INHERITANCE OF RESISTANCE OF MICE TO ENTERIC BACTERIAL AND NEUROTROPIC VIRUS INFECTIONS

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Previous studies from this laboratory have shown that certain factors regulating the resistance of mice to naturally induced *Bacillus enteritidis* infection are inborn (1). The new data to be presented show that, under the experimental conditions, resistance to *B. enteritidis* and resistance to St. Louis encephalitis virus are inherited in a similar and relatively definite manner but independently.

Materials and Technique

The general technique employed in this work has been described (1). It was pointed out that following early experiments on small numbers of mice from the Rockefeller Institute breeding stock, a special breeding colony was started in 1929 with 600 of these animals which were tested and proved free of infection. The diet was changed from a bread and milk ration, producing mortalities following injection of mouse typhoid bacilli generally high (70 per cent), but fluctuating with season, to one producing low and more stable mortalities (37 $\pm$ 1.6 per cent). Finally, *B. enteritidis* rather than *B. aertrycke* was used as the test agent. These different experimental conditions, reflected in the different levels of mortality of the two control groups, permit early and later data to be compared qualitatively (1) but not quantitatively (Hill, 2).

The breeding stock was established and has remained free of the infections under investigation and others as determined by continued testing. At the outset it would have been preferable in selecting for possible resistant strains to proceed as with the susceptibles and choose for breeding, unexposed siblings of tested litters rather than the survivors themselves. This error was committed on but a single occasion, at the commencement of the experiment, however, and the rigid measures employed for detecting infected animals were known to be effective. Only the uninfected survivors were mated and the resulting litters tested, found to be uninfected, and then placed in the breeding room. Subsequently, no survivors have been used for breeding.

The mouse colony is subjected to continued search for infectious agents. The occasional sickly mouse is sacrificed and tested for the presence of pathogenic

bacteria or virus. Feces from breeders are tested six to twelve times per year for the presence of mouse typhoid. Susceptibles and resistants are housed together after weaning time. No infection has been found; hence Hill's intimation that typhoid may persist in our colony is without basis (2).

Further Development of Susceptible and Resistant Lines

In the previous paper (1) results were given on six selections and generations of susceptibles and four selections and three generations of resistants from 1929 to June, 1932. Unselected controls showed a relatively stable mortality rate of 37 per cent. Of susceptible lines, all save 1 and 2 were discarded. These had shown a relatively stable rate of approximately 85 per cent from the outset. Other lines subsequently discarded had shown increasing mortality rates with selection. Of resistant lines, only 1 and 2 were retained. These showed rates fluctuating about a 15 to 20 per cent average.

The results of further selective breeding of susceptible lines 1 and 2 and resistant lines 1 and 2 will now be described. Selections for breeding were made as previously, from litters unexposed to infection, thereby insuring against introduction or persistence of the infection in the stock. While other workers (3–8) have bred from survivors of a test infection, our method has been to remove the first and often the second litter from the breeding room at 4 to 6 weeks of age and test each with *B. enteritidis* or virus. If the mortality was maximum or minimum as required, an additional sibling litter was selected and mated brother to sister without testing. First and second litters of the succeeding generation were then tested in the same manner and a third litter chosen for mating. Tests were run as frequently as batches of mice of different generations and different lines became available. The crude results of these tests will first be described to show the chronological progress of the work.

Previous efforts (1) to increase susceptibility and resistance by a more rigid procedure of giving resistants a 10 to 1,000 times greater dose of *B. enteritidis* than susceptibles, commenced July, 1931, were discontinued Oct. 4, 1932, without noticeable success. This result was not surprising in view of earlier tests on mice injected *per os* which had shown that changes in dosage as great as 1,000-fold did not materially influence mortality rates (9).

Subsequent tests, nine in number, from Oct. 4, 1932, to June 2, 1933, in which the standard dose, 5×10^6 of *B. enteritidis*, was given *per os* to both susceptibles

and resistants, showed an abrupt increase in mortality rates of all lines. Susceptibles previously stable at 85 per cent rose to 95 to 100 per cent, resistants from 20 per cent to 50 or 60 per cent, and unselected mice from 37 per cent to 60 or 70 per cent. This disturbing variation was due in all probability to some unknown alteration of environmental factors.

Commencing Dec. 7, 1932, and continuing through Dec. 11, 1933, the bacteria-susceptible and bacteria-resistant lines were tested for their resistance to nasally instilled louping ill virus (10). The first six of these tests have already been reported (1). Of a total of twenty-one tests with different and similar doses within and between runs, twenty showed mortality rates of susceptibles less than those of resistants. Of the total 803 susceptibles tested, 49.4 per cent died as contrasted with 91.7 per cent of 775 resistants.

It will be recalled that selections of mice for breeding had been made entirely on the basis of maximum susceptibility or resistance of progeny to *B. enteritidis*. The susceptible and resistant lines which developed were later found to be susceptible and resistant respectively to *Pasteurella avicida*, *B. friedländeri*, and pneumococci administered intranasally (1). Accordingly we came to designate the former as bacteria-susceptible (BS) lines and the latter as bacteria-resistant (BR). But now, in 1932 and 1933, when it developed that the bacteria-susceptible lines were resistant to louping ill virus and the bacteria-resistant lines susceptible, it became necessary to complicate the terminology further and to identify the one as bacteria-susceptible-virus-resistant (BSVR) lines, and the other as bacteria-resistant-virus-susceptible (BRVS) lines.

Finding the mortalities of BSVR lines to louping ill virus averaging about 50 per cent and often concentrated entirely in certain litters led us to attempt to develop sub-lines highly resistant to virus (BSVR) and highly susceptible (BSVS) respectively. Parents whose progeny had shown least and greatest mortality following nasal instillation of louping ill virus were selected for further breeding; all others in these bacteria-susceptible lines were discarded. The resulting second litters were tested, further discarding of parents practiced, and a third mating made of parents whose litters showed greatest and least mortality to virus. The resulting litters were weaned, mated brother to sister, and their progeny tested with virus.

At this point it should be restated that no animals given test bacteria or virus were ever bred or placed in the breeding room. Matings were made invariably—with the single exception in 1930 previously recorded—from sibling litters of those tested, never exposed to any infection under investigation.

Litters from parents of the sixth generation of the bacteria-susceptible (BS) lines selected on the basis of susceptibility of progeny to louping ill virus were first tested Dec. 11, 1933, with 85.5 per cent of 69 so called virus-susceptibles (BSVS) dying, as contrasted with 25.8 per cent of 97 virus-resistants (BSVR). Subsequent tests were made with encephalitis virus, St. Louis type.

Encephalitis virus, St. Louis type, was obtained, prepared, and instilled intranasally into the test mice in the same manner as louping ill virus. Its distribution in the animal following this procedure, its clinical and pathological effects have

TABLE I

Comparative Mortalities of Unselected and Selected Lines of Rockefeller Institute Mus musculus albinus Mice Following Test Injections of B. enteritidis, Louping Ill, and Encephalitis, St. Louis Type, Virus

Date	Unselected			Bacteria-susceptible lines (BS)						Bacteria-resistant lines (BR)					
	Tests with *B. enteritidis*			Tests with *B. enteritidis*			Tests with virus			Tests with *B. enteritidis*			Tests with virus		
	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
1932															
Sept. 7				134[1]	107	79.9				82[1]	9	11			
" "				45[1]	38	84.4				43[1]	7	16.3			
" "				50[1]	38	76				37[1]	8	21.6			
Oct. 4				30[2]	29	96.7				71[2]	17	23.9			
Nov. 1				86	86	100				91	20	22			
Dec. 1				45	45	100				45	26	57.8			
" 7[A]							A_5[3]	3	60				5[3]	5	100
" 19							20[4]	8	40				20	12	60
" 27							15[4]	4	26.7				20	18	90
" "							15	3	20				20	16	80
" 29							15[5]	5	33.3				20	11	55
" "							15	6	40				20	8	40
1933															
Jan. 4				41	39	95.1				102	37	36.3			
Feb. 1	28	17	60.7	24	24	100				92	49	53.3			
" 25							40[6]	18	45				40	31	77.5
Mar. 2	30	22	73.3	146	140	95.9				131	70	53.4			
" 6							39[6]	9	23.1				38	36	94.7
" 16							24[7]	8	33.3				22	21	95.5
" "							31[8]	4	12.9				30	17	56.7
" 28							44[9]	37	84.1				45	45	100
" "							4[9]	2	50				4	4	100
Apr. 4	24	16	66.7							76	46	60.5			
" 20							72[4]	37	51.4				78	78	100
" 27							70[3]	34	48.6				175	173	98.9
May 2	22	14	63.6							52	19	36.5			
" 8							58[10]	34	58.6				55	55	100
" 18							39[11]	22	56.4				78	78	100
" 29							32[12]	23	71.9				60	58	96.7
June 2	19	10	52.6							76	33	43.4			
" 8							49[9]	35	71.4				45	45	100
July 6							67[9]	44	65.7						
" 27							47[4]	22	46.8						
Aug. 16							102[4]	39	38.2						
Oct. 13				15	14	93.3				20	11	55			

TABLE I—*Concluded*

Date		Bacteria-susceptible lines (BS)					
		Sub-line BSVS Bacteria-susceptible-virus-susceptible			Sub-line BSVR Bacteria-susceptible-virus-resistant		
		Tests with virus					
1933							
Dec. 11		69[4,A]	59	85.5	97	25	25.8
1934							
Feb. 2		67[7,B]	60	89.6	45	10	22.2
Mar. 12		82[13]	59	72	5	0	0.0
" 20		90	44	48.9	7	1	14.3
May 1		90	51	56.7	67	9	13.4
" 16		90	49	54.4	90	5	5.6
June 14		81	62	76.5	66	38	57.6
" 27		88	47	53.4	72	39	54.2
July 12		190	169	88.9	124	57	46
" 20		83	34	41	91	26	28.6
Sept. 21		83	72	86.7	149	39	26.2
" "		139	105	75.5	118	33	28
Oct. 3		118	95	80.5	99	68	68.7
" 24		156	129	82.7	124	54	43.5
" "		106	102	96.2	60	10	16.7

Explanation of Symbols

[1] Dose for susceptibles 10^5, for resistants 5×10^7.
[2] " " " and resistants in remaining tests 5×10^6.
[3] " " " " " 1/20.
[4] " " " " " 1/10.
[5] " " " " " 1/15.
[6] " " " " " 1/2.
[7] " " " " " 1/50.
[8] " " " " " 1/500.
[9] " " " " " 1/30.
[10] " " " 1/20, for resistants 1/50.
[11] " " " 1/10, " " 1/80.
[12] " " " 1/10, " " 1/120.
[13] " " " and resistants in remaining tests 1/100.
A = louping ill virus employed for virus tests Dec. 7, 1932, to Feb. 2, 1934.
B = encephalitis virus employed for remainder of virus tests.

been described fully elsewhere (11). In the first test 0.03 cc. of a 1 to 50 dilution of mouse brain virus was used (Table I); subsequently the dilution was 1 to 100 and to the best of our knowledge, all variables save the continued process of selection for maximum susceptibility and resistance to this virus were kept uniform. In fifteen tests comprising 1,532 BSVS and 1,214 BSVR mice, 74.2 per cent of the former and 34.1 per cent of the latter died. Variations between tests, however, were considerable.

TABLE II

Effect of Selection on Mortality Following Test Injections

Bacteria-susceptible (BS) line 2							Bacteria-susceptible-virus-susceptible lines (BSVS)												Bacteria-susceptible-virus-resistant lines (BSVR)											
			Progeny tested with *B. enteritidis*						Progeny tested with *B. enteritidis*						Progeny tested with virus						Progeny tested with *B. enteritidis*						Progeny tested with virus			
Generation	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead
1	1	4	11	59	43	72.8																								
2	2	7	22	97	81	83.5																								
3	4	18	31	167	146	87.4																								
4	8	35	25	130	118	90.8																								
5	16	41	43	212	158	74.5																								
6	12	25	26	136	131	96.3							8	17	25	90[L]	69	76.7							4	12	21	71[L]	7	9.9
																									1	3	3	12	0	0
7	2	3	3	12	12	100							13	34	48	190[L]	122	64.2							14	36	46	192[L]	22	11.5
													4	12	14	46	21	45.7							10	14	14	45	2	4.4
8	3	3	3	16	15	93.8							6	17	25	112[L]	99	88.4							7	10	10	33[L]	5	15.2
													14	37	51	233	146	62.7							14	32	49	166	49	29.5
9							7	8	8	22	21	95.5	11	42	73	351	311	88.6	4	7	7	19	18	94.7	12	34	59	240	50	20.8
10							10	15	18	48	48	100	22	53	78	347	289	83.3	10	13	15	38	37	97.4	15	36	50	174	30	17.2
11							4	11	11	29	29	100	8	17	17	56	50	89.3	5	13	13	37	35	94.6	15	33	34	121	18	14.9
12							5	15	16	35	35	100	5	15	16	39	38	97.4												
Totals.....				829	704	84.9				134	133	99.3				1,464	1,145	78.2				94	90	95.7				1,054	183	17.4

L = louping ill virus employed. No designation = encephalitis virus.

The data on early selections in this and other lines to be described (Tables III, IV, and V) are taken from tables previously published (1).

TABLE III

Effect of Selection on Mortality Following Test Injections

Generation	Bacteria-susceptible (BS) line 1												Bacteria-susceptible (BS) line 1 × 2											
	Progeny tested with *B. enteritidis*						Progeny tested with virus						Progeny tested with *B. enteritidis*						Progeny tested with virus					
	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead
1	1	7	24	136	119	87.5																		
2	3	10	18	72	60	83.3																		
3	11	34	65	295	258	87.5							3	9	9	47	34	72.3						
4	16	76	52	270	207	76.7							1	5	3	18	16	88.9						
5	19	48	54	270	215	79.6							4	13	13	79	71	89.9						
6	11	19	19	119	104	87.4													2	5	6	26[L]	4	15.4
																			1	1	1	2	0	0
7	2	6	6	36	36	100	3	5	7	21[L]	20	95.2							1	3	3	16[L]	5	31.3
							1	1	1	3	3	100							1	2	3	4	1	25.0
8	2	5	5	29	26	89.7	3	6	8	33[L]	31	93.9												
							1	2	4	8	8	100							2	5	10	42	3	7.1
9							4	14	21	58[L]	54	93.1	2	2	2	7	6	85.7						
							1	1	1	3	3	100							4	9	10	34	2	5.9
10							3	4	4	13[L]	13	100	1	2	2	5	5	100						
							3	7	7	25	25	100							3	5	8	21	1	4.8
11							1	6	10	42	26	61.9	2	5	5	17	17	100	2	5	5	18	3	16.7
12							3	9	13	56	35	62.5												
Totals.....			1,227	1,025	83.5				262	218	83.2				173	149	86.1				163	19	11.7	

L = louping ill virus employed. No designation = encephalitis virus.

TABLE IV

Effect of Selection on Mortality Following Test Injections

Generation	Bacteria-resistant (BR) line 1												Bacteria-resistant (BR) line 2											
	Progeny tested with *B. enteritidis*						Progeny tested with louping ill virus						Progeny tested with *B. enteritidis*						Progeny tested with louping ill virus					
	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead	No. sires	No. dams	No. litters	No. progeny	No. dead	Per cent dead
1	1	8	18	75	12	16.0							1	12	7	34	11	32.4						
2	10	42	86	460	42	9.1							4	14	16	80	16	20.0						
3	32	134	142	833	130	15.6							4	13	19	119	12	10.1						
4	23	88	90	511	91	17.8	3	3	3	15	12	80.0	4	15	20	84	13	15.5	2	2	2	9	8	88.9
5	23	59	63	343	133	38.8	15	35	47	222	219	98.6	2	8	8	44	7	15.9	1	6	7	25	24	96.0
6	19	49	51	309	171	55.3	19	46	62	284	279	98.2	3	5	5	27	11	40.7	4	8	9	26	26	100.0
7	2	2	2	6	4	66.7																		
Totals.....			2,537	583	23.0				521	510	97.9				388	70	18.0				60	58	96.7	

The foregoing data, further analyzed, show the effect of selective breeding on susceptibility and resistance within each line.

Bacteria-susceptible line 2, which at the first selection and generation showed maximum mortalities to *B. enteritidis* of approximately 85 per cent and which remained so without significant change for five selections and generations, showed suddenly, at the sixth selection and generation (Table II), an abrupt rise to 96.3 per cent. This high level of mortality persisted through the eighth generation and likewise through the twelfth generation to date when selections were being made not on the basis of susceptibility to *B. enteritidis* but to virus. Thus, of 134 BSVS mice tested with *B. enteritidis*, 99.3 per cent died and of 94 BSVR, 95.7 per cent died. Selections for susceptibility to virus in the sub-line, bacteria-susceptible-virus-susceptible (BSVS), were accompanied by mortalities increasing from 76.7 per cent at the first selection in the sixth generation to 97.4 per cent at the seventh selection in the twelfth generation, among a total of 1,464 mice tested. Corresponding selections for resistance to virus in the sub-line, bacteria-susceptible-virus-resistant (BSVR), were accompanied by irregular mortalities without trend, averaging 17.4 per cent of 1,054 tested.

Bacteria-susceptible line 1 had an early history similar to line 2. Owing to subsequent events, however (Table III), it was finally discarded. Its 87.5 per cent susceptibility to *B. enteritidis*, noted at the first selection, varied between 76.7 and 100 per cent for six selections and twelve generations and averaged 83.5 per cent (Table III). Selections for high susceptibility to virus were commenced with the seventh generation mice with progeny mortalities averaging 95.2 per cent in the first test. Three further selections on eighth to eleventh generation mice did not change this high level of mortality. Progeny of eleventh generation mice, however, showed only 61.9 per cent mortality to virus, and progeny of twelfth generation mice, 62.5 per cent. No explanation for this alteration was found and the line was discarded.

Bacteria-susceptible line 1, crossed with line 2, was designated line 1 $\times$ 2. It resembled line 2 generally but because of its small numbers was discarded (Table III). Mortalities of progeny following *B. enteritidis* injection were 72.3 per cent in the third generation, 88.9 per cent in the fourth, 89.9 per cent in the fifth, 85.7 per cent in the ninth, and 100 per cent in the tenth and eleventh generations. Selection of a sub-line resistant to virus resulted in mortalities of 15.4 per cent in the first selection, sixth generation, and 16.7 per cent in the eleventh generation, or an average on a total of 163 mice of 11.7 per cent.

Bacteria-resistant line 1, following the first selection, showed a 16.0 per cent *B. enteritidis* mortality of tested progeny, and subsequent selections for four generations did not alter this general level (Table IV). Following the fifth selection, however, progeny of the fifth generation parents showed a 38.8 per cent mortality, 55.3 per cent in the sixth and 66.7 per cent in the seventh generation respectively. Progeny of the fourth generation tested for susceptibility to louping ill virus showed 80 per cent mortality. One selection was followed by an increase

in mortality of the progeny of the fifth generation to 98.6 per cent and a second selection by a mortality of sixth generation progeny of 98.2 per cent. Brother to sister matings within this line were then discontinued and matings made at random within the line.

Bacteria-resistant line 2 behaved in an entirely similar manner. Brother to sister matings were replaced by line matings as in line 1.

To summarize, mice succumbing to or surviving an enteric bacterial or a neurotropic virus infection bear progeny which tend to succumb or survive respectively. The majority of individuals subjected to this progeny test evidence intermediate degrees of resistance which can be modified by repeated selection from generation to generation. Certain of them, however, provided a sufficiently large population be tested, show an initial maximum or minimum susceptibility which remains relatively stable.

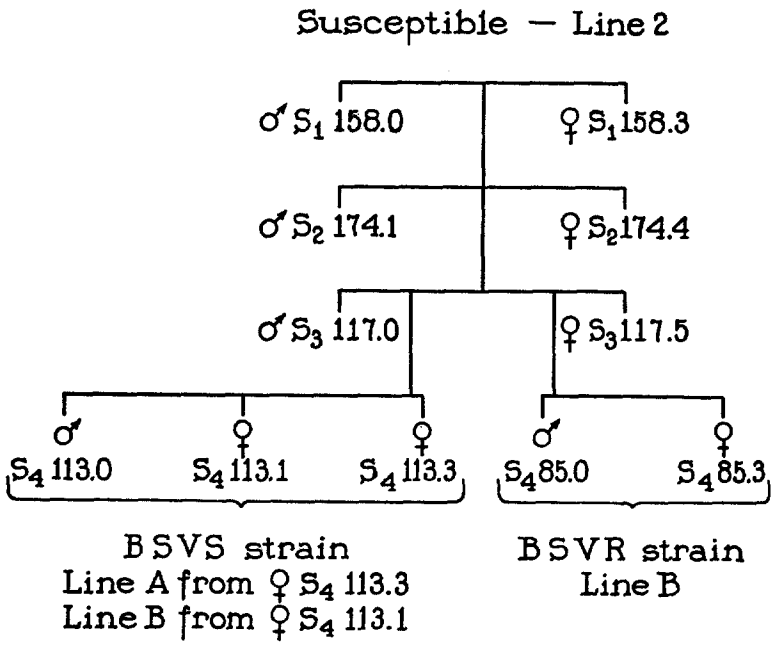
The rise in mortality rates which took place abruptly and simultaneously in all lines probably resulted from imperfect control of environmental factors.

Present Status of Inbred Lines

Rigid selection, testing, and discarding procedures on approximately 13,200 mice for twelve generations from 1930 left us in November, 1934, with three lines, one bacteria-susceptible-virus-susceptible (BSVS), one bacteria-susceptible-virus-resistant (BSVR), and one bacteria-resistant-virus-susceptible (BRVS). The first two came from identical parents and sibling progeny (Text-fig. 1) in bacteria-susceptible line 2 (Table II); the third came from the bacteria-resistant lines 1 and 2. Brother to sister breeding has been practiced throughout in the first two lines; in the BRVS line, it was replaced after the sixth generation by line breeding.

The degree of homogeneity of each line can be appraised to some extent by inspection of the two following sets of data.

The first is the results of susceptibility tests from generation to generation on the immediate forebears of the eventually selected lines and their progeny (Tables V to VII). While Tables II to IV include tests on all progeny within each line, the great majority of which were subsequently discarded, the tables to follow include only tests on siblings in the direct line and their parents.



TEXT-FIG. 1

TABLE V

Immediate Family Histories of Inbred BSVS Lines with Respect to Mortalities Following Test Injections of B. enteritidis or Virus

Generation	Line bacteria-susceptible-virus-susceptible A (BSVS)						Line bacteria-susceptible-virus-susceptible B (BSVS)					
	Progeny tested with *B. enteritidis*			Progeny tested with virus			Progeny tested with *B. enteritidis*			Progeny tested with virus		
	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
1	17	14	82.4									
2	44	36	81.8									
3	91	87	95.6									
4	26	24	92.3									
5	27	27	100				18	18	100			
6	11	11	100	12	8	66.7	20	19	95	23	16	69.6
7				24	21	87.5				34	25	73.5
8				23	23	100				37	35	94.6
9	3	3	100	207	183	88.4	8	8	100	144	128	88.9
10	37	37	100	192	168	87.5	11	11	100	155	121	78.1
11	13	13	100	32	27	84.4	16	16	100	24	23	95.8
12	23	23	100	26	25	96.2	12	12	100	13	13	100
Totals....	292	275	94.2	516	455	88.2	85	84	98.8	430	361	84.0

The bacteria-susceptible-virus-susceptible (BSVS) line for the first four generations showed mortalities to *B. enteritidis* increasing from 82.4 per cent to 92.3 per cent (Table V). At that point, two virus-susceptible lines, A and B, were selected. Both, when tested further with *B. enteritidis*, showed approximately 100 per cent mortality through the twelfth generation and both, when tested with virus, showed initial mortalities of about 68 per cent at the sixth generation, increasing to about 95 per cent at the twelfth generation and averaging 88.2 per cent and 84 per cent.

TABLE VI

Immediate Family Histories of Inbred BSVR Lines with Respect to Mortalities Following Test Injections of B. enteritidis or Virus

Line bacteria-susceptible-virus-resistant (BSVR)

Generation	Progeny tested with *B. enteritidis*			Progeny tested with virus		
	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
4	9	9	100			
5	15	15	100			
6	21	21	100	59	1	1.7
7				42	1	2.4
8				33	3	9.1
9	15	14	93.3	70	6	8.6
10	9	8	88.9	19	1	5.3
11	37	35	94.6	46	6	13.0
Totals.....	106	102	96.2	269	18	6.7

The bacteria-susceptible-virus-resistant line (Table VI), commenced at the fourth generation, showed thereafter through the eleventh generation an average mortality to *B. enteritidis* of 96.2 per cent without significant variation and mortalities to virus from 1.7 per cent to 13.0 per cent, averaging 6.7 per cent of 269 tested mice.

TABLE VII

Immediate Family Histories of Inbred BRVS Line with Respect to Mortalities Following Test Injections of B. enteritidis or Virus

Line bacteria-resistant-virus-susceptible (BRVS)

Generation	Progeny tested with *B. enteritidis*			Progeny tested with virus		
	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
1	4	0	0			
2	16	0	0			
3	22	0	0			
4	82	14	17.1			
5	29	5	17.2	20	19	95
6	43	15	34.9	39	38	97.4
Totals.....	196	34	17.3	59	57	96.6

The bacteria-resistant-virus-susceptible line (Table VII) showed mortalities to *B. enteritidis* increasing from 0 per cent to 34.9 per cent and averaging 17.3 per cent and mortalities to virus relatively stable at 96.6 per cent.

The three inbred lines have shown percentage mortalities to *B. enteritidis* and virus averaging as follows: BSVS, 95.2 and 86.3 per cent; BSVR, 96.2 and 6.7 per cent; BRVS, 17.3 and 96.6 per cent. High susceptibility proved relatively stable throughout, while high resistance appeared to fluctuate from generation to generation.

The second set of data comprised repeated tests with *B. enteritidis* and virus on divided litters of each line.

TABLE VIII

Mortalities of Inbred Lines Following Injections of B. enteritidis or Encephalitis Virus in Divided Litters

Date	Bacteria-susceptible-virus-susceptible line (BSVS)						Bacteria-susceptible-virus-resistant line (BSVR)						Bacteria-resistant-virus-susceptible line (BRVS)					
	Tests with *B. enteritidis*			Tests with virus			Tests with *B. enteritidis*			Tests with virus			Tests with *B. enteritidis*			Tests with virus		
	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
1934																		
Nov. 2	19	19	100	10	10	100	19	19	100	10	1	10	7	0	0.0	5	5	100
1935																		
Jan. 29	49	48	98	49	46	93.9	50	47	94	50	8	16	50	8	16	50	46	92
Oct. 1	47	46	97.9	51	50	98	43	41	95.3	40	8	20	58	13	22.4	67	65	97
Nov. 25	12	12	100	15	15	100	12	12	100	22	1	4.5	11	2	18.2	10	9	90
Totals...	127	125	98.4	125	121	96.8	124	119	96.0	122	18	14.8	126	23	18.3	132	125	94.7

Litters aged 5 weeks from each strain were divided each into two approximately equal batches. One batch was given *B. enteritidis per os*; the other encephalitis virus *per nares*. Four such consecutive tests on divided litters are recorded in Table VIII.

The average mortalities (Table VIII) were as follows: BSVS line, *B. enteritidis* 98.4 per cent, virus 96.8 per cent; BSVR line, *B. enteritidis* 96 per cent, virus 14.8 per cent; BRVS line, *B. enteritidis* 18.3 per cent, virus 94.7 per cent. These figures closely approximate the average figures for twelve generations given above (Tables V to VII),

except for a 10 per cent higher level of susceptibility to virus in the BSVS and BSVR lines, and are taken to represent the susceptibilities of the lines used in the cross-breeding experiments which follow.

A final comparison of strains was made with respect to their ability to resist different quantities of the infectious agent. *B. enteritidis* was administered *per os* in doses varying from 5×10^8 to 5×10^3 each to batches of 10 to 20 mice of each strain. Encephalitis virus was instilled intranasally in dilutions of 10^{-1} to 10^{-5} to similar batches. The result was in accord with previous findings that the mice of a given line react similarly to a certain range of dosage of organisms. The susceptible lines showed high mortalities following doses as little as 1/100th of the standard and the resistant lines showed low mortalities following doses 100 times the standard. Consequently the resistant lines, both bacterial and virus, were considered to withstand 1,000 to 10,000 times the dose fatal for susceptibles.

Cross-Breeding Experiments

The three lines were now regarded as sufficiently stable to carry out cross-breeding and backcross experiments for the purpose of analyzing the mechanism of inheritance.[1] The susceptibility level has remained close to 95 per cent, and the resistance level 10 to 20 per cent (Tables V to VIII). Previous cross-breeding of the bacteria-resistant with the white-face susceptible strain (1) had shown resistance to be dominant in the F_1 progeny, and a segregation of resistance and susceptibility in the backcross progeny on the basis of inheritance. The following tests were made on both bacterial factors and virus factors.

Jan. 4, 1935. A. 2 BSVS males were mated to 8 BSVR females in cages containing 1 male to 4 females, respectively. 2 BSVR males were likewise mated to 8 BSVS females. B. Secondly, 2 BSVS males were mated with 8 BRVS females and 2 BRVS males with 8 BSVS females. C. Thirdly, 2 BSVR males were mated with 8 BRVS females and 2 BRVS males with 9 BSVR females.

[1] Variance in mortalities of progeny from each line to *B. enteritidis* and virus infections was tested by the Lexian ratio formula and found to be well within normal expectancy; $P = >0.10$. A similar test on litters from the BSVR × BRVS cross-mating described below gave the same result.

June 11, 1935. Similar matings were made with A, 2 BSVS males and 8 BSVR females and 2 BSVR males and 8 BSVS females, and with C, 4 BSVR males with 20 BRVS females and 4 BRVS males with 18 BSVR females.

F_1 progeny of these matings, when 4 to 6 weeks of age, were tested with the standard dose of *B. enteritidis*, *per os*, 5×10^6 organisms, or encephalitis virus *per nares*, 0.03 cc. of a 10^{-2} dilution. The litters were divided for the most part, one-half receiving the bacteria, the other half the virus. A few litters were tested with virus alone or with both bacteria and virus in a manner described below.[2] All methods gave similar results.

The results of tests on a total of 1,448 F_1 progeny comprising 253 litters are summarized in Table IX. F_1 progeny of the A crossing, BS (98 per cent) VS (97 per cent) with BS (96 per cent) VR (15 per cent) lines, would be expected, on the basis of resistance to virus being dominant, to be bacteria-susceptible-virus-resistant. Actually, of 179 tested with *B. enteritidis*, 86.6 per cent succumbed,

[2] Three methods of double injection were compared: A, giving bacteria and virus at the same time, B, bacteria followed 5 weeks later by virus, and C, virus followed by bacteria. F_1 BRVR mice, whose mortalities to bacteria and virus averaged 26.2 per cent and 16.7 per cent respectively according to the divided litter and single injection tests (Table IX), were treated as follows: A. 20 were given the standard dose of encephalitis virus *per nares*, 0.03 cc. of a 1 to 100 dilution, followed immediately by the standard dose of *B. enteritidis*, *per os*, 5,000,000 bacilli. B. 10 received *B. enteritidis* followed by virus 5 weeks later. C. 10 received virus followed by bacteria 5 weeks later. As controls, 3 BRVS mice were given virus and 3 BSVR were given *B. enteritidis*. All controls succumbed promptly. Of the A batch, 70 per cent succumbed, a percentage more than double that encountered by the single injection method. Of the B mice, 30 per cent succumbed to *B. enteritidis* and 28.6 per cent of the survivors to virus. Of the C mice, 30 per cent succumbed to virus and 14.3 per cent of the survivors to *B. enteritidis*, a result not at too great variance with the 26 per cent virus and 17 per cent *B. enteritidis* mortalities expected.

In a further test, 46 BRVR mice were given C virus followed by *B. enteritidis* with resulting mortalities of 10.9 per cent and 22.0 per cent respectively; 51 were given B *B. enteritidis* followed by virus with resulting mortalities of 29.4 per cent and 8.3 per cent respectively. As controls, 4 BRVS mice were given virus followed by *B. enteritidis* with 75 per cent and 0 per cent mortalities resulting, and 11 *B. enteritidis* followed by virus with 18.2 per cent and 88.9 per cent fatalities. 8 BSVR mice were given virus followed by *B. enteritidis* with 12.5 per cent and 85.7 per cent mortalities, as expected. The B method of giving *B. enteritidis* followed by virus may activate a quiescent bacterial carrier state; both B and C methods introduce a slight selective effect. The C method of virus followed by bacteria, however, has consistently approximated the effects of single injection tests and is now used to test the resistance of selected strains.

TABLE IX

Mortalities of F_1, F_2, and Backcross Progeny Following Test Injections with B. enteritidis and Encephalitis Virus

Date mated	Matings—Cross-breeding	No. litters tested	Total No. progeny	Tests with *B. enteritidis*			Tests with encephalitis virus		
				No. injected	No. dead	Per cent dead	No. injected	No. dead	Per cent dead
1935									
Jan. 4	A. 2 ♂ BSVS × 8 ♀ BSVR	13	62	14	12	85.7	54	20	37.0
	2 ♂ BSVR × 8 ♀ BSVS	22	99	36	32	88.9	80	34	42.5
June 11	2 ♂ BSVS × 8 ♀ BSVR	16	86	60	48	80.0	65	12	18.5
	2 ♂ BSVR × 8 ♀ BSVS	18	107	69	63	91.3	78	22	28.2
Total F_1 progeny		69	354	179	155	86.6	277	88	31.8
Jan. 4	B. 2 ♂ BSVS × 8 ♀ BRVS	34	250	86	14	16.3	173	130	75.1
	2 ♂ BRVS × 8 ♀ BSVS	25	132	34	12	35.3	99	71	71.7
Total F_1 progeny		59	382	120	26	21.7	272	201	73.9
Jan. 4	C. 2 ♂ BSVR × 8 ♀ BRVS	29	172	67	14	20.9	127	26	20.7
	2 ♂ BRVS × 9 ♀ BSVR	18	90	44	13	29.5	58	13	22.4
June 11	4 ♂ BSVR × 20 ♀ BRVS	45	243	166	40	24.1	158	20	12.7
	4 ♂ BRVS × 18 ♀ BSVR	33	207	132	40	30.3	141	22	15.6
Total F_1 progeny		125	712	409	107	26.2	484	81	16.7
Total F_2 progeny		71	265	265	71	26.8	191	67	35.1
	Matings—Backcross								
1935									
Sept. 17	A. 2 F_1 ♂ (BSVR ♂ × BSVS ♀) × 9 ♀ BSVS	5	14	6	4	66.7	14	7	50.0
	5 F_1 ♀ (BSVS ♂ × BSVR ♀) × 1 ♂ BSVS	8	46	20	19	95.0	46	25	54.3
Total backcross progeny		13	60	26	23	88.5	60	32	53.3
Sept. 17	A. 2 F_1 ♂ (BSVS ♂ × BSVR ♀) × 8 ♀ BSVR	7	27	21	21	100.0	27	4	14.8
	10 F_1 ♀ (BSVR ♂ × BSVS ♀) × 2 ♂ BSVR	13	70	52	39	75.0	70	14	20.0
Total backcross progeny		20	97	73	60	82.2	97	18	18.6
Sept. 17	C. 2 F_1 ♂ (BSVR ♂ × BRVS ♀) × 8 ♀ BRVS	14	103	54	12	22.2	103	44	42.7
	4 F_1 ♀ (BRVS ♂ × BSVR ♀) × 1 ♂ BRVS	8	58	33	3	9.1	58	23	39.7
Total backcross progeny		22	161	87	15	17.2	161	67	41.6
Sept. 17	C. 2 F_1 ♂ (BRVS ♂ × BSVR ♀) × 9 ♀ BSVR	6	47	42	20	47.6	47	5	10.6
	11 F_1 ♀ (BSVR ♂ × BRVS ♀) × 3 ♂ BSVR	12	81	57	29	50.9	81	15	18.5
Total backcross progeny		18	128	99	49	49.5	128	20	15.6

and of 277 tested with virus 31.8 per cent succumbed. F_1 progeny of the B crossing, BS (98 per cent) VS (97 per cent) with BR (18 per cent) VS (95 per cent), should be bacteria-resistant and virus-susceptible on the basis of resistance to bacteria being dominant (1). Actually 21.7 per cent of 120 succumbed to *B. enteritidis* and 73.9 per cent of 272 to virus. F_1 progeny of the C crossing, BS (96 per cent) VR (15 per cent) with BR (18 per cent) VS (95 per cent) lines, would be expected to be resistant to both bacteria and virus. On testing, 26.2 per cent of 409 mice given *B. enteritidis* and 16.7 per cent of 484 given virus succumbed.

TABLE X

Comparison of Experimental Data of Cross-Breeding Tests with Results Expected on Basis of Single Factor Type of Inheritance

Symbols	Three tested lines of mice
A = bacteria-resistant (BR) a = bacteria-susceptible (BS)	$\frac{aa}{bb}$ = bacteria-susceptible (BS—98.4 per cent) virus-susceptible (VS—96.8 per cent)
B = virus-resistant (VR) b = virus-susceptible (VS)	$\frac{aa}{BB}$ = bacteria-susceptible (BS—96.0 per cent) virus-resistant (VR—14.8 per cent)
	$\frac{AA}{bb}$ = bacteria-resistant (BR—18.3 per cent) virus-susceptible (VS—94.7 per cent)

First generation matings

Genetic formulae	Expected results	Experimental data (per cent mortality)
1. $\frac{aa}{bb} \times \frac{aa}{BB} = \frac{aa}{bB}$	Bacteria-susceptible / Virus-resistant	Bacteria test—86 per cent mortality / Virus " —31 " " "
2. $\frac{aa}{bb} \times \frac{AA}{bb} = \frac{aA}{bb}$	Bacteria-resistant / Virus-susceptible	Bacteria " —21 " " " / Virus " —73.9 " " "
3. $\frac{aa}{BB} \times \frac{AA}{bb} = \frac{Aa}{Bb}$	Bacteria-resistant / Virus-resistant	Bacteria " —26.2 " " " / Virus " —16.7 " " "

Second generation matings

Genetic formulae	Expected results	Experimental data (per cent mortality)
F_2 from F_1 of 3, $\frac{aA}{Bb} \times \frac{aA}{Bb}$	Bacteria-susceptible— 25 per cent / Virus-susceptible — 25 " "	Bacteria test—26.8 per cent mortality / Virus " —35.1 " " "
Backcross F_1 of 1, $\frac{aa}{bB} \times \frac{aa}{bb}$	Bacteria-susceptible—100 " " / Virus-susceptible — 50 " "	Bacteria " —88.5 " " " / Virus " —53.3 " " "
$\frac{aa}{bB} \times \frac{aa}{BB}$	Bacteria-susceptible—100 " " / Virus-susceptible — 0 " "	Bacteria " —82.3 " " " / Virus " —18.6 " " "
Backcross F_1 of 3, $\frac{aA}{Bb} \times \frac{AA}{bb}$	Bacteria-susceptible— 0 " " / Virus-susceptible — 50 " "	Bacteria " —17.2 " " " / Virus " —41.6 " " "
$\frac{aA}{Bb} \times \frac{aa}{BB}$	Bacteria-susceptible— 50 " " / Virus-susceptible — 0 " "	Bacteria " —49.5 " " " / Virus " —15.6 " " "

The tests of F_1 progeny show that susceptibility to *B. enteritidis* and to encephalitis virus is controlled basically by inherited factors, with resistance dominant over susceptibility. Moreover, progeny from a given cross-breeding, A, B, or C, reacted similarly regardless of whether male or female parent carried resistance or susceptibility factors, indicating absence of sex linkage of the inherited factors.

Backcross matings for segregation of resistance factors according to heredity were carried out in the following manner.

Sept. 17, 1935. F_1 progeny from the A mating, having proved susceptible to bacteria and resistant to virus, BS (87 per cent) VR (32 per cent), were backcrossed to both the original bacteria-susceptible-virus-susceptible, BS (98 per cent) VS (97 per cent), and bacteria-susceptible-virus-resistant, BS (96 per cent) VR (15 per cent), lines. In the first instance in which the backcross progeny would be expected on the basis of a simple type of inheritance to be highly susceptible to bacteria and 50 per cent susceptible to virus, 88.5 per cent of 26 tested with *B. enteritidis* and 53.3 per cent of 60 tested with virus succumbed (Tables IX and X). In the second instance in which the backcross progeny should be susceptible to bacteria and resistant to virus, 82.2 per cent of 73 tested with bacteria and 18.6 per cent of 97 tested with virus succumbed.

F_1 progeny from the C mating having proved resistant to both bacteria and virus, BR (26.2 per cent) VR (16.7 per cent), were backcrossed to the original BR (18 per cent) VS (95 per cent) and BS (96 per cent) VR (15 per cent) lines. In the first instance, the backcross progeny would be expected to be resistant to bacteria and 50 per cent susceptible to virus. This expectancy was approximated by actual mortalities of 17.2 per cent of 87 tested with bacteria and 41.6 per cent of 161 tested with virus (Tables IX and X). In the second instance, the backcross progeny should be 50 per cent susceptible to bacteria and resistant to virus. Actually 49.5 per cent of 99 succumbed to *B. enteritidis* and 15.6 per cent of 128 to encephalitis virus.

Finally, F_1 mice derived from the C matings, BSVR $\times$ BRVS, which on testing proved resistant to both bacteria and virus, BR (26.2 per cent) VR (16.7 per cent), were mated brother to sister. The F_2 progeny resulting were tested for the most part with both bacteria and virus by the method described below. Of 265 tested with encephalitis virus, 71 or 26.8 per cent died and of the 191 survivors tested with *B. enteritidis*, 67 or 35.1 per cent died. Similar rates were obtained when batches were injected with one agent only, or when litters were divided and one agent given to one lot, and the other to the remainder.

The results of the cross-breeding tests support the selective breeding data in indicating that resistance to these infections is controlled basically by inherited factors and confirm our previous findings (1)

that the factors are not sex-linked and that resistance is dominant over susceptibility. Besides, they demonstrate that the factors regulating resistance to *B. enteritidis* are not related to those regulating resistance to encephalitis virus. Finally, they indicate that the mechanism of this inheritance may be relatively simple, since the mortality percentages of F_1, F_2, and backcross mice approximate for the most part those expected on the basis of two single factor crossings (Table X).

Development of BRVR Line

Throughout the breeding and testing of the three strains for resistance and susceptibility to *B. enteritidis* and encephalitis virus, no evidence of a strain highly resistant to both agents was at hand. BRVR mice were encountered, however, when the BSVR strain was crossed with BRVS (Tables IX and X). Moreover, these doubly resistant F_1 mice, when bred *inter se*, showed an average mortality rate in the neighborhood of that to be expected on the basis of a two single factor type of inheritance. Hence it was assumed that among these F_2 progeny, a pure BRVR strain might be present. To segregate this possible strain from heterozygous BRVR reactors, the following procedure was adopted.

A number of F_2 litters survived the double injection test of *B. enteritidis* followed 5 weeks later by virus. Their parents (F_1) were selected, mated again, and the resulting litters, comprising 42 males and 147 females, siblings of those tested, were mated brother to sister for F_3 progeny to be tested. 122 F_3 litters, totaling 610 individuals, were tested, with an average 30 per cent mortality to *B. enteritidis* and 25 per cent to virus. Certain of these litters survived *in toto* the double injection. The F_2 parents of these were then mated to the original BSVS strain on the supposition that the latter strain was doubly recessive and that the cross-mating would disclose the presence of homozygous F_2 individuals.

These experiments are now in progress and certain lines are emerging which to date are resistant to both *B. enteritidis* and virus.

High Susceptibility of All Selected Strains to Rabies Virus

To louping ill and St. Louis encephalitis viruses, the selected mouse strains react in a consistent manner. To mouse passage rabies virus, however, they all proved highly susceptible (12). The following protocol is illustrative of the reaction.

Batches of twelve of each of the BSVS, BSVR, and BRVS strains were injected as follows: intracerebrally with 0.03 cc. diluted 1 to 100,000 and 1 to 500,000; intralingually with 0.03 cc. diluted 1 to 100 and 1 to 1,000, and into the calf muscle, with 0.03 cc. diluted 1 to 10 and 1 to 30. Thus, 60 mice of each strain were compared for susceptibility to different doses and by different routes. The rabies strain employed, No. 1, passage 70, was obtained from the hippocampal lobe of a dog suffering with rabies and was subsequently passed through 70 mice by intracerebral injection of brain tissue.

All of the 12 BSVS and 11 of the 12 BSVR mice succumbed following the intracerebral injection of the 1 to 100,000 dilution and following the smaller intracerebral dose of 1 to 500,000 dilution, 8 of 12 BSVS and 5 of 11 BSVR mice died. The lingual injection of 1 to 100 dilution of virus was uniformly fatal to all; 1 to 1,000 killed 7 of 12 BSVS and all BSVR mice. Injection into the calf muscle of 1 to 10 dilution was fatal to all alike; less virus, 1 to 30 dilution, killed 9 of 12 BSVS and 11 of 12 BSVR mice.

No significant differences in mortality between strains in this or other tests were apparent following injection of moderate or minimum doses of virus by natural or artificial routes.

DISCUSSION

The groundwork of experimental knowledge of the inheritance of resistance to infectious disease was laid by workers with plant diseases. Biffen, in 1905 (13 *a*), crossbred strains of wheat resistant to yellow rust with strains susceptible and by testing F_1 and F_2 progeny under field conditions, demonstrated its dependence upon a single factor type of inheritance with susceptibility dominant. His homozygous strains were not completely resistant or susceptible, respectively, but mainly of high or low resistance. He noted that modifications in amount of available nitrogen increased the susceptibility of genetically resistant individuals. Burkholder, in 1918 (13 *b*), and McRostie, in 1919 (13 *c*), studying the inheritance of resistance of beans to anthracnose, employed non-infected greenhouse stock and controlled dosage by inoculating each individual in the greenhouse with a similar amount of the more pure strain of the infecting agent. Each found resistance inherited and based upon a single factor mechanism with resistance dominant. Many plant infections have now been studied with techniques aiming at once at naturalness of mode of infection consistent with adequate control. Results are compared with those of a field test. Resistance is found to be inherited in some instances

on a multiple and in some on a single factor basis with resistance dominant (resistance of wheat to (*a*) stem rust, (*b*) leaf rust, (*c*) bunt; resistance of (*d*) barley to rusty blotch, (*e*) cabbage to yellows, (*f*) maize to rust, (*h*) oats to loose smut) more frequently than susceptibility (13 *d* to *k*). Inborn resistance to one infection generally proved independent of resistance to another. No anatomical or physical mechanism has thus far been proved causally related to resistance or susceptibility.

Workers on infectious diseases of animals have paid little attention until recently to the possible regulation of resistance by inborn factors and to the differentiation of individuals according to these factors. Common practice shows that if a batch of animals is given an injection of a virulent agent by some artificial route, the great majority succumb within a few hours. Again, if less virulent agents, smaller doses, or more natural routes of infection are used, a percentage of a random batch of individuals may survive, but if the test is run in duplicate or repeated, the percentage of survivors varies in a random manner. Greenwood and Topley have experience with this sort of result and attribute differences in survival rate and in fate of individuals to uncontrolled errors of technique (14).

An experimental attack on the question in fowl and rodents was undertaken by Frateur in 1924 (3), Roberts and Card in 1926 (4), Lambert and Knox in 1928 (5), Irwin in 1929 (6), Schott in 1932 (7), and Gowen and Schott in 1933 (8). All bred for resistance from survivors of a highly artificial infection and noted a progressively declining mortality. They crossed the selected survivors with either the original unselected stock or with a susceptible strain and tested F_1, F_2, and backcross progeny. Frateur (3) and Gowen and Schott (8) interpreted their figures as suggesting a single factor type of Mendelian inheritance with resistance dominant; the remainder accounted for their data on a multiple factor basis. These workers employed materials and techniques, however, which render their results difficult of interpretation from an infectious disease point of view. In the first place, the test infection is suspected of persisting in their stock since survivors are used for breeding. The presence of the infection involves risks of dam and sire infecting each other and of a part of the litter dying from the infection, leaving survivors for later tests which

may prove resistant (*a*) because of their selection through previous infection or because possessed of (*b*) an active or (*c*) a passive immunity. Irwin (6) and Gowen and Schott (8) especially have endeavored to minimize the significance of a transfer of passive immunity in influencing their data, but the probability remains that the persistent infection is a factor in enhancing the resistance of their test progeny. In the second place, resistance has been tested to highly artificial infections,—intraperitoneal injections of large doses, bacteria of low natural infectivity,—without determining experimentally whether such tests are in fact a measure of the resistance of the individual to the infection in nature. Indeed, experiment has often shown the contrary to be the case in that resistance of animals to bacterial infections differs according to the portal of entry employed, and animals susceptible by an artificial route are not necessarily as susceptible by a natural route, and *vice versa*. In the present state of knowledge, therefore, it is important for the worker to indicate clearly the type of resistance he is studying and determine in each instance the degree to which this resistance is a measure of resistance in nature.

Our investigations in inborn resistance commenced (1923 (9)) when it was found that batches of animals bred in the laboratory in an effort to control all possible environmental variables, if exposed to infectious agents in a way simulating nature, differed from batches of uncontrolled mice of the sort discussed by Greenwood and Topley (14) in responding as a group in a relatively predictable manner. Moreover, the survival of some individuals, as contrasted with the death of others under apparently similar and controlled conditions, took on a possible significance. Although the reaction of a given individual of the group could not be predicted, the differences in individual response were regarded as possibly due not to technical irregularities, but to differences in their degree of inborn resistance.

This idea was supported by experiments showing that certain strains of mice suffered consistently higher mortalities than others following *per os* instillation of mouse typhoid bacilli (15) and also following exposure to a naturally spreading herd infection (16). Selective breeding experiments (17) showed that progeny of individuals surviving a *per os* instillation of mouse typhoid bacilli suffered less mortality, and progeny of individuals succumbing early to the infec-

tion suffered greater mortalities following the test infection than the unselected controls. In these tests, however, the original population was too small to insure the selection of individuals with the widest possible differences in inborn resistance characteristic of the strain, and was too small to insure that the selected lines would provide a sufficient number of fertile dams. Then too, the resistant lines were bred from survivors, and the bread and milk diet produced mice whose mortalities fluctuated with season. The findings, however, in spite of their limitations, pointed consistently toward the presence of innate differences in resistance. Consequently, the breeding experiments were repeated (1) with an original population of 600 to cover the susceptibility range of the strain and allow for selection of optimum breeders with progeny exhibiting the desired maximum and minimum mortalities. Moreover, a diet was used which gave relatively stable mortality percentages in unselected controls. Here, although an error was committed in making the first selection for resistants from survivors rather than from the unexposed progeny of mice which were later proved survivors, these selected survivors were proved free of infection before introducing them into the colony and thereafter selections were made from unexposed sibling litters. The colony has remained free of infection from the outset.

The present experiments in their entirety demonstrate that the individual constituents of any sizable population of mice in which environmental variables have been controlled as far as possible differ widely in their innate resistance to infectious agents. This difference is of the order of 1,000 lethal doses or of mortalities to a standard dose of 95 per cent as contrasted with 15 per cent. These innate differences in the resistance of individual mice were brought out by the progeny test and by the development of lines from certain individuals by selective breeding. This procedure segregated at the outset individuals whose resistance was maximum or minimum, respectively, remaining unchanged for twelve generations. The resistance of the majority, however, was intermediate, increasing or decreasing on repeated selection from generation to generation. Crossing the initially highly susceptible and resistant lines and testing F_1, F_2, and backcross progeny resulted in percentage mortalities in the neighborhood of those expected on the basis of a single factor type of Men-

delian inheritance for resistance to *B. enteritidis* and to encephalitis virus. Resistance proved dominant in each instance. Moreover, the histories of the direct descendants in each line (Tables V to VII) support the theory of a single factor type of inheritance, since mortalities in succeeding generations showed mainly no definite progress with selection but proved relatively stable. Consequently, we regard the above as evidence of a single main factor type of inheritance with possibly a number of small modifiers.

Individuals inherently resistant or susceptible to one infectious or toxic agent may or may not prove likewise resistant or susceptible to another. Previously we noted that survivors of mouse typhoid or individuals fed on McCollum ration were relatively resistant not only to a subsequent injection of an antigenically different strain of mouse typhoid but to $HgCl_2$ (18, 19). This indicated to us that resistance was conditioned not only by specific immunity but by non-specific factors as well. The association of resistance to mouse typhoid and to $HgCl_2$ was considered not as necessary nor as an indication of a pan-resistance, as Hill (2) has inferred. Rather the parallel was one of chance. This supposition is borne out by the present studies in which from hybrid stock, lines were segregated with various combinations of resistance and susceptibility, namely, BSVS line, susceptible to two enteric and three respiratory tract bacterial and three virus infections, BRVR line, resistant to all save rabies, BSVR line, susceptible to the bacterial and resistant to two of the three tested virus infections, and the BRVS line, resistant to the bacterial and susceptible to the virus infections. And finally, the cross-breeding of these strains and testing of progeny brought out the independence of genetic factors governing resistance to *B. enteritidis* and encephalitis virus, respectively. It follows that the amount of inherent resistance displayed by an individual to an infectious agent cannot be taken without experiment as a measure of its resistance to another.

The effect of unforeseen environmental variables on the manifestation of genetic factors is disturbing in carrying out this sort of experiment. Control measures are frequently inadequate to prevent variations in results such as the sudden increase of 15 to 20 per cent in mortality percentages in all lines recorded in the present experiments.

The expression of genetic factors is conditioned by the expression of somatic factors and the experiments can achieve at best but a measure of the summation effect of both under conditions in which the environmental ones have been controlled as far as possible.

In concluding this portion of the discussion, we point out again the close parallelism between our findings and those of workers on plant diseases.

The thesis of variability of host resistance and its regulation by inborn and environmental factors has both particular and general bearing upon experimentation in infectious disease. The particular effect of innate factors on type of clinical disease and tissue changes is exemplified in studies on susceptible and resistant mice following oral administration of *B. enteritidis* (1), and nasal administration of pneumococci (20) and encephalitis virus (21). Again the rôle of innate resistance factors is being investigated in experimental epidemiology, for example, in the matter of determining the status of survivors of an epidemic. Are they inherently resistant at the outset and spared from the ravages of the epidemic agent, or are they differentiated only by the chance exposure to subinfectious doses which have immunized them, or do both processes participate?

Greenwood and Topley have studied the question in herds of infected mice to which normal animals were added daily (14). Data on cage age of mice at death were set out in the form of life tables, but their analyses indicate merely that in mouse populations of this sort as well as in human populations, the mode of action of host factors remains in the field of conjecture. The lack of control in these experiments merely complicates the simple problem long familiar to immunologists, namely, are survivors of a relatively natural test infection resistant to a subsequent exposure because of innate resistance or acquired specific immunity factors, or both? The question in this simple test or in the herds of English mice cannot be answered until the resistance of individuals in the herd at the beginning of the experiment is measurable. One experiment fulfilling this requirement has been reported (1). In each of thirty-six tests, 5 mice of known 37 per cent mortality following standard test were given the stomachal instillation of *B. enteritidis* and placed in a cage with 5 or 3 mice 85 per cent susceptible and with a similar number 15 per cent susceptible.

The infection was allowed to spread from the infected to the two classes of contacts hitherto unexposed. Of the 194 susceptibles, 70 per cent succumbed, as contrasted with 12 per cent of resistants. This left the surviving populations at the close of the epidemic composed of 70 per cent of individuals known at the outset to be innately highly resistant. This indicates clearly that survivors of this type of experiment are largely selected resistants. The remaining question of what sort of mice are subclinically immunized and how readily is subclinical immunization accomplished is now under study. At present we find immunization difficult under natural conditions with susceptibles and readily accomplished with resistants.

In general, the concept of control of host variables enforces a conservative attitude in judging seemingly contradictory results of different workers unless their test animals are comparable in all respects. And finally, standardized animals, like pure reagents in chemistry, should provide a means of elucidating many of the quantitative problems in infectious disease.

CONCLUSIONS

Under the conditions specified, there may be selected promptly from a hybrid stock of mice, of which 40 to 50 per cent die following a standard dose of *B. enteritidis* or St. Louis encephalitis virus, lines in which as high as 95 per cent and as low as 15 per cent succumb. Three lines,—one bacteria-susceptible-virus-susceptible, one bacteria-susceptible-virus-resistant, and one bacteria-resistant-virus-susceptible,—are regarded as remaining relatively stable after approximately twelve generations of selection and brother to sister or line inbreeding.

Crossing susceptible with resistant lines and testing F_1, F_2, F_3, and backcross progeny resulted in mortality percentages in the neighborhood of those expected on the basis that resistance to *B. enteritidis* and to encephalitis virus is each inherited independently on a single factor basis with resistance dominant over susceptibility.

A bacteria-resistant-virus-resistant line is being developed from a cross between bacteria-susceptible-virus-resistant and bacteria-resistant-virus-susceptible lines.

All selected lines proved uniformly susceptible to a strain of mouse passage rabies virus.

Miss Alfhild Johnson assisted with the technical part of these experiments.

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