A GENETIC STUDY OF SUSCEPTIBILITY TO EXPERIMENTAL TUBERCULOSIS IN MICE INFECTED WITH MAMMALIAN TUBERCLE BACILLI*

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The role of inherited susceptibility or resistance in experimental tuberculosis has been investigated in several species of laboratory animals. In 1921, Wright and Lewis (1) reported that pronounced differences with respect to survival time existed in the response to mammalian tubercle bacilli among inbred strains of guinea pigs and various crossbred individuals derived from them; families within these lines exhibited different types of skin lesions following intracutaneous infection (2). Lurie *et al.* also observed hereditary differences among rabbits with regard to resistance to infection with both bovine and human tubercle bacilli (3). Long and Vogt (4) reported that two strains of mice, C albino and C57B1, differed in their susceptibility to bovine tubercle bacilli. Later studies have shown that different mouse strains do indeed differ widely in their response to experimental tuberculosis infection (5–9).

The present paper is a report of further investigations on the genetic factors that influence the response of mice to infection with mammalian tubercle bacilli. Information already at hand (5) concerning the greater susceptibility of inbred C57B1 mice than that of an inbred Swiss line determined the choice of these two strains for detailed genetic analysis.

Materials and Methods

Mouse Strains.—Pedigreed stock of both strains of mice was obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The C57B1/6 (a strain of black mice hereafter referred to as C57B1) belonged to the F_{46} to F_{49} generations, and the Swiss to the F_{46} to F_{50}. Brother-sister inbreeding of these two separate strains was continued at the Rockefeller Institute. Environmental conditions were kept as constant as possible although air-conditioning was not available in the breeding rooms. Breeding animals were housed in metal boxes containing cedar shavings and were maintained on a diet of Purina laboratory chow and water *ad libitum*.

At least 24 hours before inoculation, mice were transferred from the breeding quarters to an

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isolation laboratory designed specifically for the experimental study of tuberculosis in animals. The mice were housed, 2 together, on wire grids in metal boxes and were given thereafter a diet of Rockland mouse pellets and water *ad libitum*.

Infection Tests.—Mycobacterium tuberculosis var. *bovis* (Vallée strain) was used in all experiments. In general, the maintenance of the culture of tubercle bacilli and the infection of mice were carried out by techniques previously described (10). The culture, transferred weekly in tween albumin liquid medium, was replaced every 4 months by a new isolate of Vallée obtained from the lungs of experimentally infected mice, in order to insure maximum virulence. Each mouse received intravenously (*via* a lateral caudal vein) a standard inoculum, prepared from cultures incubated for 8 days, and consisting of 0.2 ml of a 1/50 culture dilution in 0.1 per cent aqueous bovine albumin. Plate counts, made on oleic acid albumin agar at the time of each infection test, demonstrated that the infective dose of organisms per mouse contained approximately 3×10^6 viable units. Mice were checked daily to record the deaths, and the presence of tuberculous infection was verified by necropsy. Survival time after infection was used as the criterion of susceptibility or resistance.

EXPERIMENTAL RESULTS

Influence of Mouse Strain upon Survival Time after Infection with Tubercle Bacilli.—The difference in susceptibility to tuberculous infection between the C57B1 and Swiss mice (5) was confirmed in a large number of tests carried out with animals 4 to 6 weeks of age infected intravenously with 0.2 ml of a 1/50 culture dilution of *M. tuberculosis* Vallée. The results of experiments extending over 3 years are presented in Table I and summarized in Table II. In these and all subsequent tables, results for males and females are presented together, since in none of the experiments reported in this paper could any marked difference be found that could be attributed to sex.

Of the C57B1 mice, the very large majority (72.9 per cent) died between 21 and 30 days after infection, though a few were still surviving in the 81 to 90 day period. In general, the greatest mortality among the C57B1 animals was observed several weeks before deaths began to occur among mice of the Swiss strain. The mean day at death (28.1 ± 0.6) differed significantly (Table X) from the mean for the Swiss mice (55.3 ± 0.6). As described elsewhere, the difference between the two strains was reflected also in the pulmonary lesion (5). Mice dying within 30 days after infection exhibited large elevated discrete "tubercles", whereas the lesions of animals dying at the later period were flat and often confluent.

Possible Role of Latent Corynebacterial Infection.—During the early phase of the genetic studies, several animals from the Swiss strain developed fatal corynebacterial pseudotuberculosis following intravenous inoculation with tubercle bacilli. The intercurrent infection of Swiss mice with *Corynebacterium kutscheri* was self-limited and did not recur during the investigations reported in the present paper. This episode, however, raised the possibility that corynebacteria might affect the course of the tuberculous disease. A subsequent study of experimental infection with *C. kutscheri* showed that Swiss mice were ex-

TABLE I

*Survival Time of Swiss and C57B1 Mice Infected with M. tuberculosis**

Date exp.	No. mice	Time of death (5-day intervals) after infection													
		16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85
Swiss mouse strain															
4/24/58	15						3	5	6	1					
11/5/58	12								2	5	3	1	1		
12/3/58	36							1	14	15	6				
12/22/58	40						1	7	10	13	5	1	3		
11/13/59	8							3	1	2	2				
11/24/59	10			1			2	1	5	1					
Total	121			1			6	17	38	37	16	2	4		
C57B1 mouse strain															
6/19/56	33	1	10	12	3		2		2		1	1			1
6/26/57	48	1	30	9	5	1		1			1				
4/24/58	12		6	5											1
11/5/58	13	1		7	1		2			1		1			
12/3/58	14		2	8	1	1		1							1
12/22/58	42	1	24	13	3	1									
8/12/59	14		2	1	5	4	1				1				
11/13/59	15			2	7	6									
11/24/59	49	9	34	6											
12/17/59	22	1	19	1	1										
Total	262	14	127	64	26	13	5	2	2	1	3	2			3

* Each mouse received intravenously 0.2 ml of a 1/50 culture dilution.

TABLE II

Survival Time of Swiss and C57B1 Mice Infected with M. tuberculosis (Vallée)*
(Summary of Data Presented in Table I)

Strain	Total No. mice	Per cent dying at intervals (days) after infection								Mean ± SE
		16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	
C57B1	262	5.3	72.9	14.9	2.8	1.1	1.9	0.0	1.1	28.1 ± 0.6
Swiss	121	0.0	0.8	0.0	19.0	62.0	14.9	3.3	0.0	55.3 ± 0.6

* Each mouse received intravenously 0.2 ml of a 1/50 culture dilution.

tremely susceptible, and C57B1 mice strikingly resistant, to corynebacterial disease (11). The resistance of the latter strain was correlated with a naturally occurring latent infection with *C. kutscheri* (12). In agreement with these findings, repeated tests throughout the period of the genetic studies of tuberculosis revealed that mice of the C57B1 strain were healthy carriers of *C. kutscheri*, and that Swiss mice in our laboratory were free of this agent.

Experimental tuberculosis in mice can be enhanced by a superimposed or current infection with either of two pneumotropic viruses, PVM or PR8 (13). Evidence has also been presented that latent corynebacterial disease can be activated by superimposed infections such as salmonellosis (14), or infectious ectromelia (15). For these reasons it was necessary to determine whether the susceptibility of C57B1 mice to experimental tuberculosis was the result, in part at least, of activation of their preexisting latent corynebacterial infection. To investigate this problem, mice were examined bacteriologically for possible evocation of corynebacteria from the latent state during *in vivo* multiplication of virulent tubercle bacilli.

C57B1 mice, 4 to 6 weeks old, were injected intravenously with 0.2 ml of a 1/50 culture dilution of bovine tubercle bacilli and were then sacrificed at 1, 3, 9, 15, or 22 days after infection. The homogenates of kidneys, spleen, liver, and lungs from individual mice were plated in duplicate onto OA agar (for enumeration of tubercle bacilli) and onto PF agar (for enumeration of *C. kutscheri*) (11). A similar procedure was carried out with animals that had not received tubercle bacilli. No growth of *C. kutscheri* or of its avirulent form (11, 12) was obtained from organ homogenates of any mice, not even from animals sacrificed just before death (22 days after infection), at which time one might have expected secondary invasion of the tissues by potentially virulent organisms of endogenous origin. As can be seen in Table III, rapid multiplication of tubercle bacilli, culminating in death of animals within 22 days, occurred in all organs. Gross inspection of the organs revealed progressive tuberculous disease without any evidence of the characteristic renal and hepatic lesions caused by corynebacteria (11, 12).

Thus it is apparent that experimental infection of C57B1 mice with tubercle bacilli did not activate latent *C. kutscheri*, and that the great susceptibility of these animals to tuberculous infection is not the result of concurrent infection with corynebacteria.

Factors Affecting Survival after Tuberculous Infection.—The difference in susceptibility to experimental tuberculosis between C57B1 and Swiss mice was altered when the conditions of infection were changed, as demonstrated in the following experiments:

Effect of age of mice at time of infection: Mice of various ages were inoculated with 0.2 ml of a 1/50 culture dilution of *M. tuberculosis*. The results of testing the groups are presented in Fig. 1 and Table IV. The mathematical calculations are based on

mice surviving 16 days or more after infection, since mice dying earlier, with rare exceptions, showed no lesions in the lung, and death was therefore attributed to extraneous causes. The relative susceptibilities of C57B1 and Swiss mice tested when 4 to 6 weeks old are shown in the top frame of Fig. 1. The mean survival time after

TABLE III

Bacteriologic Studies on C57B1 Mice after Infection with M. tuberculosis (Vallée)*

Time sacrificed postinjection	No. of colonies recovered from organ homogenates			
	Kidneys	Spleen	Liver	Lungs
days				
1	12‡	1100	c	190
	23	2100	1200	250
	0	1000	3800	200
	17	1500	1×10^4	100
3	25	3600	2×10^4	180
	28	4400	1×10^4	220
	20	3000	1×10^4	c
	28	3600	1×10^4	450
9	600	6×10^4	c	c
	890	9×10^4	2×10^5	3×10^4
	c	c	c	2×10^4
	c	9×10^4	2×10^5	3×10^4
15	9000	$>10^5$	$>10^5$	$>10^5$
	6000	9×10^4	$>10^5$	$>10^5$
	8200	6×10^4	$>10^5$	$>10^5$
	1000	5×10^4	$>10^5$	$>10^5$
22§	8000	$>10^4$	$>10^5$	$>10^5$
	8000	$>10^4$	c	$>10^5$
	10000	$>10^4$	$>10^5$	$>10^5$

* Each mouse received intravenously 0.2 ml of a 1/50 culture dilution.

‡ The figures represent the number of colonies obtained on OA agar inoculated with 0.025 ml of homogenate. Multiply (× 200) to obtain total population per organ.

§ Three other animals, dead at this time period, were not examined.

c, contaminated.

infection was 33.9 ± 2.1 days for C57B1 mice and 50.5 ± 1.5 days for Swiss mice. When tested at 4 to 10 months of age, the C57B1 mice proved much more resistant. At this time they behaved like the Swiss mice, the means being practically the same (51.2 ± 1.8 for the Black and 51.3 ± 2.9 for the Swiss). From this point on, in every group tested, the susceptibilities of the strains were reversed, and sometimes the difference between them is mathematically significant. For example, in mice infected between 11 and 15 months of age, the mean survival time of the C57B1 was 51.8 ±

1.2 days and of the Swiss it was 45.8 ± 1.1. Compared by means of the t test, $P < 0.001$ (Table IV). Here the phenomenon of early deaths became prominent. This latter finding does not represent a specific increase in susceptibility to tuberculosis but rather

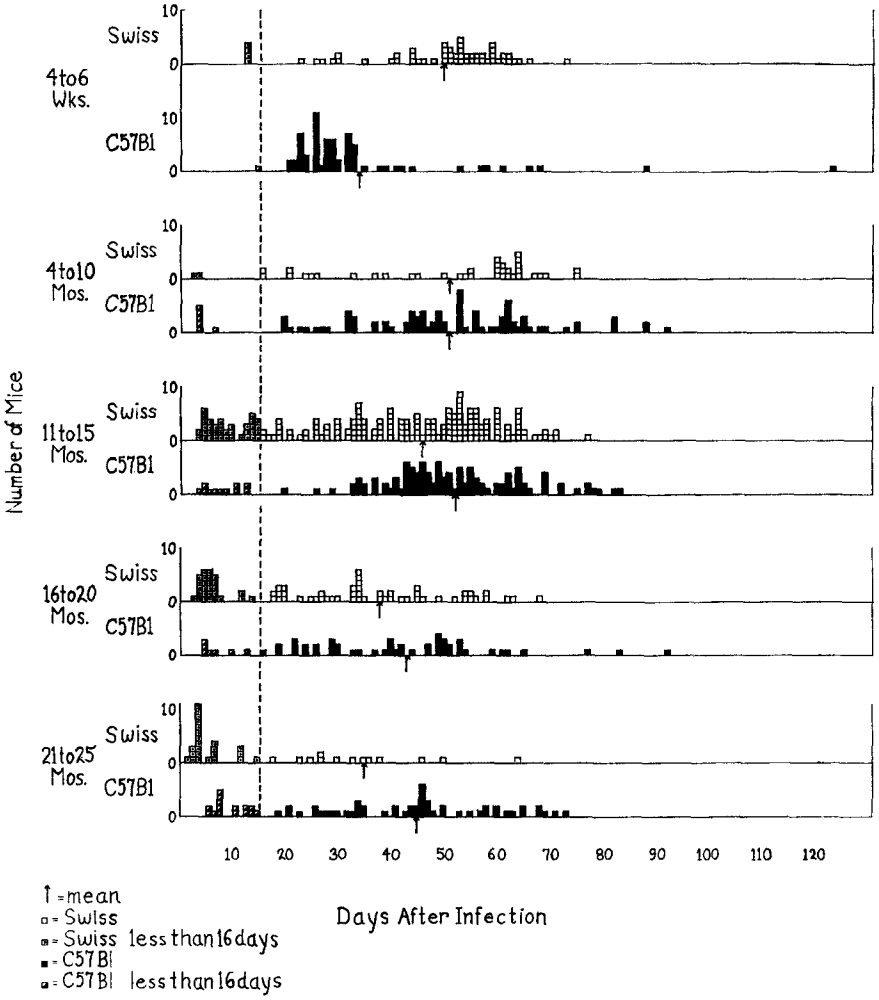

FIG. 1. Survival time of C57B1 and Swiss mice infected at different ages. Each mouse received intravenously 0.2 ml of a 1/50 culture dilution of *M. tuberculosis* (Vallée).

a generalized decrease in resistance to various stressful agents, death being triggered by the insult of the challenge infection. The occurrence of early deaths was again noteworthy in the next two age groups (16 to 20 months and 21 to 25 months). In both of these the reversed susceptibility was maintained. Though the difference between strains is not significant in the 16 to 20 month age group, it is significant in mice in-

fected at 21 to 25 months where $P < 0.02 > 0.01$. It is also significant when the two groups are combined ($P < 0.01$). When the data for the three latter groups are combined, that is including all mice infected at 11 months or older, the mean survival time for the C57B1 was 48.1 ± 1.0 days and for the Swiss the mean was 43.2 ± 1.0, and by the t test for significance $P < 0.001$. Although some of these differences are mathematically significant, their extent was in no case as great as it was in the young mice when the susceptibilities were reversed.

Thus in comparing the susceptibility of mouse strains to experimental tuberculosis, it is important to take into account the age of the animals at the time of infection.

TABLE IV

Comparison of Survival Time of C57B1 and Swiss Mice Infected at Different Ages

Age at infection	Strain	No. mice	Survival time (days) Mean ± SE	t	df	P value
4 to 6 wks.	Swiss	52	50.5 ± 1.5	6.14	116	<0.001
	C57B1	66	33.9 ± 2.1			
4 to 10 mos.	Swiss	37	51.3 ± 2.9	0.02	121	>0.90
	C57B1	86	51.2 ± 1.8			
11 to 15 mos.	Swiss	149	45.8 ± 1.1	3.55	256	<0.001
	C57B1	109	51.8 ± 1.2			
16 to 20 mos.	Swiss	49	37.8 ± 1.9	1.61	95	>0.10
	C57B1	48	42.8 ± 2.4			
21 to 25 mos.	Swiss	13	34.8 ± 3.5	2.41	64	<0.02
	C57B1	53	45.3 ± 2.0			
16 to 25 mos.	Swiss	62	37.2 ± 1.7	2.91	161	<0.01
	C57B1	101	44.1 ± 1.5			
11 to 25 mos.	Swiss	211	43.2 ± 1.0	3.47	419	<0.001
	C57B1	210	48.1 ± 1.0			

That age has an effect upon the outcome of tuberculous infection has also been reported for a strain of DBA mice (8).

Effect of decreasing the infective dose: C57B1 and Swiss mice, 4 to 6 weeks of age, were inoculated intravenously with either 0.2 ml of a 1/50 culture dilution or with 0.2 ml of a 1/500 culture dilution.

As results were obtained in three experiments in two consecutive years, the combined data are presented together in Table V.

Strain differences observed with the standard dilution of 1/50 were consistent with those reported previously. The mean survival time for C57B1 animals (24.9 ± 1.1 days) was shorter than that for the Swiss (54.5 ± 0.9), though the range of the former (16 to 81 days) overlapped that of the albino (28 to 83 days). Survival was naturally prolonged in both strains when the infective inoculum was reduced tenfold, but the

effect was more striking in the case of C57B1 mice than of the Swiss mice; the mean survival time was 125.1 ± 4.8 days for the former as against 89.9 ± 2.6 days for the latter. Furthermore, one C57B1 individual lived 202 days, whereas all Swiss mice were dead by the 113th day.

Thus, the relative susceptibilities of C57B1 and Swiss strains to experimental tuberculosis are dependent upon the numbers of organisms injected.

In Vivo Multiplication of Tubercle Bacilli.—Attempts were made to determine whether the differences exhibited by the two strains, regardless of the size of the infective dose, were related to the multiplication of the infecting organisms during the early phase of infection. In the following experiment, *in*

TABLE V

Effect of Size of Infective Inoculum on Survival Time of Swiss and C57B1 Mice after Infection with M. tuberculosis (Vallée)

Dilution of culture	Mouse strain	Total No. Mice	Time of death (10 day periods) after infection*																				Mean ± SE	SD ± SE
			16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–150	151–160	161–170	171–180	181–190	191–200	201–210		
1:50	C57B1	63	9	50	2	1				1													24.9 ± 1.1	8.5 ± 0.8
	Swiss	46		1		4	35	6		1													54.5 ± 0.9	6.2 ± 0.6
1:500	C57B1	75		3	2	2	1	2			3	5	11	12	8	8	4	3	3	6	1	1	125.1 ± 4.8	41.4 ± 3.4
	Swiss	41		1		2			2	14	14	4	4										89.9 ± 2.6	16.6 ± 1.8

* Each mouse received intravenously 0.2 ml of indicated culture dilution.

vivo multiplication of tubercle bacilli within the organs of mice receiving a small inoculum was followed during the 2 week period after infection.

Mice, 4 to 6 weeks old, of both strains were infected intravenously with 0.2 ml of a 10^{-3} culture dilution of the Vallée culture and were then sacrificed at 1, 2, 7, or 14 days after injection. Enumeration of living tubercle bacilli present in homogenates of kidneys, spleen, liver, and lungs from individual animals was carried out as described previously, homogenization of the organs being accomplished by the use of a teflon grinder (10).

As seen in Table VI, there was no marked difference between the two mouse strains with regard to the numbers of colonies of tubercle bacilli recovered from various organs during the 2 week period following infection. The strain differences were therefore not correlated with differences in the initial rate of bacterial multiplication *in vivo*. These findings support the view expressed earlier that the high resistance to tuberculosis of certain mouse strains depends not upon a greater ability to prevent the infection from becoming established, but rather upon their ability to retard the progression of the disease (5).

Similar results have been obtained with virulent as well as attenuated strains of tubercle bacilli in comparative studies of the course of the infective process in C57B1 and other mouse strains (9, 16).

TABLE VI

*Multiplication of M. tuberculosis (Vallée) in Swiss and C57B1 Mice**

Time sacrificed postinjection	No. of colonies isolated from organ homogenates							
	Swiss mice				C57B1 mice			
	Kidneys	Spleen	Liver	Lungs	Kidneys	Spleen	Liver	Lungs
days								
1	0	5‡	22	0	0	5	22	0
	0	11	22	0	0	6	21	0
	0	11	13	0	0	7	19	0
2	0	11	37	0	0	15	37	0
	1	11	45	0	0	23	31	0
	1	15	47	1	0	9	c	0
7	5	900	400	8	0	270	180	10
	14	1100	1100	c	1	160	200	2
	6	1000	20	9	3	9000	430	16
14	150	4700	2100	1000	20	4600	900	2800
	33	2700	1700	420	c	11000	1100	1000

* Each mouse received intravenously 0.2 ml of a 10^{-3} culture dilution.

‡ The figures represent the number of colonies of tubercle bacilli obtained from 0.025 ml of homogenate. Multiply (×200) to obtain total bacterial population per organ.

c, contaminated.

Breeding Tests

Genetically Designed Breeding of C57B1 and Swiss Strains: Comparison of the F_1 and Backcross Generations to the Parental Strains with Respect to Survival after Infection with Tubercle Bacilli.—The foregoing experiments have revealed some of the factors that influence the outcome of tuberculous infection in C57B1 and Swiss mice. The standard conditions of infection chosen for the genetic studies that follow should be restated: (*a*) all mice were no more than 4 to 6 weeks of age when tested; (*b*) each animal was injected intravenously with 0.2 ml of a 1/50 culture dilution of *M. tuberculosis* Vallée; and (*c*) Rockland mouse pellets and water were supplied *ad libitum.* Under these conditions, the C57B1 strain can be classified as relatively susceptible to experimental tuberculosis; the Swiss strain, as relatively resistant.

The genetic relationship indicated by the contrasting response to infection as shown by the C57B1 mice compared with Swiss mice was further tested by making reciprocal crosses between the strains and comparing the survival time of the progeny after infection with tubercle bacilli. Control groups from the C57B1 and Swiss stock were inoculated with each experimental group.

The results of the crosses are set forth in Tables VII and VIII and illustrated in Fig. 2, which present also the data from the backcrosses to be described later.

The analysis of the data is based upon mice living less than 120 days after injection of the standard inoculum. Occasionally individuals survived for as long as 10 months. Inasmuch as no evidence of disease was noted when mice living

TABLE VII

Survival Time after Infection of Mice of Swiss Strain, F_1 (C57B1 × Sw), and Backcrosses to Swiss*

Group	No. mice	Survival time		
		Range	Mean ± SE	SD ± SE
		days	*days*	*days*
Swiss stock	121	28–74	55.3 ± 0.6	6.6 ± 0.4
F_1	238	20–106	70.3 ± 1.0	15.5 ± 0.7
1st BC	89	21–111	72.4 ± 1.4	12.8 ± 1.9
2nd BC	102	20–103	52.5 ± 1.5	15.6 ± 1.1
3rd BC	226	16–109	49.7 ± 0.9	13.7 ± 0.6
4th BC	210	20–78	46.8 ± 0.6	10.0 ± 0.5

* All mice were injected intravenously with 0.2 ml of 1/50 culture dilution of *M. tuberculosis* (Vallée).

longer than 120 days were finally sacrificed, it cannot be determined whether they exhibited complete resistance to infection or whether the inoculum had been ineffectively administered. In those groups in which such mice occurred, the number of survivors usually did not exceed 1 to 2 per cent of the total number infected, but in the 2nd and 3rd backcrosses to the C57B1, for some unexplained reason, it was 6 and 11 per cent respectively. In the 4th backcross, however, it fell again to about 3 per cent. In mice surviving less than 16 days death could not be attributed to tuberculosis; such individuals are not shown on the chart, and have been omitted from the calculations.

The mice in these experiments were of different colors. The line of Swiss used carries the genes for albino, black, and agouti, and when crossed with the black strain (C57B1) produces black agouti (the color of the common house mouse) offspring in the F_1 generation. The progeny from the backcross of F_1 mice to the Swiss were of two kinds, black agouti (like the F_1 parent), or albino (like the Swiss): and from the backcross in the other direction they were either black agouti or black (like the C57B1 parent).

The black agouti offspring in the backcross appeared to be somewhat more resistant than the black, and there was some suggestion that females were more susceptible than males, but since there was no mathematically significant difference attributable to either coat color or sex, no allowance for these characteristics is made in the tabulations.

The data show clearly the difference between strains as already recorded in Tables I and II. It is also evident that the F_1 exhibited even greater resistance than the resistant parental Swiss stock. This was true irrespective of the way

TABLE VIII

Survival Time after Infection of Mice of Strain C57B1, F_1 (C57B1 × Sw), and Backcrosses to C57B1*

Group	No. of mice	Survival time			Ratio No. mice dead 16–41 days:42–120 days
		Range	Mean ± SE	SD ± SE	16–41:42–120
		days	*days*	*days*	
C57B1	262	16–85	28.1 ± 0.6	9.9 ± 0.4	
F_1	238	20–106	70.3 ± 1.0	15.5 ± 0.7	
1st BC	48	20–41	29.4 ± 0.7	4.7 ± 0.5	
	76	54–115	77.8 ± 1.6	14.0 ± 1.1	0.6 : 1
2nd BC	97	19–41	26.9 ± 0.5	4.9 ± 0.4	
	42	45–90	63.5 ± 1.7	11.3 ± 1.2	2.3 : 1
3rd BC	136	18–41	25.3 ± 0.4	4.2 ± 0.3	
	21	42–120	68.0 ± 4.6	21.1 ± 3.3	6.5 : 1
4th BC	176	18–39	24.2 ± 0.3	3.8 ± 0.2	
	13	42–112	58.9 ± 5.4	19.6 ± 3.8	13.5 : 1

* Each mouse received intravenously 0.2 ml of a 1/50 culture dilution.

the cross was made; the data revealed that no special influence was exerted by either sire or dam on the resistance of the F_1 as a whole, or when divided according to sex; therefore the results of the crosses are not given separately. The mean survival time for all F_1 mice tested was 70.3 ± 1.0 days, although there was great variability. Since the original strains were highly inbred, it might have been expected that the F_1 would be as nearly uniform as one of the parent strains; actually the F_1 variability was greater than either, the standard deviation being 15.5 ± 0.7 days, compared with 9.9 ± 0.4 for the C57B1, and 6.6 ± 0.4 days for the Swiss.

The overdominance exhibited by animals of the F_1 generation suggests that

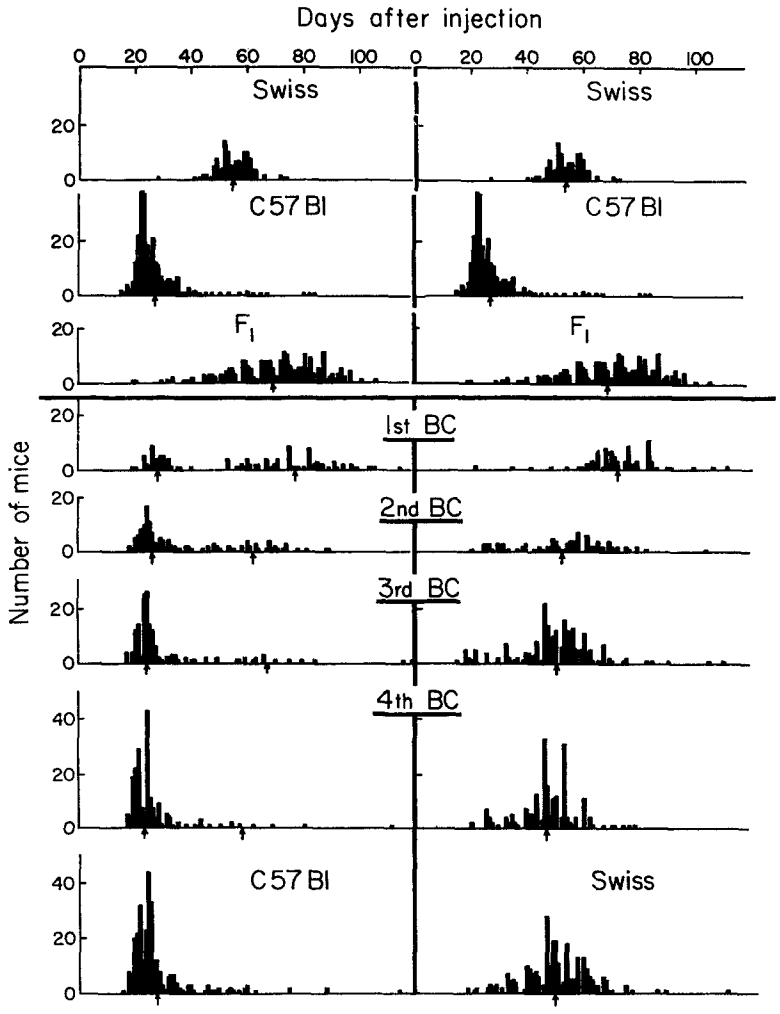

FIG. 2. Survival times after infection. Male and female mice, 4 to 6 weeks of age, were inoculated intravenously with 0.2 ml of a 1/50 culture dilution of *M. tuberculosis* (Vallée). Frames one and two: Swiss and C57B1 mice tested 1956 to 1959. Frame three: F_1 (Swiss × C57B1). Frames four through seven: 4 successive backcrosses; left, to strain C57B1, right, to Swiss strain. Frame eight: Swiss and C57B1 mice tested 1960 to 1963.

a number of genes might be operative. Possibly, factors for resistance may have been contributed by the susceptible stock and were more effective in the new combination of genes; epistasy may have contributed to the result. Another hypothesis is that heterosis accounts for the increased resistance. Hybrid vigor might play an important role in combatting an infection such as tuberculosis

by endowing the F_1 generation with greater capacity than that possessed by the inbred lines to adapt to a variety of conditions.

In support of the view that the F_1 hybrids were more vigorous, data may be cited with regard to their productivity as measured by the number of living young per litter at birth. Table IX shows that mothers of the F_1 hybrid generation had an average of 7.3 ± 0.4 mice per litter, which is significantly larger than the average litter size from mothers from the Swiss ($P < 0.01$) or the C57B1 ($P < 0.001$) sublines used in these experiments.

To test further for genetic control of susceptibility, backcrosses were produced by mating F_1 males and females with mice of both the Swiss and C57B1 parental strains. In the results of the first backcross to the Swiss in Fig. 2 and Table VII, it can be seen that the progeny were as resistant as the F_1's, the mean survival time of the backcross mice (72.4 ± 1.4 days) being slightly

TABLE IX

Number of Live Young Born in First Litters of Swiss, C57B1, and F_1 Mice

Group	No. of litters	No. of mice per litter	Comparison of groups		
		Mean ± SE	t	df	*P* value
Swiss	92	5.9 ± 0.2			
			3.22	130	<0.01
F_1	40	7.3 ± 0.4			
			4.67	150	<0.001
C57B1	112	5.3 ± 0.2			

higher, though not significantly so (Table X). In contrast, mice from the backcross in the opposite direction behaved quite otherwise (Fig. 2 and Table VIII). They fell into two groups separated by a period of 13 days during which no deaths occurred. One group in this 1st backcross, in which the deaths ranged from the 54th to the 115th day after infection, had a mean survival time of 77.8 ± 1.6 days, with standard deviation of 14.0 ± 1.1 days. This is higher than, and significantly different from, that of the F_1 generation ($P < 0.001$, Table X). The other group, with an earlier mortality and narrower range of survival (from the 20th to the 41st day after infection) had a mean survival time of 29.4 ± 0.7 days with a standard deviation of 4.7 ± 0.5 days. This mean survival time differs from that of the first group by 48.4 days; in fact, the response of this second group to infection was similar to that of the parental C57B1 strain, and the difference between their mean survival values is not statistically significant (Table X). The separation of these backcross progeny into two groups indicates a segregation of genetic factors and suggests the dominance of a gene or genes for resistance deriving from the Swiss. The number of individuals in

the first backcross dying between 20 to 41 days *versus* those dying between 54 to 115 days are in a ratio of 0.6:1. While this does not differ significantly from a simple Mendelian ratio for a single pair of genes, other interpretations, such as a larger number of genes with linkage, are possible.

The interpretation of the high mean survival time of the 1st group (over 41 days) is not clear. What part might be attributed to heterosis and what part reflects genetic influences cannot be decided, especially since in collateral experiments also, carried out at this time, the survival time of the C57B1 stock was somewhat elevated, and as already noted, the mean of the first backcross to the Swiss also was high.

TABLE X

Comparison of the Mean Survival Time of Various Groups of Mice after Infection with M. tuberculosis (Vallée): Swiss, C57B1, F_1, and 1st Backcrosses, 1956 to 1959

Groups compared		t	df	*P* value
Swiss	*vs.* C57B1	27.11	381	<0.001
"	*vs.* F_1	10.12	357	<0.001
F_1	*vs.* BC to Swiss	1.11	325	>0.20
Swiss	*vs.* "	12.58	208	<0.001
C57B1	*vs.* F_1	36.52	498	<0.001
"	*vs.* BC to C57B1 (54–120 days)	34.79	336	<0.001
F_1	*vs.* "	3.75	312	<0.001
BC to C57B1 (20–41 days)	*vs.* "	23.06	122	<0.001
"	*vs.* C57B1	0.89	308	>0.30

Progeny tests were not undertaken at this time, but backcrosses in both directions were continued in order to obtain additional information regarding the heritability of the type of response to challenge by *M. tuberculosis*.

In carrying out this plan, males and females from the first backcross were mated, each in the same direction as before, with individuals of the parent strains, and this procedure was continued by crossing hybrids of each succeeding backcross generation with the appropriate parental stock until progeny were obtained from the 4th backcross. About 20 crosses were made in each case, to yield roughly 100 to 200 progeny to be tested for susceptibility to tubercle bacilli. For various reasons, not every mating contributed to each test group. The results of the tests are presented in Fig. 2 and Tables VII and VIII.

Referring to the data from the series of crosses back to the Swiss, it can be seen that the mean survival time of animals from the 2nd backcross was considerably less than that of the F_1's, and less than that of the 1st backcross generation in this direction, being approximately at the level of the parent stock, and not significantly different from it ($P > 0.05$). This suggests the loss of heterosis and of the effects of the postulated epistatic genes. Tests of the 3rd

backcross gave essentially similar figures. In the 4th backcross the mean was lower than that of the original Swiss stock (tested 1956 to 1959). However, as previously stated, every experiment carried out during this study included control mice from both parental strains. Swiss mice from later inbred generations, tested 1960 to 1963 (mean 49.1 ± 0.7 with SD of 11.8 ± 0.5), and represented in the bottom frame in Fig. 2, had a mean survival time that also, in response to some unknown factor, had decreased slightly but significantly below that originally shown. The mean of the 210 mice comprising the 4th backcross generation did not correspond to that of either of the large groups of Swiss mice, but when compared to the mean survival time (45.9 ± 1.0 with SD 11.7 ± 0.7 days) of the 134 Swiss mice tested at exactly the same time (1962 to 1963), there was no significant difference (Table XI).

On the basis of a single factor, expectation for heterozygotes in the 4th backcross would be 1 in 16 mice. If more genes were involved, progress toward

TABLE XI

Comparisons of the Mean Survival Times of Several Groups of Mice

Groups compared	t	df	*P* value
4th BC to C57B1 1960–1963 *vs.* C57B1 Control 1960–1963	1.61	478	>0.10
Swiss Control 1956–1959 *vs.* Swiss Control 1960–1963	5.43	397	<0.001
4th BC to Swiss 1962–1963 *vs.* " " "	2.29	486	<0.05
" " " *vs.* Swiss Control 1962–1963	0.75	342	>0.40

homogeneity would be slower. Under the conditions of the experiment, the degree of genetic uniformity attained cannot be stated. However, the 4th backcross population responded to infection in the same way as did the inbred Swiss mice tested at the same time.

With respect to the series of backcrosses to C57B1 mice, the general pattern set by the 1st backcross to that stock was repeated with a continued shift of survival values toward that of the original C57B1 parent stock. The ratio of the number of mice surviving 41 days or less to those living more than 41 days postinfection increased in the successive backcrosses. The ratio of the 2 classes changed from 0.6:1 in the 1st backcross, to 2.3:1 in the 2nd, 6.5:1 in the 3rd, and 13.5:1 in the 4th (Table VIII).

A further comparison may be made between the 4th backcross treated as a whole, and its C57B1 controls. Again the degree of genetic uniformity actually attained by the backcross group is unknown, however the mean survival time for mice living less, plus those living more than 41 days was 26.5 ± 0.8 with standard deviation of 10.8 ± 0.6 days, which does not differ significantly from the control stock tested in 1960 to 1963 (mean 28.2 ± 0.7 with standard deviation of 11.8 ± 0.5 days) (Table XI). Earlier and later controls are similar.

Thus it is apparent that by the 4th backcross to the C57B1 and, as already seen in the 4th backcross to the Swiss, groups of mice have been obtained that behaved in response to infection with tubercle bacilli as did the original parental stocks. By means of a controlled breeding program, the influence of heredity has been demonstrated.

On the assumption that a single pair of genes is involved, according to genetic theory, the proportion of resistants in successive backcrosses to the recessive is reduced by half in each generation, and the ratio of susceptibles to resistants would be 1:1, 3:1, 7:1, and 15:1. It can be seen that the ratio of mice with survival time of 41 days or less to those surviving more than 41 days, that was found experimentally in the four backcrosses is not unlike the theoretical expectation for one pair of genes.

However, to conclude that only one pair of genes was involved would be unwarranted, since genetic identification of the type of animal used for backcrossing could not be made, and an unwitting selection of the more susceptible mice as parents could account for some of the results obtained.

Furthermore, the use of the 41st day after infection as a dividing line, though suggested by the data, is an arbitrary one, since the deviations from the mean among the highly inbred C57B1 and among the presumably genetically uniform F_1 class go beyond this point in both directions.

Other types of analysis are possible. For example, comparisons may be made of successive backcross generations, each group considered as a whole. The data at hand support the single gene pair hypothesis, but they too are open to the objection of unconscious bias in the choice of parents for the crosses. Further experiments are under way, and the study of the genes concerned is being continued.

In discussing the mode of inheritance, it may be recalled that previous studies (5) have indicated that a number of inbred strains of mice can be placed in a graded series with respect to susceptibility to tuberculous infection. Such a gradation argues for the existence of a number of genes, with different combinations or frequencies characterizing the different strains.

Although no definite statement can be made concerning the number of genes involved, the overall picture presented by the experiments reported here shows the influence of heredity. Proceeding from the relatively high resistance shown by mice of the F_1 generation, directed backcrossess produced groups of mice that exhibited the susceptibility or resistance to infection with tubercle bacilli that was shown by the parent stocks.

DISCUSSION

The foregoing results have demonstrated that hereditary factors influence the susceptibility of mice to experimental tuberculosis; the difference in host response to infection has been analyzed in the present study with two inbred strains, the Swiss and the C57B1.

As with many other characters, the expression of genetic differences could be demonstrated only under certain well defined environmental conditions. One of the factors shown to be of importance was the age of the animals at the time of infection. In fact, the susceptibility of the two strains cited was apparently reversed when mice infected at 1 year or more of age were compared with those infected when young. The size of the challenge dose also proved of great importance. C57B1 mice were more susceptible than Swiss mice when the infective inoculum was large, but they survived longer than the latter following infection with a small dose. Thus, the variables of infective dose and age of host must be controlled in order to define the genetic differences and to classify mice as resistant or susceptible. The fact that the C57B1 strain is latently infected with corynebacteria, which when activated give rise to fatal pseudotuberculosis, does not seem to affect the course of experimental infection with tubercle bacilli.

The analysis of the genetic factors involved in susceptibility to tuberculous infection was based upon results of crossbreeding experiments. The data obtained were sufficiently clear cut to justify certain conclusions which have been stated in the body of the paper. It may be of interest to compare the results of these crossbreeding experiments in mice with those of earlier studies with families or strains of guinea pigs or rabbits.

Various techniques have been used to demonstrate strain differences in susceptibility in different species of animals. Whereas we infected mice by the intravenous route, and used survival time postinfection as the criterion of susceptibility, Wright and Lewis (1) used survival time after intraperitoneal or subcutaneous infection of guinea pigs with either bovine or human tubercle bacilli. In contrast, Lurie *et al.* (3), working with rabbits, took advantage of the fact that quantitative inhalation of human tubercle bacilli results in discrete lesions in the lung. The ratio of the number of inhaled bacilli to the number primary foci produced was used as a measure of resistance.

In both mice and guinea pigs the breeding experiments gave evidence that a gene or genes for resistance were dominant, but in rabbits the F_1 hybrids obtained by mating resistant and susceptible strains were intermediate in susceptibility. The view that hybrid vigor might have played a part in the overdominance seen in the F_1 generation of the mice studied by us was supported by the evidence from litter size. No account of backcrosses of guinea pig families was given, but the single backcross in rabbit experiments led to the conclusion that the genes concerned with the inheritance of resistance are multiple and additive in nature. In mice, the number of genes has not been determined. Work with small mammals has the advantage that large scale experiments are feasible. In the present report, the ample data obtained from identified generations of mice have provided the following evidence in regard to constitutional influence. Beginning with inbred strains with contrasting susceptibilities to tuberculous disease, intermingling their genes in an F_1 generation, and backcrossing the hybrids according to a definite scheme, it has been possible to

separate out again, groups of mice showing susceptibilities characteristic of the original strains. The conclusion can therefore be drawn that genetic factors influence susceptibility to experimental tuberculosis in mice.

SUMMARY

A study has been made of the genetic aspects of the difference between two inbred strains of mice (C57B1/6 and Swiss) in response to experimental infection with mammalian tubercle bacilli. Males and females, 4 to 6 weeks of age were inoculated intravenously with 0.2 ml of a 1/50 culture dilution of *Mycobacterium tuberculosis* var. *bovis* (Vallée strain) grown in tween albumin medium. Mean survival time for C57B1 animals was 28.1 ± 0.6 days and for Swiss, 55.3 ± 0.6 days postinfection. The characteristic survival time of the two strains was reversed in mice receiving a smaller infective dose. The age of mice at the time of inoculation also affected the results of infection: both C57B1 and Swiss, inoculated at 12 months of age, died at the same rate, but when inoculated at older ages, C57B1 survived slightly longer.

Bacteriologic studies demonstrated that there was no significant difference between the two mouse strains with regard to the numbers of viable units of tubercle bacilli recovered from various organs during the 2 week period following infection with a 10^{-3} culture dilution of Vallée. Moreover, the standard infective inoculum (1/50 culture dilution) did not activate corynebacterial pseudotuberculosis in C57B1 mice, a strain known to be latently infected with *Corynebacterium kutscheri*; rapid multiplication of tubercle bacilli occurred, but no corynebacteria were recovered.

When C57B1 and Swiss strains were crossed, survival tests after infection with the standard inoculum demonstrated that mice of the F_1 generation were more resistant than either parent. Whether the overdominance was due to a new combination of parental genes for resistance or to heterosis was not determined. The increased litter size of the F_1 mice, an evidence of increased vigor, supports the view that heterosis was involved. In backcrosses to the resistant strain (Swiss), survival time gradually became stabilized at approximately the parental level. In the 1st backcross to the susceptible strain (C57B1), survival times fell into two classes indicating segregation of genes, with perhaps dominance of genes from the Swiss. After repeated backcrosses to C57B1, mice of the 4th backcross generation had a survival time essentially the same as that of the original parental strain.

On the basis of having obtained progeny characterized by the original parental susceptibilities after genetic tendencies had been intermingled by crossbreeding, it was concluded that hereditary factors influenced the response of mice to experimental infection with *M. tuberculosis*. The number of genes was not determined.

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BIBLIOGRAPHY

1. Wright, S., and Lewis, P. A., Factors in the resistance of guinea pigs to tuberculosis, with special regard to inbreeding and heredity, *Am. Naturalist*, 1921, **55**, 20.
2. Lewis, P. A., and Loomis, D., Ulcerative types as determined by inheritance and as related to natural resistance against tuberculosis: an experimental study on inbred guina pigs, *J. Exp. Med.*, 1928, **47**, 449.
3. Lurie, M. B., Zappasodi, P., Dannenberg, A. M., Jr., and Weiss, G. H., On the mechanism of genetic resistance and its mode of inheritance, *Am. J. Human Genet.*, 1952, **4**, 302.
4. Long, E. R., and Vogt, A. B., Relation of sex to the course of experimental tuberculosis in mice, *Am. Rev. Tuberc.*, 1941, **44**, 196.
5. Pierce, C. H., Dubos, R. J., and Middlebrook, G., Infection of mice with mammalian tubercle bacilli grown in Tween albumin liquid medium, *J. Exp. Med.*, 1947, **86**, 159.

6 *a*. Grumbach, A., Der erbliche Einfluss auf Überlebensdauer, Organlokalisation und-Manifestation im Tuberkuloseversuch, *Schweiz. Z. Path. und Bakt.*, 1949, **12**, 614.

6 *b*. Grumbach, A., Der erbliche Einflus auf Überlebensdauer, Organlokalisation und-Manifestation im Tuberkuloseversuch an C3H-Mäusen, *Schweiz. Z. Allgem. Path. und Bakt.*, 1952, **15**, 715.

6 *c*. Grumbach, A., Tuberkuloseversuch an genetisch reinem Mäusematerial (C3H), *Bull. Schweiz. Akad. Med. Wiss.*, 1953, **9**, 167.

6 *d*. Grumbach, A., and Beer, H., Tuberkuloseversuche an genetisch bekanntem Mäusematerial Uberlebensdauer von zwei homozygoten Mäusestämmen und der F-1-Generation ihrer Hybriden, *Acta. Genet. Basel*, 1962, **12**, 103.

7. Gray, D. G., and Mattinson, M. W., Detection of small numbers of tubercle bacilli from dispersed cultures, using mice, guinea pigs, and artificial media, *Am. Rev. Tuberc.*, 1952, **65**, 572.
8. Grumbach, F., Contribution à l'étude du terrain dans la tuberculose expérimentale: influence de la constitution héréditaire et de l'âge des souris, *Proc. Intern. Congr. Microbiol. 6th* Rome, 1953, **4**, 331.
9. Gray, D. F., Graham-Smith, H., and Noble, J. L., Variations in natural resistance to tuberculosis, *J. Hyg.*, 1960, **58**, 215.
10. Pierce, C. H., Dubos, R. J., and Schaefer, W. B., Multiplication and survival of tubercle bacilli in the organs of mice, *J. Exp. Med.*, 1953, **97**, 189.
11. Pierce-Chase, C. H., Fauve, R. M., and Dubos, R., Corynebacterial pseudotuberculosis in Mice. I. Comparative susceptibility of mouse strains to experimental infection with *Corynebacterium kutscheri*, *J. Exp. Med.*, 1964, **120**, 267.
12. Fauve, R. M., Pierce-Chase, C. H., and Dubos, R., Corynebacterial pseudotuberculosis in Mice. II. Activation of natural and experimental latent infections, *J. Exp. Med.*, 1964, **120**, 283.
13. Volkert, M., Pierce, C., Horsfall, F. L., and Dubos, R. J., The enhancing effect

of concurrent infection with pneumotropic viruses on pulmonary tuberculosis in mice, *J. Exp. Med.*, 1947, **86,** 203.

14. Wolff, H. L., On some spontaneous infections observed in mice. I. *C. kutscheri* and *C. pseudotuberculosis*, *Antonie van Leeuwenhoek, J. Microbiol. and Serol.*, 1950, **16,** 105.
15. Lawrence, J. J., Infection of laboratory mice with *Corynebacterium murium*, *Australian J. Sc.*, 1957, **20,** 147.
16. Sever, J. L., and Youmans, G. P., The enumeration of nonpathogenic viable tubercle bacilli from the organs of mice, *Am. Rev. Tuberc.*, 1957, **75,** 280.