

TABLE I.

Protocols of the Mice Used in Experiment 1.

Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.
	1922					
1	Mar. 21	A, few C*	0	——	0	Well.
	" 22	Bacilli ingested.				
	" 23	A, few C	0	0	0	Well.
	" 25	" " "	0	0	0	"
	" 27	" " "	0	0	0	"
	" 29	" " "	0	+ (1 col.).	0	"
	" 31	" " "	0	+	0	"
	Apr. 3	——	——	++	——	"
	" 6	C = A	0	+ (1 col.).	0	"
	" 12	" = "	+	0	0	"
	" 18	A, few C	0	0	——	"
	" 25	" " "	0	0	1:20 to 1:640 ++†	"
	May 9	" " "	0	0	1:20 to 1:640 ++†	"
	" 17	——	——	——	1:20 to 1:5,120 ++ 1:10,240—	"
	" 22	A, few C	0	0	——	"
2	Mar. 21	" " c	0	——	0	"
	" 22	Bacilli ingested.				
	" 23	A, few C	+	0	0	Well.
	1922					
4	Apr. 3	A	0	0	——	Sick.
	" 6	" = C	0	+ (1 col.).	——	"
	" 12	" = "	0	+ (1 ").	1:20 to 1:40 ++†	"
	" 18	" = "	++	0	——	Fair.
	" 25	" = "	0	0	1:20 to 1:640 ++†	Well.
	May 9	" few "	0	0	1:20 to 1:10,240 ++†	"
	" 17	——	——	——	1:20 to 1:10,240 ++ 1:20,480—	"
	" 22	A, few C	0	0	——	"
5	Mar. 21	" = "	0	——	0	"
	" 22	Bacilli ingested.				
	" 23	A = C	++	0	0	Well.
	" 25	" few " and c	+	0	0	"
	" 27	A = C	+	++	0	Sick.
	" 28	——	——	——	——	D.‡§
6	" 21	A = C	0	——	0	Well.

	Mar. 25	A, few c	0	0	0	Well.		Mar. 22	Bacilli ingested.				
	" 27	" " "	0	0	0	"		" 23	A = C	++	0	0	Well.
	" 29	" " "	0	0	0	"		" 25	" = "	+	0	——	"
	" 31	" " C and c	0	0	0	"		" 27	" = "	++	0	0	"
	Apr. 3	A, few C and c	0	0	——	"		" 29	"	0	+	0	Sick.
								" 31	C, few A and c	+	+	0	"
	" 6	A	0	0	0	"							
	" 12	C	0	0	0	"		Apr. 3	C = A	0	0	——	"
	" 18	A, few C	0	0	0	"		" 6	" = "	0	+ (1 col.).	0	Fair.
	" 25	" " "	0	0	0	"		" 12	" few "	++	0	0	"
	May 9	" " "	0	0	0	"		" 18	"	0	0	——	"
	" 22	" " "	0	0	——	"		" 25	——	——	0	0	"
								May 9	——	——	0	1:20 to 1:40 ++†	"
3	Mar. 21	" " "	0	——	0	"							
	" 22	Bacilli ingested.						" 16	——	——	——	——	D. ‡ §
	" 23	A, few C	++	0	0	Well.	7	Mar. 21	A, few c	0	——	0	Well.
	" 25	C " A	0	0	0	"		" 22	Bacilli ingested.				
	" 27	" " "	++	++	0	Sick.							
	" 28	——	——	——	——	D. ‡ §		" 23	A	0	0	0	Well.
								" 25	C, few A	0	0	0	"
4	" 21	A = C	0	——	0	Well.		" 27	A	++	0	0	"
	" 22	Bacilli ingested.						" 29	" few C	++	0	0	"
								" 31	" " "	++	0	0	"
	" 23	A = C	0	0	0	Well.		Apr. 3	" " "	0	0	——	"
	" 25	" = "	0	0	0	"		" 6	" " "	++	0	0	"
	" 27	" = "	0	0	0	"		" 12	" " "	0	0	0	"
	" 29	——	——	+	0	Sick.		" 18	"	0	0	——	"
	" 31	A = C	+ (1 col.).	++	——	"		" 25	" few C	0	0	0	"
								May 9	——	——	——	——	D. ‖

* C indicates colon colonies; c, coccus colonies; A, *acidophilus* colonies; ——, test not performed; 0, test negative; +, 1 to 10 colonies; ++, 10 to 50 colonies; +++, more than 50 colonies; −, test negative. † Further dilutions not made. ‡ Autopsy lesions typical of mouse typhoid. § Heart's blood culture positive. ‖ No lesions. Heart's blood negative.

TABLE I—*Continued.*

Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.	Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.
	1922							*1922*					
8	Mar. 21	A, few c	0	——	0	Well.	11	Apr. 12	A	0	0	0	Well.
	" 22	Bacilli ingested.						" 18	" few C	0	0	——	"
								" 25	" = "	0	0	0	"
	" 23	A, few c	0	0	0	Well.		May 9	" few "	0	0	0	"
	" 25	" " "	0	0	0	"		" 22	" = "	0	0	——	"
	" 27	" " C and c	++	++	0	Sick.	12	Mar. 21	" few c	0	——	0	"
	" 28	——	——	——	——	D.‡§		" 22	Bacilli ingested.				
9	" 21	A, few C and c	0	——	0	Well.		" 23	A, few c and C	++	0	0	Well.
								" 25	" = C = c	+	0	0	"
	" 22	Bacilli ingested.						" 27	A, few c " " "	0	0	0	"
	" 23	A, few c	0	0	0	Well.		" 29	"	0	+	0	"
	" 25	" " "	0	0	0	"		" 31	" few c	0	+	0	"
	" 27	" " "	0	0	0	"		Apr. 3	"	0	+	——	Fair.
	" 29	" " "	0	0	0	"		" 6	" few C	0	+	0	Sick.
	" 31	" = C	++	0	0	"		" 12		+	0	1:20 to 1:40	Fair.
	Apr. 3	——	——	0	——	"			" = "			++†	
	" 6	A	0	0	0	"		" 18	——	0	0	——	"
	" 12	"	0	0	0	"		" 25		——	0	1:20 to 1:640	"
	" 18	——	——	0	——	"			A, few C			++†	
	" 25	A, few C	0	0	0	"		May 9		0	0	1:20 to 1:10,240	Well.
	May 9	" " "	0	0	0	"						++	
	" 22	" " "	0	0	——	"			" " "			1:20,480 –	
								" 22	and c	0	0	——	"

10	Mar. 21	A = C	0	——	0	Well.	13	Mar. 21	A = C	0	——	0	Well.
	" 22	Bacilli ingested.						" 22	Bacilli ingested.				
	" 23	A, few c	0	0	0	Well.		" 23	A = C	0	0	0	Well.
	" 25	" " "	0	0	0	"		" 25	C, few A	+	0	0	"
	" 27	" " "	++	0	0	"		" 27	" " "	+	0	0	"
	" 29	" " "	0	0	0	"		" 29	" = "	0	0	0	"
	" 31	" " "	0	0	0	"		" 31	" = "	++	+	0	"
	Apr. 3	" = C	0	0	——	"		Apr. 3	A, few C	0	0	——	"
	" 6	"	0	0	0	"		" 6	"	0	0	0	"
	" 12	" few C	0	0	0	"		" 12	"	0	0	0	"
	" 18	" " "	0	0	——	"		" 18	"	0	0	——	"
	" 25	" " "	0	0	0	"		" 25	"	0	0	0	"
	May 9	" = "	0	0	0	"		May 9	" few C	0	0	0	"
	" 22	" = "	0	0	——	"		" 22	"	0	0	——	"
11	Mar. 21	" few c	0	——	0	"	14	Mar. 21	" few c	0	——	0	"
	" 22	Bacilli ingested.						" 22	Bacilli ingested.				
	" 23	A, few c	0	0	0	Well.		" 23	A, few c	0	0	0	Well.
	" 25	" " "	0	0	0	"		" 25	" = C	——	+ (1 col.).	0	Sick.
	" 27	" " "	0	0	0	"		" 27	——	——	0	0	"
	" 29	" " "	0	0	0	"		" 29	——	——	+	0	"
	" 31	" " "	0	0	0	"		" 31	——	——	++	——	"
	Apr. 3	"	0	0	——	"		Apr. 3	——	——	++	——	"
	" 6	"	0	0	0	"		" 4	——	——	——	——	D.§

either destroyed the bacilli at once or passed them with the feces for a period of time. Blood cultures and agglutination tests from these animals were persistently negative. The mice remained quite normal throughout the 8 weeks observation. (2) A group which, besides passing the bacilli with the stools for an irregular period of time, showed small numbers of specific organisms in the blood stream for 2 or 3 weeks. Agglutinins of high titer appeared after 3 weeks; the animals, after showing typical symptoms of mouse typhoid, recovered completely. (3) A third group which passed bacilli in large numbers with the stools, showed positive blood cultures, and, after the usual incubation period of 5 days, succumbed in varying intervals of time.

Mouse 13, which had a few bacilli in the blood on one occasion but no agglutinins, was a connecting link between Groups 1 and 2; similarly, Mice 6 and 14, which succumbed after the appearance of agglutinins, might be placed between Groups 2 and 3.

This experiment, then, analyzed the wide variations in host susceptibility to a fixed dose of *Bacillus pestis caviæ*.

It is fair to assume that during any epidemic the virus in question is at its highest level of virulence, that a vigorous strain has been selected which is well adapted to the biological medium of the host. It was therefore desirable that the virulence of the strain should be at its maximum, so that phenomena subsequent to its ingestion would be analogous to those occurring during an experimental epizootic of mouse typhoid. A rapid series of animal passages *per os* was then conducted with this aim in view.

Also, throughout this work, the strain of *Bacillus pestis caviæ*, isolated from the first animal to die in the above series of animal passages, has been transferred continually from series to series in the following manner. An 18 hour heart's blood broth culture diluted 1:200 is injected by stomach tube into at least ten mice weighing about 16 to 18 gm. each. The first animal to die is then autopsied and the heart's blood culture, after proper precautions, is injected into the next series, as described above.

Experiment 2 brings together for comparison and study, as Experiment 1, three series of normal mice injected *per os* with the active strain.

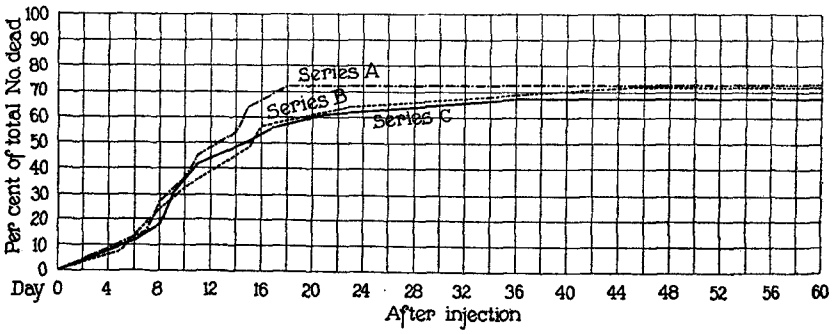
Experiment 2.—Series A: 16 normal mice from the breeding room, weighing about 18 gm. each, were placed in separate jars. The regular diet of bread and milk was continued. The animals were then given by stomach tube an 18 hour heart's blood broth culture, diluted 1:100, of the active strain from the first mouse to die in a routine passage series. Blood cultures were not taken.

Series B: 1 month later 12 normal mice from the breeding room were treated in the same way. Results of stool and blood cultures, agglutinin reactions, and condition of the animals were recorded.

Series C: 1 week later 11 normal mice from the breeding room were injected and studied in a similar manner.

Text-fig. 1 shows the mortality curves of the three series.

About 30 per cent of the mice in each series survived. They all belonged to Group 1, which passed the bacilli for a few days with the stools but showed no positive blood cultures and no agglutinins. They remained well throughout the entire 8 weeks of observation.



TEXT-FIG. 1. Mortality curves of the mice used in Experiment 2.

The striking similarity of the mortality curves (Text-fig. 1) leads one to regard them as being more or less constant under the given conditions; that is, as representing the balance between microbic virulence and collective host susceptibility. If reliable, they may explain many phenomena of experimental mouse typhoid epizootics and may change not a few unknown variables to constants in the mathematical study of epidemiology.

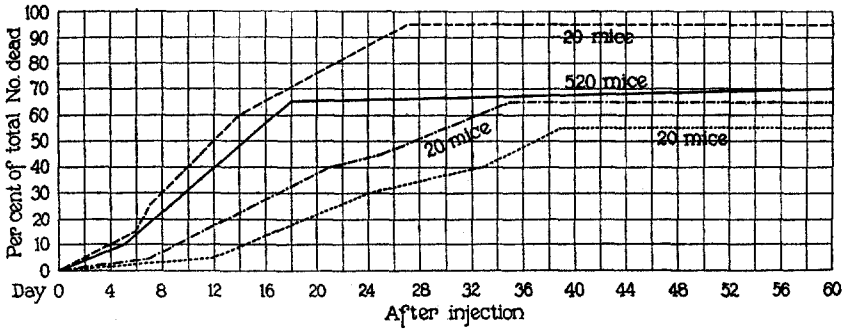
Therefore, we desired to become familiar with this curve and to determine any factors referable to host or bacteria which might alter its characteristics. By varying the host and keeping the microbic potentialities constant and later by varying the microbe and keeping

the host potentialities constant, the two factors responsible for these equilibrium curves were analyzed.

A standard host factor was arbitrarily chosen as being at least 10 mice from The Rockefeller Institute breeding room, of the same age, weighing 16 to 18 gm. each, fed on bread and milk, and living in separate glass battery jars. These mice at no time during this series of experiments showed bacilli of the paratyphoid-enteritidis group in their feces, nor did they show blood agglutinins for this group in a dilution of 1:20 to 1:80. A standard microbe factor was arbitrarily chosen as being a 16 to 24 hour heart's blood broth culture taken from the first mouse to die in any routine series such as described above. All mice and all infecting cultures, unless otherwise stated, conformed to the above specifications.

Host Factor Standardized, Microbe Factor Standardized.

It was hoped, of course, under the standard experimental conditions described above, that the mortality or equilibrium curves would



TEXT-FIG. 2. Variation in the mortality curves with the host and microbe factors standardized.

continue to be similar from series to series. And this has been true in ten series totalling 520 control mice. However, during June and July, two series of 20 mice each followed a more gradual curve and one series of 20 mice followed a more abrupt curve (Text-fig. 2). A possible explanation for these fluctuations will be discussed later, but since 90 per cent of the mice followed a similar death curve over

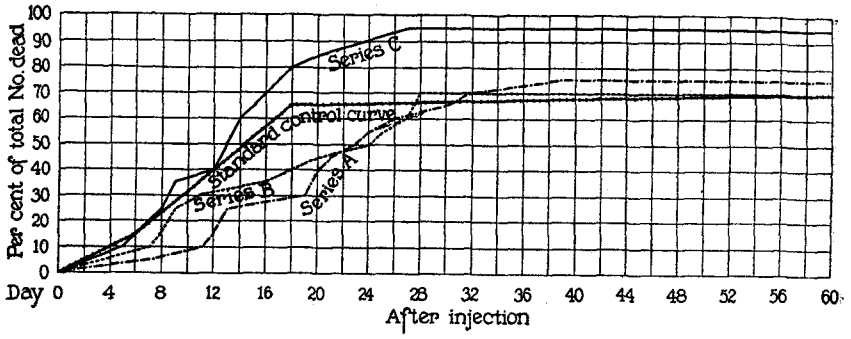
a period of 8 months and since there has been no persistent tendency to vary, the solid curve in Text-fig. 2 has been taken for a normal control.

Host Factor Varied, Microbe Factor Standardized.

The striking variation in susceptibility of mice to mouse typhoid infection has been indicated in the general work on epizootics.[2] Text-fig. 2 in this paper shows that about 20 to 30 per cent of the mice in any control series are refractory to infection *per os* and Experiment 1 reveals that these survivors may show no evidence of disease or may recover from the infection and develop agglutinins of high titer. This refractory state does not seem to be related to the normal intestinal flora;[6] it can, however, be somewhat modified by injuring the intestinal wall with ox bile.[7]

Experiment 3 was devised to bring out any possible variation in the mortality curve due to differences in age and weight of the mice employed.

Experiment 3.—20 mice, 6 weeks old, weighing 10 gm. each, called Series A, 20 mice, 1½ years old, weighing 27 gm. each, called Series B, and 20 mice, 12 weeks old, weighing 18 gm. each, called Series C, were placed in separate jars. They were all injected *per os* with an 18 hour heart's blood broth culture diluted 1:200 from the first mouse to die in a previous current series of 20. Blood cultures were taken at irregular intervals; agglutination tests were done after 6 weeks; the condition of the animals was noted; duration of life was recorded; and fatal cases were autopsied (Table II).



TEXT-FIG. 3. Mortality curves of the mice used in Experiment 3.

[6] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 21.
[7] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 33.

TABLE II.

Protocols of the Mice Used in Experiment 3.

June 22, 1922. Bacilli ingested.

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.	Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	June 23.	June 26.	June 30.	July 8.	July 18.	Aug. 14.	Aug. 14.		June 23.	June 26.	June 30.	July 8.	July 18.	Aug. 14.	Aug. 14.
A1	0	0	0	0	0	1:20 —	0	B13	0	0	0	0	D.*† July 10.		
A2	0	0	+ (1 col.).	+	D.*† July 12.			B14	0	0	0	0	0	1:20 —	0
A3	0	0	+ (1 ").	0	D.*† July 12.			B15	0	0	0	D.*† July 8.			
A4	0	+	++	D.*† July 5.				B16	0	0	0	+	0	D.*† July 20.	
A5	0	0	++	D.*† July 3.				B17	0	0	+++	D.*† July 1.			
A6	0	0	0	0	0	1:40 —	0	B18	0	0	+	D.*† July 1.			
A7	0	0	0	0	0	1:20 —	0								
A8	0	0	++	+	D.*† July 11.			B19	0	+	D.*† June 29.				
A9	0	0	0	0	0	1:20 to 1:320 ++	0	B20	0	0	0	+ (1 col.).	D.*† July 17.		
						1:640—		C1	0	0	+ (1 col.).	+	++	D.*† July 19.	
A10	0	0	0	+ (1 col.).	0	D.*† July 31.		C2	0	0	+	D.*† July 3.			
A11	0	0	+ (1 col.).	+	0	1:20—	0								
A12	0	0	0	0	0	D.*† July 24.		C3	0	0	+	++	D.*† July 9.		
A13	0	0	0	+	D.*† July 13.			C4	0	0	0	++	D.*† July 10.		
A14	0	0	0	0	0	D.*† July 22.									

A15	0	+	++	D.*† July 3.				C5	0	0	+	D.*† July 6.				
A16	0	0	0	+	D.*† July 15.			C6	0	0	+	D.*† July 5.				
A17	0	0	+	0	D.*† July 18.			C7	0	0	+	0	D.*† July 12.			
A18	0	++	D.*† June 29.					C8	0	0	D.*† June 30.					
A19	0	0	+	D.*† July 5.				C9	0	0	+	D.*† July 9.				
A20	0	0	+	0	D.*† July 16.			C10	0	0	+++	D.*† July 1.				
B1	0	0	0	0	+	D.*† July 20.		C11	0	0	0	+	++	D.*† July 19.		
B2	0	0	+	D.*† July 3.				C12	0	+ (1 col.)	D.*† July 1.					
B3	0	0	0	0	0	1:20−	0	C13	0	0	+	D.*† July 5.				
B4	0	0	+ (1 col.).	0	0	1:20−	0									
B5	0	0	++	+	++	D.*† July 19.		C14	0	++	D.*† June 28.					
B6	0	+	+ (1 col.).	0	0	1:20−	0	C15	0	0	D.*† June 30.					
B7	0	0	0	0	D.*† July 13.			C16	0	+	D.*† June 28.					
B8	0	+	D.*† June 30.					C17	0	0	0	0	0	1:20 −	0	
B9	0	0	0	0	0	1:20−	0	C18	0	0	+	D.*† July 7.				
B10	0	0	0	+	D.*† July 16.			C19	0	+++	D.*† June 28.					
B11	0	0	+ (1 col.).	+	+ (1 col.).	1:20−	0	C20	0	0	+ (1 col.).	D.*† July 6.				
B12	0	D.*† June 25.														

* Autopsy lesions typical of mouse typhoid. † Heart's blood culture positive.

TABLE III.

Protocols of the Mice Used in Experiment 4.

June 22, 1922. Bacilli ingested.

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.	Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	June 23.	June 26.	June 30.	July 8.	July 18.	Aug. 14.	Aug. 14.		June 23.	June 26.	June 30.	July 8.	July 18.	Aug. 14.	Aug. 14.
Penn. 1	0	0	0	0	0	D.*† July 26.		N. J. 6	0	0	+ (1 col.).	+ (1 col.).	D.*† July 13.		
" 2	0	0	0	0	0	1:20 —	0	" " 7	0	0	+ (1 ").	+ (1 ").	0	1:20 to 1:640++	0
" 3	0	0	0	0	0	1:20 —	0							1:1,280—	
" 4	0	0	0	0	0	1:20 —	0								
" 5	0	0	0	0	0	1:20 —	0	" " 8	0	0	++	D.*† July 1.			
" 6	0	0	0	0	0	1:20 —	0								
" 7	0	0	0	0	0	1:20 —	0	" " 9	0	0	++	D.*† July 5.			
" 8	0	0	0	0	0	1:20 —	0								
" 9	0	0	0	0	0	1:20 —	0	" " 10	0	+	D.*† June 29.				
" 10	0	0	0	+ (1 col.).	0	1:20 —	0								
" 11	0	0	0	0	0	1:20 —	0	" " 11	0	+ (1 col.).	++	+ (1 col.).	0	1:20 —	0
" 12	0	0	0	0	0	D.‡ July 21.		" " 12	0	+	++	D.*† July 1.			
" 13	0	0	0	0	0	1:20 —	0	" " 13	0	0	+	D.*† July 7.			
" 14	0	0	0	0	D.‡ July 9.			" " 14	0	0	+	0	0	1:20 —	0
" 15	0	0	0	0	D.*† July 16.			" " 15	0	0	0	+	D.*† July 13.		
" 16	0	0	0	+	D.*† July 18.			" " 16	0	0	+	++	D.*† July 10.		

Penn. 17	0	0	0	0	0	1:20 —	0	N. J. 17	0	0	+	+++	D.*† July 10.		
" 18	0	0	0	0	0	1:20 —	0								
" 19	0	0	0	0	0	1:20 —	0	" " 18	0	0	+ (1 col.).	0	0	1:20 —	0
" 20	0	0	0	0	0	1:20 —	0	" " 19	0	0	+	0	D.*† July 15.		
" 21	0	0	0	0	0	1:20 —	0								
" 22	0	0	0	0	0	D.‡ July 21.		" " 20	0	0	D.*† June 29.				
" 23	0	0	0	0	0	1:20 —	0	" " 21	0	0	+ (1 col.).	0	0	D. *† July 24.	
" 24	0	0	0	0	0	1:20 —	0								
" 25	0	0	0	0	0	D.*† July 22.		" " 22	0	0	D. *† June 30.				
" 26	0	0	0	0	0	D.‡ Aug. 1.		" " 23	0	0	+ (1 col.).	+ (1 col.).	0	D.*† July 28.	
N. J. 1	0	0	+	+	D.*† July 17.			" " 24	0	+ (1 col.).	+	D. *† July 5.			
" " 2	+	0	0	D. *† July 8.				" " 25	0	0	+	D.*† July 3.			
" " 3	0	0	+ (1 col.).	0	0	1:20 —	0	" " 26	0	+	+	D.*† July 6.			
" " 4	0	0	0	0	0	1:20 —	0								
" " 5	0	+ (1 col.).	+	D.*† July 9.				" " 27	0	0	0	0	0	1:20 —	0
								" " 28	0	0	+	+	0	1:20 —	0

* Autopsy lesions typical of mouse typhoid. † Heart's blood culture positive. ‡ No lesions. Heart's blood negative.

From Text-fig. 3 it may be seen that no series was similar to the standard control curve adopted from ten series totalling 520 young adult mice. Series C, which should correspond to the standard control curve, showed an unexplainable abrupt rise with a decided increase in normal mortality rate, while Series A and B, following the standard curve more closely, showed the flattening encountered during the early summer experiments. An explanation for these fluctuations will be discussed later. The curves, however, do present evidence that young, adult, and old mice of these experiments show no striking difference in susceptibility to mouse typhoid infection.

From Table II it may be seen that most of the survivors in Series A, B, and C showed no positive blood cultures, no agglutinins, and remained well throughout the experiment. Mouse A 9, although blood cultures were negative, showed agglutinins in low titer; Mice A 11, B 4, B 6, and B 11 showed positive blood cultures but no agglutinins.

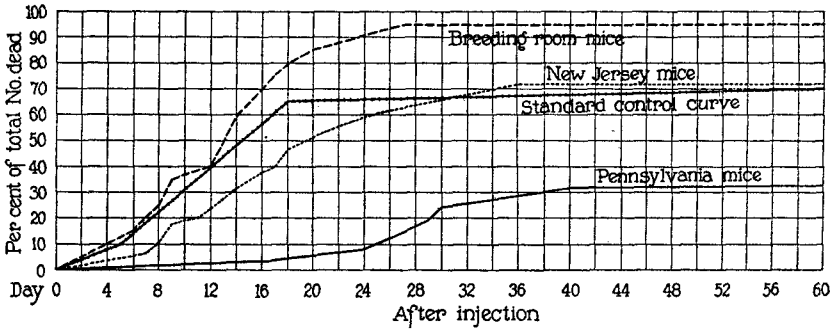
Experiment 4 was designed to bring out any possible variation in the mortality curve due to differences in breed of mice.

Experiment 4.—26 mice from a Pennsylvania breeder and 28 mice from a New Jersey breeder were placed in separate jars. Stool cultures, examined twice, were negative; blood cultures were negative; and agglutination tests were negative in dilutions of 1:20 and 1:40. The injection was carried out simultaneously with Experiment 3, consequently the same bacterial suspension and same controls were used. Subsequent procedures were similar to those employed in Experiment 3.

Text-fig. 4 shows that different breeds of mice vary in their susceptibility to mouse typhoid infection. While the New Jersey mice followed a curve quite similar to that of the 520 standard controls, the Pennsylvania mice were more refractory to infection.

Table III analyzes this difference. While nearly all the survivors in the Pennsylvania series showed negative blood cultures and agglutinins throughout the experiment, the New Jersey mice were less resistant. Not only did a much higher per cent succumb, but the survivors followed a different course. Nos. 4 and 27 were entirely refractory; Nos. 3, 11, 14, 18, and 28 showed positive blood cultures, but no agglutinins; No. 7 showed positive blood cultures and homologous agglutinins.

In brief, then, observation and experiment thus far have shown that 20 to 30 per cent of any series of mice taken from The Rockefeller Institute breeding room are not susceptible to mouse typhoid infection from a certain strain of *Bacillus pestis caviæ*. These mice may discharge the bacilli for a few days with the feces, but at no time are blood cultures positive nor are agglutinins demonstrable. An additional 5 or 10 per cent of the mice show symptoms of disease and positive blood cultures but survive and may later develop agglutinins. The remaining, approximately 70 per cent, pass the ingested bacilli *per rectum*, show positive blood cultures, and die in a more or less constant ratio, relative to time. Age appears not to affect susceptibility; source of stock or breed, on the other hand, is an important factor.



TEXT-FIG. 4. Mortality curves of the mice used in Experiment 4.

In general, susceptibility must be considered a relative and graded property, for although individuals appear to fall into rough groups, a study of many protocols will show occasional intermediate and varied responses to the bacteria by the host.

The agglutination phenomenon is no criterion of immunity; a surviving mouse may or may not give a positive reaction.

Microbe Factor Varied; Host Factor Standardized.

After becoming familiar with the general range of host variability, it was desired to vary the microbe factor and to determine its effect upon the mortality curves. The effect of dosage variation was first tried.

TABLE IV.

Protocols of the Mice Used in Experiment 5.

June 7, 1922. Bacilli ingested.

Mouse No.	Blood cultures.								Agglutinins.	Blood culture.
	June 8.	June 9.	June 10.	June 12.	June 17.	June 21.	June 27.	July 5.	July 18.	July 20.
Series 1.										
1, male.	+ (1 col.).	+ (1 col.).	+ (1 col.).	+	+	+	D. * † June 23.			
2, "	0	0	0	+	0	0	D. * † June 23.			
3, "	0	0	+	++	++	+	D. * † June 22.			
4, "	0	0	0	+ (1 col.).	0	0	0	0	D.* † July 15.	
5, "	0	0	0	++	D. * † June 18.					
6, "	0	0	0	0	+	0	0	+ (1 col.).	D.* † July 13.	
7, "	0	0	0	+	+	D. * † June 21.				
8, "	0	0	0	0	0	0	0	0	1:20 to 1:1,280 ++ 1:2,560 −	0
9, "	0	0	0	+	++	D.* † June 19.				
10, "	0	0	0	0	0	D.* † June 22.				
11, female.	0	0	0	0	0	0	0	0	1:20 −	0
12, "	0	0	0	0	++	D.* † June 19.				

13, female.	0	0	0	+	D.*† June 18.					
14, "	0	0	0	+	0	0	0	0	1:20 −	0
15, "	0	0	0	0	0	+	0	D.*† July 5.		
16, "	0	0	0	0	0	0	0	D.*† June 30.		
17, "	0	0	0	+	0	0	0	0	1:20 to 1:320 ++ 1:640 −	0
18, "	+ (1 col.).	++	+++	D.*† June 12.						
19, "	0	+	+++	D.*† June 12.						
20, "	0	0	0	0	0	0	0	0	1:20 to 1:80 ++ 1:160 −	0
Series 2.										
1, male.	0	0	0	0	+	++	D.*† June 23.			
2, "	0	0	0	+	+	+	+	++	D.*† July 10.	
3, "	0	0	0	+ (1 col.).	0	0	D.*† June 28.			
4, "	0	0	0	0	+ (1 col.).	+ (1 col.).	0	0	D.*† July 11.	
5, "	0	0	0	0	D.*† June 16.					
6, "	0	0	0	0	+ (1 col.).	+ (1 col.).	0	D.*† July 1.		

* Autopsy lesions typical of mouse typhoid. † Heart's blood culture positive.

TABLE IV—*Continued.*

Mouse No.	Blood cultures.								Agglutinins.	Blood culture.
	June 8.	June 9.	June 10.	June 12.	June 17.	June 21.	June 27.	July 5.	July 18.	July 20.
7, male.	0	0	0	0	++	++	D.*† June 27.			
8, "	0	+	+	0	0	0	0	0	1:20 —	0
9, "	0	0	0	0	0	0	0	0	D.* July 10.	
10, "	0	0	0	0	0	0	0	0	1:20 —	0
11, female.	0	0	0	+	++	++	+ (1 col.).	+	D.*† July 13.	
12, "	0	0	+ (1 col.).	0	0	0	0	0	D. July 18.	
13, "	0	0	0	0	0	0	0	0	1:20 —	0
14, "	+ (1 col.).	0	0	++	++	D.*† June 18.				
15, "	0	0	0	+	+	+	D.*† June 24.			
16, "	0	0	0	0	0	0	D.*† June 24.			
17, "	0	0	0	0	+	+	0	D.*† July 3.		
18, "	0	0	0	0	+	++	D.*† June 28.			
19,	0	0	0	0	++	D.*† June 19.				
20, "	0	0	0	+ (1 col.).	0	0	0	+ (1 col.)	D.*† July 7.	

Series 3.										
1, male.	0	0	0	0	0	0	0	0	1:20 —	0
2, "	0	0	0	0	0	0	0	0	1:20 —	0
3, "	0	0	0	0	+ (1 col.).	+	D.*† June 28.			
4, "	0	0	0	0	0	0	0	0	1:20 —	0
5, "	0	0	0	0	0	0	0	0	1:20 —	0
6, "	0	0	0	+ (1 col.).	+ (1 col.).	+	+	++	D.*† July 8.	
7, "	0	+	+	++	D.*† June 15.					
8, "	0	0	0	0	0	++	D.*† June 24.			
9, "	0	0	0	0	0	0	0	0	1:20 —	0
10, "	0	0	0	+ (1 col.).	+ (1 col.).	0	0	0	D.*† July 6.	
11, female.	0	0	0	0	++	++	D.*† June 26.			
12, "	0	0	0	0	0	+ (1 col.).	+	D.*† July 3.		
13, "	0	0	0	0	++	D.*† June 19.				
14, "	0	0	0	0	++	D.*† June 19.				
15, "	0	0	0	0	++	D.*† June 19.				
16, "	0	0	0	0	0	0	0	0	D.*† July 12.	
17, "	0	0	0	0	0	0	+	D.*† July 2.		

TABLE IV—*Continued.*

Mouse No.	Blood cultures.								Agglutinins.	Blood culture.
	June 8.	June 9.	June 10.	June 12.	June 17.	June 21.	June 27.	July 5.	July 18.	July 20.
18, female.	0	0	+ (1 col.).	++	D.*† June 14.					
19, "	0	0	0	0	0	0	0	0	1:20−	0
20, "	0	0	0	0	0	0	0	0	1:20 to 1:5,120++ 1:10,240−	0
Series 4.										
1, male.	0	0	0	++	D.*† June 15.					
2, "	0	0	0	0	0	0	0	0	1:20−	0
3, "	0	0	0	0	+	+	0	0	1:20 to 1:80 ++ 1:160−	D.*† July 24.
4, "	0	0	0	0	0	0	+	0	1:20−	0
5, "	0	0	0	0	0	0	0	0	1:20−	0
6, "	0	0	0	0	0	0	0	0	D.*† July 19.	
7, "	0	0	0	0	D.*† June 17.					
8, "	0	0	0	0	0	0	0	0	1:20 to 1:80 ++ 1:160−	0
9, "	0	0	0	++	D.*† June 15.					
10, "	0	0	0	0	+	+	+	D.*† July 4.		
11, female.	0	0	0	0	0	0	0	0	1:20−	0

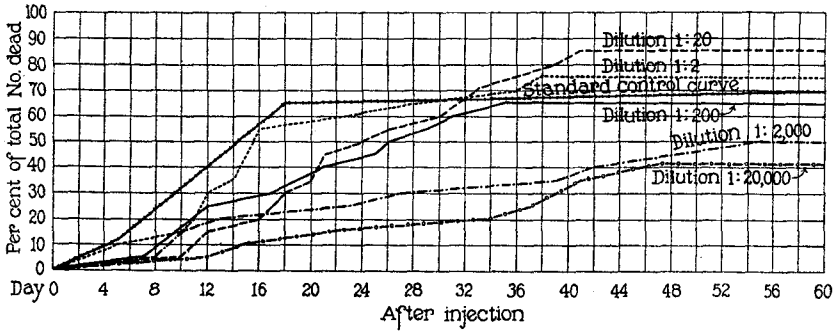
12, female.	0	0	0	0	0	0	0	0	D.*† July 16.	
13, "	0	0	0	0	0	0	0	0	1:20—	0
14, "	0	0	0	0	0	0	0	0	1:20—	0
15, "	0	0	0	0	0	0	0	0	1:20—	0
16, "	0	0	0	0	0	0	0	0	1:20—	D. Aug. 2.
17, "	0	0	0	0	0	D.*† June 20.				
18, "	0	0	0	0	0	0	0	0	1:20—	0
19, "	0	0	0	0	0	0	0	0	1:20—	0
20, "	0	0	0	0	+ (1 col.).	+	0	D.*† June 30.		
Series 5.										
1, male.	0	0	0	+ (1 col.).	+	D.*† June 22.				
2, "	0	0	0	0	0	0	0	0	1:20—	0
3, "	0	0	0	0	0	0	0	0	D.*† July 11.	
4, "	0	0	0	0	0	0	0	0	1:20—	0
5, "	0	0	0	0	0	0	0	D. June 29.		
6, "	0	0	0	0	0	0	0	0	1:20—	0
7, "	0	0	0	+ (1 col.).	+	+	+	0	D.*† July 14.	
8, "	0	0	0	0	0	0	0	0	1:20—	0
9, "	0	0	0	0	0	0	0	0	D. July 18.	
10, "	0	0	0	0	0	0	0	0	1:20—	0

TABLE IV—*Concluded.*

Mouse No.	Blood cultures.								Agglutinins.	Blood culture.
	June 8.	June 9.	June 10.	June 12.	June 17.	June 21.	June 27.	July 5.	July 18.	July 20.
11, female.	0	0	0	0	0	0	0	0	1:20—	0
12, "	0	0	0	0	++	D.*† June 19.				
13, "	0	0	0	0	0	0	0	0	1:20—	0
14, "	0	0	0	0	0	0	0	0	1:20—	0
15, "	0	0	0	0	0	+	0	0	1:100 to 1:3,200 ++ 1:6,400—	0
16, "	0	0	0	0	0	0	0	0	1:20—	D.*† July 24.
17, "	0	0	0	0	0	0	0	0	1:20—	0
18, "	0	0	0	0	0	0	0	0	D.*† July 18.	
19, "	0	0	0	0	0	0	0	0	1:20—	0
20, "	0	0	0	0	0	0	0	0	1:20—	0

Experiment 5.—100 mice from the breeding room, weighing 16 to 18 gm. each, were placed in separate jars. An 18 hour heart's blood broth culture from the first mouse to die of a previous current series was used for the injections. It was completely agglutinated in its homologous serum diluted 1:10,000; intraperitoneally it killed mice in 5 days at a dilution of 1:10,000; there were approximately 1 billion organisms per cc. in the broth culture.

Series 1, 10 males and 10 females, was given *per os* a dilution of 1:2 (0.5 cc. of the undiluted broth culture); Series 2, 10 of each sex, received a dilution of 1:20; Series 3, a dilution of 1:200; Series 4, a dilution of 1:2,000; and Series 5, a dilution of 1:20,000. Blood cultures were taken at short intervals; the condition of the animals was noted; agglutination tests were done after 5 weeks; duration of life was charted; and autopsies were performed on fatal cases.



TEXT-FIG. 5. Mortality curves of the mice used in Experiment 5.

Text-fig. 5 presents the results of this experiment in a striking manner. The curves of Series 1, 2, and 3 are grouped about the standard control curve and, except for a flattening, are roughly similar to it and to each other. The curves of Series 4 and 5, however, similar to each other, have a very gradual slope. It seems, therefore, that the massive lethal dose of a 1:200 dilution or less selects a relatively constant number of susceptibles, roughly 70 to 80 per cent of the total number used. And a dilution of 1:2,000 to 1:20,000 selects about 40 to 50 per cent. These observations, together with the results of Experiment 1, would indicate that the susceptibility of a population to mouse typhoid is a relative and graded property. Just how enduring this quality may be has not been determined, but so far it is clear that regardless of dosage 20 to 30 per cent of these mice are immune to *Bacillus pestis caviæ* infection *per os*.

TABLE V.

Agglutination Titers of the Cultures Used in Experiment 6.

Culture No.	Serum dilution.											
	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:20,480	1:40,960
1	++	++	++	++	++	++	++	++	++	++	+1	−
2	++	++	++	++	++	++	++	++	++	++	−	−
3	++	++	++	++	++	++	++	++	++	++	+1	−
4	++	++	++	++	++	++	++	++	++	++	−	−
5	++	++	++	++	++	++	++	++	++	++	+1	−

++ indicates complete agglutination; +1, marked agglutination, supernatant fluid not clear; −, no agglutination.

From Table IV it is seen that while the survivors of Series 2, 3, 4, and 5 fall regularly into the groups indicated above, those surviving the largest dose usually showed positive blood cultures and agglutinins.

Experiment 6 was planned to compare the infectivity of the current strain with carrier cultures, chronic septicemia cultures, and a laboratory stock culture which had not had an animal passage for 2½ years.

Experiment 6.—100 mice from the breeding room, weighing 16 to 18 gm. each, were placed in separate jars. 20 were injected *per os* by stomach tube with Culture 1, 4 billion organisms per cc., diluted 1:200; 20 were similarly injected with Culture 2, 3 billion organisms per cc.; 20 with Culture 3, 2 billion organisms per cc.; 20 with Culture 4, 1 billion organisms per cc.; and 20 with Culture 5, 8 billion

TABLE VI.

Intraperitoneal Titers of the Cultures Used in Experiment 6.

Culture No.	Broth dilution.				
	1:20	1:200	1:2,000	1:20,000	1:200,000
	days	*days*	*days*	*days*	*days*
1	1, 1	1, 3	3, 4	5, 40+	4, 8
2	1, 1	1, 3	3, 7	3, 7	5, 7
3	1, 1	1, 1	4, 4	3, 6	4, 40+
4	1, 1	3, 4	5, 5	4, 8	5, 8
5	1, 1	2, 3	6, 6	7, 8	7, 20

Two mice were injected for each dilution. The figures refer to the duration of life after injection. Survivors were discarded after 40 days.

organisms per cc. Agglutinations were done on the five cultures (Table V); a numerical estimate was made of the number of organisms per cubic centimeter in the 18 hour broth cultures; and intraperitoneal titrations were done (Table VI). The animals injected *per os* were observed daily. Blood cultures were taken; duration of life was recorded; and autopsies were performed on the fatal cases (Table VII).

Culture 1 was an 18 hour heart's blood broth culture from the first animal to die in a previous routine series of 20 mice. Culture 2 was an 18 hour tail blood broth culture from a mouse which had been injected *per os* 5 weeks previously. Cultures 3 and 4 were obtained from the stools of two separate mice which had been infected 5 weeks previously. Culture 5 was an 18 hour broth culture of the stock original strain which had not had an animal passage on artificial media for 2½ years.

TABLE VII.

Protocols of the Mice Used in Experiment 6.

June 30, 1922. Bacilli ingested.

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	July 1.	July 5.	July 7.	July 10.	July 17.	Aug. 15.	Aug. 15.
Series 1.							
1	+	+	+++	D.*† July 8.			
2	0	0	+ (1 col.).	+	D.*† July 16.		
3	0	++	+++	D.*† July 8.			
4	0	0	+	++	D.*† July 17.		
5	0	0	+	+	+	D.*† July 26.	
6	0	+ (1 col.).	0	+	D.*† July 13.		
7	0	++	D.*† July 7.				
8	0	0	0	+	0	1:20 —	0
9	0	0	0	0	0	1:20 —	0
10	0	++	++	++	D.*† July 11.		
11	0	+	++	D.*† July 10.			
12	0	+ (1 col.).	+	++	D.*† July 13.		

13	0	0	0	0	0	1:20 —	0
14	0	++	++	D.*† July 9.			
15	0	0	+	+	+	D.*† July 28.	
16	0	+ (1 col.).	+	0	0	1:20 —	0
17	0	0	+	+	D.*† July 17.		
18	0	0	0	0	0	1:20 —	0
19	0	0	+ (1 col.).	+	+ (1 col.).	1:20 —	0
20	0	+	++	++	D.*† July 13.		
Series 2.							
1	0	0	+ (1 col.).	+	D.*† July 14.		
2	0	0	+	+	D.*† July 13.		
3	0	0	+	D.*† July 10.			
4	0	+	+	+	+	1:100 to 1:400 ++ 1:800 —	0
5	0	+	+	+	D.*† July 15.		
6	0	0	+ (1 col.).	0	0	1:20 —	0
7	0	0	+ (1 ").	0	0	1:20 to 1:320 + 1:640 —	0
8	0	+++	D.*† July 6.				

* Autopsy lesions typical of mouse typhoid. † Heart's blood culture positive.

TABLE VII—*Continued.*

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	July 1.	July 5.	July 7.	July 10.	July 17.	Aug. 15.	Aug. 15.
9	0	++	D.*† July 7.				
10	0	0	+	+	D.*† July 13.		
11	0	0	0	+ (1 col.).	D.*† July 16.		
12	0	++	D.*† July 7.				
13	0	0	+ (1 col.).	0	0	1:20 to 1:640 ++ 1:1,280 −	0
14	0	0	+	+	0	1:20 −	0
15	0	+	D. *† July 6.				
16	+	0	+	+	0	1:20 to 1:1,280++ 1:2,560 −	0
17	0	0	0	0	0	D.*† Aug. 10.	
18	0	0	+	+ (1 col.).	+	1:20 −	0
19	0	0	+ (1 col.).	+	+	1:20 −	0
20	0	0	+ (1 ").	+	0	D.*† Aug. 7.	
Series 3.							
1	0	0	+ (1 ").	0	0	1:20 −	0
2	0	+	0	+	0	1:20 −	0

3	0	++	+++	D.*† July 8.			
4	0	++	D.*† July 8.				
5	0	0	+	D.*† July 10.			
6	0	+++	D.*† July 6.				
7	0	0	+ (1 col.).	0	+	D.*† July 28.	
8	0	D.*† July 6.					
9	0	0	+ (1 col.).	+	+	D.*† July 20.	
10	0	+	+	+++	D.*† July 13.		
11	0	0	0	0	0	1:20 —	0
12	0	+	0	+	+	1:20 —	0
13	0	+	++	D.*† July 10.			
14	0	+	D.*† July 7.				
15	0	+	+	0	D.*† July 13.		
16	0	++	+++	D.*† July 8.			
17	0	+ (1 col.).	0	0	0	D.*† July 24.	
18	0	0	0	0	0	1:20 —	0
19	0	0	+ (1 col.).	0	0	1:20 —	0
20	0	0	0	0	0	1:20 —	0

TABLE VII—*Continued.*

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	July 1.	July 5.	July 7.	July 10.	July 17.	Aug. 15.	Aug. 15.
Series 4.							
1	0	+	+	+	D.*† July 12.		
2	+	++	++	D.*† July 9.			
3	+ (1 col.).	+	D.*† July 10.				
4	0	0	0	+	+	D.*† July 24.	
5	0	0	0	0	0	1:20 —	0
6	0	0	+	++	D.*† July 14.		
7	0	0	+	+ (1 col.).	0	1:20 —	0
8	0	+ (1 col.).	+	D.*† July 10.			
9	0	0	+ (1 col.).	+ (1 col.).	0	1:20 —	0
10	0	0	+	+	+ (1 col.).	1:20 to 1:80 ++ 1:160 —	0
11	0	++	D.*† July 7.				
12	0	0	0	+	0	D.*† July 24.	
13	0	+ (1 col.).	+ (1 col.).	+	D.*† July 15.		
14	0	+	+++	D.*† July 8.			

15	0	+	+	++	D.*† July 14.		
16	0	0	0	+	D. *† July 16.		
17	0	0	0	+	0	1:20 —	0
18	0	+ (1 col.).	0	+	+	D.*† July 22.	
19	0	0	0	0	0	1:20 —	0
20	0	0	++	D.*† July 9.			
Series 5.							
1	0	0	0	0	0	1:20 —	0
2	0	+	+++	D.*† July 8.			
3	0	0	0	0	0	1:20 —	0
4	0	0	+	+	D.*† July 17.		
5	0	0	0	0	0	1:20 —	0
6	0	+	+++	D.*† July 11.			
7	0	0	0	+ (1 col.).	0	1:20 —	0
8	0	0	0	+ (1 ").	0	1:20 —	0
9	0	0	0	0	0	1:20 —	0
10	0	0	+	D.*† July 9.			
11	0	0	+	D.*† July 10.			
12	0	0	0	0	0	1:20 —	0
13	0	+ (1 col.).	+	+	D.*† July 13.		

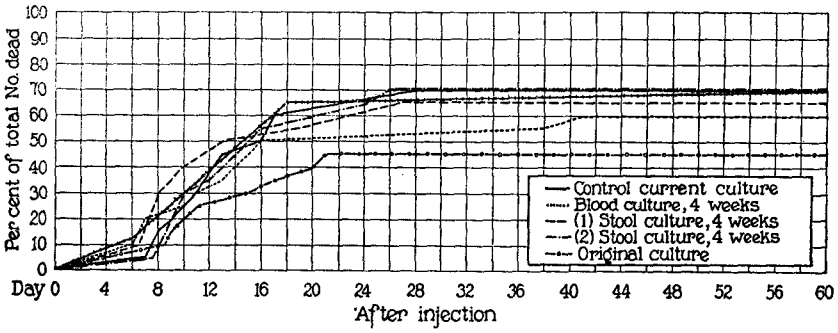
TABLE VII—*Concluded.*

Mouse No.	Blood cultures.					Agglutinins.	Blood culture.
	July 1.	July 5.	July 7.	July 10.	July 17.	Aug. 15.	Aug. 15.
14	0	+	++	D.*† July 8.			
15	0	+ (1 col.).	+ (1 col.).	0	0	1:20 –	0
16	0	+ (1 ").	+	+	+	D.*† July 20.	
17	0	0	0	0	0	1:20 –	0
18	0	0	0	+	0	1:20 –	0
19	0	+ (1 col.).	+	+	D.*† July 15.		
20	0	0	+	+	+ (1 col.).	D.*† July 21.	

The mortality curves of the current culture and the two stool carrier cultures follow the standard control curve very closely (Text-fig. 6). The series injected with the chronic septicemia culture and the series injected with the original stock strain show somewhat more gradual slopes.

The agglutination titer and intraperitoneal titer of all five series were about the same (Tables V and VI).

Table VII analyzes the host reaction to the various strains. The survivors of Series 1, 3, 4, and 5 showed for the most part no positive blood cultures and no agglutinins; the survivors of Series 2, however, usually showed positive blood cultures and agglutinins.

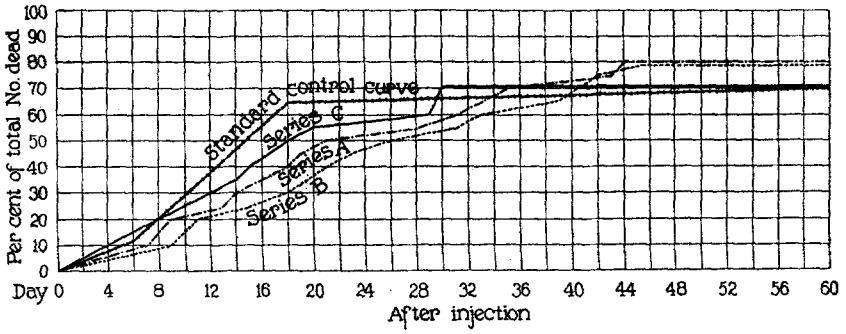


TEXT-FIG. 6. Mortality curves of the mice used in Experiment 6.

Experiment 7 was planned to compare the infectivity of a culture taken from an animal early in the disease with cultures taken shortly after death and 6 days after death.

Experiment 7.—A series of 10 mice was infected as usual with an 18 hour heart's blood broth culture from the first mouse to die of a preceding series. 4 days later, blood cultures on 6 of these mice were positive and a culture from 1 of the mice (No. 8) was used to infect 20 mice *per os* in a dilution of 1:200, 3 billion colonies per cc., and 8 mice intraperitoneally in dilutions of 1:100, 1:1,000, 1:10,000, and 1:100,000. 3 days later, or 7 days after injection, the original mouse (No. 8) died and was again cultured. The resulting culture, 10 billion colonies per cc., was injected into a new series precisely as above. The dead mouse was then kept aerobically at room temperature for 6 days and cultured again. The resulting culture, 1 billion per cc., was injected as above.

From Text-fig. 7 it may be seen that the mortality curves of these series vary but little from the standard control curve. Also the intraperitoneal virulence titration was similar in the three series.



TEXT-FIG. 7. Mortality curves of the mice used in Experiment 7.

DISCUSSION.

The concept of variation in host susceptibility to infection has had need of experimental demonstration and measurement.

Fluctuation of microbic virulence, on the other hand, has engaged the attention of bacteriologists and immunologists for the past decade and has occasioned an enormous amount of experimental study. Such investigation with microbes gaining entrance by an abnormal portal of entry to a foreign host or growing on artificial media results in considerable variation, but the few experiments detailed above, in which bacteria were used which gain entrance to their native host by the normal portal of entry, elicit so far, under natural conditions, but little evidence for change in bacterial virulence.

Concerning the unexplained fluctuations in the standard control curves, this much may be said. During the summer a small outbreak of mouse typhoid occurred among the normal breeding stock. And it was at this time that the mice used in the experiments detailed above followed more acute or more gradual mortality curves. Quite possibly, the animals may have been incipient cases or may have acquired some degree of resistance which affected the death rate. Evidence for this hypothesis will be presented at another time.

CONCLUSIONS.

Mice bred at The Rockefeller Institute vary in their susceptibility to mouse typhoid infection caused by a certain strain of *Bacillus pestis caviæ.*

This graded variation may be roughly analyzed 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.

The strain of *Bacillus pestis caviæ* employed throughout a 10 month series of experiments has shown no permanent change in virulence. Blood cultures taken from infected mice early in disease, shortly after death, and 6 days after death, chronic stool carrier cultures, and chronic septicemia cultures, all show approximately the same degree of virulence.