CORYNEBACTERIAL PSEUDOTUBERCULOSIS IN MICE

I. Comparative Susceptibility of Mouse Strains to Experimental Infection with Corynebacterium kutscheri*

By CYNTHIA H. PIERCE-CHASE, Ph.D., ROBERT M. FAUVE,‡ M.D., and RENE DUBQS, Ph.D.

(*From The Rockefeller Institute*)

(Received for publication, April 10, 1964)

Differences in host response to infection are generally attributed to the classical immunologic phenomena, conditioned by genetic and environmental factors. At the beginning of this century, however, there was much interest in the fact that resistance to infection could result from the persistence of viable forms of the homologous pathogen in the tissues of the host. The resistance to infection due to such persistence was then commonly termed "premunition" or "infection immunity." Although infection immunity was emphasized chiefly with regard to syphilis, relapsing fever, and malaria, it is almost certain that the phenomenon applies to a large variety of other infectious processes, and is of large importance not only in individual but also in herd immunity (1). As will be seen, another interesting aspect of microbial persistence with consequent herd immunity is that it introduces a complicating factor in the analysis of the genetic determinants of resistance to infection.

The fact that most pathogens, if not all, can persist in the body in a non-active state for prolonged periods of time has given rise to an extensive and rather confusing terminology, which has been discussed in detail with respect to viral agents (2). The expressions "carrier state," "latent, dormant, or inapparent infections," "masking and unmasking of infectious agents," etc. have never been clearly defined, and it is often difficult to differentiate the shades of meaning which they are supposed to convey (3). More recently, the word "persister" has been introduced to designate microbial agents which persist in the body despite the efforts to eliminate them either by chemotherapy or by immunological reactions (4, 5).

Many experimental and clinical observations reveal that while microbial persistence is usually accompanied by increased resistance to superinfection, it can also constitute a source of potential danger because the latent infection often evolves into overt disease when the resistance of the host is lowered by physiological disturbances. Thus, infec-

* This investigation was supported in part by a research grant (AI-3790) from The National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service, Bethesda.

‡ Present address: Institut Pasteur, Garches, Seine et Oise, France.

tion immunity and endogenous microbial disease constitute the two opposite manifestations of a single phenomenon which is obviously of great theoretical and practical importance. Unfortunately, there is no understanding of the factors which determine whether infection will remain latent or become activated. Nothing is known, furthermore, of the state in which the microorganisms persist in the host, nor of the humoral or cellular mechanisms which permit them to survive, and thereby to increase resistance to superinfection.

The present and following papers describe results obtained with a specific disease entity, corynebacterial pseudotuberculosis of mice caused by *Corynebacterium kutscheri*, which has aspects bearing directly upon the aforementioned problems.

The original reports of Kutscher (6) and Bongert (7), describing a diphtheroid as an etiologic agent which produces pseudotuberculosis in mice, have been followed by numerous publications demonstrating that many outbreaks of endogenous infection in mouse colonies are caused by *Corynebacterium kutscheri*, particularly when animals are under conditions of physiological stress.[1]

During an investigation in this laboratory of the genetic factors which affect the course of experimental tuberculosis in mice (9), an intercurrent corynebacterial infection was observed in animals from one of the two inbred strains under study. Preliminary experiments revealed that normal mice of these two strains, namely Swiss Lynch and C57Bl/6, differed markedly in their response to experimental infection with *C. kutscheri*, the causative agent isolated from the diseased animals. Various other strains of mice were then compared with respect to survival time and to the extent of bacterial multiplication occurring *in vivo* following inoculation with *C. kutscheri*. These observations are described in the present report and serve to present a laboratory model for the study of certain phenomena associated with latent infection which will be presented in the following paper (8).

Material and Methods

Bacterial Culture.—

Isolation and Identification of Strain.—The culture of *Corynebacterium kutscheri* used in the following experiments was first isolated from a pulmonary abscess occurring unexpectedly in a Swiss Lynch mouse previously inoculated with *Mycobacterium tuberculosis* (Vallée strain). Mice of the highly inbred Swiss Lynch strain have been shown to be relatively resistant to experimental tuberculosis (10). Pathologic examination of the animal just mentioned, dying earlier than predicted after infection with tubercle bacilli, revealed pulmonary abscesses similar to those seen in mouse tuberculosis. Moreover, Ziehl-Neelsen–stained impression smears demonstrated the presence of pleomorphic bacilli having a slight degree of acid fastness. The disease, however, was not tuberculosis. It was proven, by means of cultural studies, to be corynebacterial pseudotuberculosis.

Duplicate plating of the pathologic material onto oleic acid–albumin agar (OA) (11) and onto beef heart infusion containing 1 per cent Pfanstiehl peptone, 0.5 per cent NaCl, and 1.5 per cent agar (PF) yielded no tubercle bacilli on OA and a pure culture of corynebacteria on PF. Repeated subcultures of these newly isolated organisms were made into PF broth from single colonies picked from serial transfers on PF agar in order to insure purity of the culture.

[1] References to this particular aspect of the problem are presented in the following paper (8).

Biochemical tests were carried out in the following media: Mueller's tellurite-serum agar; bacto-urea base concentrate; and carbohydrate redi-discs obtained from Pennsylvania Biological Laboratories, Inc., Philadelphia and used comparatively in three different media at pH 7.3, namely: (*a*) veal infusion broth, (*b*) PF broth previously fermented by 4 hour growth of *Escherichia coli*, and (*c*) a standard carbohydrate free basal medium consisting of 1.0 per cent proteose peptone No. 3, 0.5 per cent bacto-beef extract, and 0.5 per cent NaCl. Fermentation tubes containing one redi-disc in 3.0 ml of medium (1.1 per cent carbohydrate) were inoculated with 0.1 ml PF broth culture or a saline suspension of growth from PF agar. Growth and acid production were determined after 24, 48, and 72 hours of incubation by withdrawing small aliquots and testing with bromthymol blue. The final acidity in tubes giving positive tests was pH 5.1, as tested by bromcresol purple and bromcresol green. Identical results were obtained with all three media. The presence of catalase was determined by suspending sediment growth from PF broth or a loopful of growth from PF agar into a well containing 0.1 ml of 30 per cent hydrogen peroxide.

Identification of the organism as *C. kutscheri* was based upon the following properties: bacterial and colonial morphology typical of corynebacteria, non-motility, reduction of potassium tellurite, presence of catalase and urease, production of acid to pH 5.1 but no gas in fermentation tests with dextrose, levulose, maltose, mannose, salicin, and sucrose; negative fermentations with adonitol, arabinose, dulcitol, galactose (24 hour reading of pH 6.8–7.0 which returned to pH 7.3 upon further incubation), inositol, inulin, lactose, mannitol, melibiose, melizitose, raffinose, rhamnose, sorbitol, sorbose, starch, trehalose, and xylose. The pathogenicity for mice is discussed later in this present report.

Four other strains of corynebacteria isolated from mice in other laboratories were used in comparative virulence studies. One of these strains, *Corynebacterium cardezium* (CC), was kindly supplied by Dr. Ioulios A. Iossifides (12). With the exception of CC, all strains of corynebacteria exhibited biochemical characteristics identical with our own laboratory strain of *C. kutscheri*. The strain, CC, grew more slowly and differed slightly in fermentation properties (13). Suspensions of all 5 strains agglutinated to equivalent titers in rabbit antiserum prepared according to the Wittler technique (14) against our own strain.

Maintenance of Culture.—The medium used in the majority of experiments was PF agar or broth at pH 7.4. Difco veal infusion agar or broth could be used instead. Stock cultures were maintained on PF agar slants incubated 24 hours at 37°C and then stored in the refrigerator. To obtain cultures for infection experiments, at least 2 serial transfers were made into PF broth. Fresh isolates were made occasionally from the lesions of infected animals, but this was not necessary to maintain the virulence of the strain. Numerous serial transfers of 0.1 ml of 24 hour broth cultures did not decrease the infectiveness of the bacteria.

Enumeration of Viable Units for Infection.—Inocula for infection *via* the intravenous route were prepared from broth cultures incubated at 37°C for 24 hours. Serial tenfold dilutions were made in an aqueous solution of 0.1 per cent bovine albumin (fraction V). For each experiment, the numbers of viable bacterial units injected were determined by plating dilutions of 10^{-5}, 10^{-6}, and 10^{-7} onto agar and recording the number of colonies appearing after incubation. The enumeration procedure revealed that 24 hour cultures yielded an approximate constant population of 3×10^8 viable units per ml. An infective dose of 0.2 ml of 10^{-4} culture dilution administered intravenously (*i.e.* approximately 60,000 viable units of *C. kutscheri* per animal) was used for most of the experiments. The numbers of viable units in other dilutions of culture can be calculated proportionately.

Strains of Mice.—

The majority of experiments were conducted with the 2 inbred strains of mice, Swiss Lynch and C57Bl/6, both raised at The Rockefeller Institute by Dr. Clara J. Lynch. They were main-

tained on a diet of Purina laboratory chow (free of antibiotics) and water given *ad libitum*. The sources of other strains tested are indicated for individual experiments (Tables IV and V). A comprehensive listing of the origin of inbred mouse strains and of commercially available animals can be found elsewhere (15, 16).

All animals were housed, 4 or 5 per box, on cedar shavings in metal boxes with wire grid tops; the boxes were kept in an enclosed cabinet exhausted into an exterior duct under slight negative pressure. The temperature varied from 72–75°F and the relative humidity from 20 to 26 per cent. All animals were placed on a diet of Rockland mouse pellets and water *ad libitum* 24 hours prior to infection and were maintained on this regimen throughout the course of experiments.

Intravenous injections of *C. kutscheri* were made into the lateral caudal veins. Mice were examined daily and were autopsied either at death or upon termination of the experiment. Verification of the etiologic role of *C. kutscheri* was achieved by observing the presence of gross abscesses in the kidneys, liver, or lungs, by demonstrating typical corynebacteria in impression smears stained by Giemsa, and by occasional bacteriologic isolation. A detailed description of corynebacterial pseudotuberculosis and its causative agent can be found elsewhere (17, 18).

Enumeration of Viable C. kutscheri from Infected Organs.—

Mice were sacrificed with chloroform at various intervals of time after the intravenous injection of *C. kutscheri*. The kidneys, lungs, spleen, and liver were removed aseptically, the gall bladder being discarded. Individual organ homogenates were prepared in 2 per cent aqueous bovine albumin by means of teflon homogenizers (obtained from Tri-R Instruments, Long Island City, New York), and serial tenfold dilutions were made in 0.1 per cent aqueous bovine albumin; 0.025 ml of dilution was plated onto PF agar according to a previously described technique (19). The numbers of colonies obtained after incubation at 37°C for 24 hours allowed calculation of the numbers of viable units of *C. kutscheri* present in the host organs.

EXPERIMENTAL RESULTS

Response of a Susceptible Mouse Strain (Swiss Lynch) to Intravenous Infection with C. kutscheri.—The severity of disease produced in the Swiss Lynch mouse strain by infection with *C. kutscheri* differs from case to case when the agent is administered *per os*, intraperitoneally, subcutaneously, or *via* aerosol. In contrast, intravenous inoculation elicits a more uniform response. This can be measured by death rate, and by the presence of gross pearly abscesses in the lungs, kidneys, and liver. Microscopic examination of stained smears prepared from lesions reveals corynebacteria located both intra- and extracellularly.

The results in Table I are typical of those obtained in experiments carried out in the course of 5 years. Regardless of age or sex of mice, an inoculum of 0.2 ml of 10^{-4} culture dilution given intravenously produces 100 per cent mortality, the majority of animals dying between the 4th and 7th day postinjection.

Comparison of Mouse Strains with Regard to Intravenous Infection with C. kutscheri.—The striking difference in response to infection between the Swiss Lynch and C57Bl/6 mouse strains is illustrated in Table II. Whereas all Swiss mice inoculated with a 10^{-4} culture dilution of *C. kutscheri* died, all animals of the C57Bl/6 strain survived the infection. Moreover, the mice of this latter group showed no evidence of gross disease when sacrificed at 4 to 6 weeks after

inoculation and, as in the case of Swiss mice, age and sex were without effect upon the outcome. As will be described subsequently however, sacrifice of C57Bl/6 animals during the 1st week after injection revealed the presence of gross abscesses, particularly in the kidneys, thus providing evidence that infection with extensive disease had initially occurred.

TABLE I

*Infection of Swiss Lynch Mice with Corynebacterium kutscheri**

Age at infection	No. mice		Time of death postinoculation, in days								
	Male	Female	3	4	5	6	7	8	9	10	11
wks.											
4	17			2‡	3	8	3				1
		19		4	6	6	2	1			
6	18		1	3	4	3	7				
		14	1	3	1	2	7				
8	33		1	2	9	6	12		2	1	
		30		1	9	11	8	1			
12	17			1	3	6	4	1	1		1
		8	1			5	1		1		
24	22			4	2	11	4		1		
		5			2		1			2	
32	8		1		4	2		1			
Total No. mice.....	115	76	5	20	43	61	49	4	5	3	2

* All mice were injected intravenously with 0.2 cc of a 10^{-4} culture dilution.

‡ Each figure refers to numbers of mice dying on day indicated.

Effect of Inoculum Size on Survival of Susceptible and Resistant Mouse Strains.— As can be seen in Table III, the survival time following intravenous injection (0.2 ml) of dilutions of *C. kutscheri* varied with the inoculum size. It was found that the resistance of C57Bl/6 mice was not absolute, since it could be overcome by inocula larger than that administered by injection of a 10^{-4} culture dilution. Even with the 10^{-1} dilution, however, C57Bl/6 animals died more slowly than the Swiss animals. As shown previously, the infective dose contained in 0.2 ml of a 10^{-4} dilution allowed 100 per cent survival of C57Bl/6 mice, but produced 100 per cent mortality in Swiss mice. In fact, as few as 600 infectious units of *C. kutscheri* (the dose contained in 0.2 ml of a dilution of 10^{-6}) was sufficient to initiate a fatal infection in the latter animals.

TABLE II

*Comparative Susceptibility of Swiss Lynch and C57Bl/6 Mice to Infection with Corynebacterium kutscheri**

Age when injected	Swiss Lynch		C57Bl/6	
	Males	Females	Males	Females
wks.				
4	17‡	19	20‡	32
6	18	14	18	15
8	33	30	21	19
12	17	8	19	8
24	22	5	36	23
32	8	0	9	50
Total No. mice.....	115	76	123	147
	All died		All survived	

* All mice were injected intravenously with 0.2 ml of 10^{-4} culture dilution of *C. kutscheri*.
‡ Figures indicate numbers of mice tested.

TABLE III

Effect of Size of Infective Inoculum of C. kutscheri upon Survival of Swiss Lynch and C57Bl/6 Mice*

Culture dilution	Mouse strain	No. mice	Time of death postinoculation, in days														No. surviving
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10^{-1}	Swiss	24	19‡	5													0
	C57Bl/6	30		1	26	3											0
10^{-3}	Swiss	64			5	33	5	19	1		1						0
	C57Bl/6	21						1	1		4				1		14
10^{-4}	Swiss	191			5	20	43	61	49	4	5	3	2				0
	C57Bl/6	270															270
10^{-5}	Swiss	22			2			8	2	2		3	2			1	2
10^{-6}	Swiss	71						4	12			23	5		1		31

With the exception of animals receiving 10^{-4} dilution (see Table II), all mice were 5 to 6 weeks of age when injected.

* All mice injected intravenously with 0.2 cc of indicated culture dilution.

‡ Number of mice dying on day indicated.

Resistance of C57Bl/6 Mouse Strains Obtained from Different Laboratories.— Mice of the C57Bl/6 strain are widely used and are maintained in a highly in-

bred state in many laboratories. Comparative tests with animals of this strain obtained from various sources were therefore carried out to ascertain whether the resistance to experimental infection with *C. kutscheri* was a general property of C57Bl/6 mice. As seen in Table IV, the survival of all mice following an intravenous inoculation of 10^{-4} culture dilution provides evidence that the resistance of C57Bl/6 animals to this infection is indeed a general characteristic. It is of interest to note, furthermore, that mice from a line of C57Bl/6 in which

TABLE IV

*Sources of C57Bl/6 Mice Tested and Found Resistant to C. kutscheri**

Source of mice	No. tested	
	Males	Females
Dr. C. J. Lynch, The Rockefeller Institute	123	147
Roscoe B. Jackson Memorial Laboratories, Bar Harbor	53	50
" " " " " , 1st generation raised at The Rockefeller Institute)	6	6
Dr. J. J. Bittner, University of Minnesota, Minneapolis	6	6
" " " " , (1st generation raised at The Rockefeller Institute)	12	8
Dr. G. L. Wolff, Institute for Cancer Research, Philadelphia	10	10
Millerton Farms, Millerton, New York	10	10
Darwin Laboratories, Brooklyn, New York	20	10
Dr. C. Haagensen, College of Physicians and Surgeons, Columbia University, New York	40	0
National Institutes of Health, Bethesda	4	1
Roscoe B. Jackson Memorial Laboratories (ragged)‡	28	21
" " " " " (non-ragged)	28	22

* All mice survived intravenous inoculation with 0.2 cc of 10^{-4} culture dilution of *C. kutscheri*.

‡ Mutant ragged carried in heterozygous condition after backcrossing 6 to 10 generations to C57Bl/6. The authors are indebted to Dr. E. L. Green for supplying mice from this line

the dominant mutant "ragged" was carried in heterozygous condition after 6 to 10 generations of backcrossing were as uniformly resistant as the homozygous C57Bl/6 stock. (The mutant was obtained by the incorporation of nine genes from other stocks into a C57Bl background.)

Response of Other Mouse Strains.—The lethal effect of intravenous inoculation of a 10^{-4} culture dilution of *C. kutscheri* was determined for mice of various strains; the majority of animals were 4 to 6 weeks old at the time of injection. As shown in previous experiments, deaths occurred for the most part 4 to 7 days' postinfection, and there was no apparent effect of age or sex upon the final outcome of infection. The findings for each mouse strain are summarized in Table V, reference being made to Table IV.

Strains from 30 different sources were studied, representing different genetic stocks or in a few instances different colonies of the same inbred strain. Six strains were shown to be highly susceptible, 3 to be partially susceptible, and 21 to be highly resistant. Surviving animals were uniformly free of gross evidence

TABLE V

Comparative Susceptibility of Mouse Strains to Infection with C. kutscheri*

Strain	Source	No. total	
		Dead	Tested
Swiss Lynch	Dr. C. J. Lynch, The Rockefeller Institute	191	191
Swiss R/J	Roscoe B. Jackson Memorial Laboratories	50	50
A/Jax	" " " " "	20	20
Princeton	Dr. J. B. Nelson, The Rockefeller Institute	91	91
RFVL	Rockefeller Foundation Virus Laboratories, New York	9	11
CF_1	Carworth Farms, New City, New York	13	41
CF_1 (SPF)	" "	22	24
Rockefeller Swiss	The Rockefeller Institute	2	20
NCS‡	" " "	4	30
CFW	Carworth Farms	0	20
ICR Swiss	Millerton Farms	0	41
Balb/C	Dr. J. S. Henderson, The Rockefeller Institute	0	40
BSVS	Dr. H. A. Schneider, The Rockefeller Institute	0	10
BRVR	" " " " " " "	0	20
RIII	Dr. C. Haagensen, College of Physicians and Surgeons, Columbia University	0	10
YBR/He	Dr. C. J. Lynch, The Rockefeller Institute	0	10
DBA/2	" " " " " " "	0	43
"	Roscoe B. Jackson Memorial Laboratories	0	30
DBA/212	Dr. A. Goldfader, Francis Delafield Hospital, New York	0	10
C57Bl/6	Dr. C. J. Lynch, The Rockefeller Institute	0	270
"	(For other breeding colonies, see Table IV)	0	361

Appreciation is extended to Dr. Dan H. Moore of The Rockefeller Institute for arrangements to obtain mice from the laboratories of Dr. Haagensen and Dr. Goldfader.

* All mice were inoculated intravenously with 0.2 cc of 10^{-4} culture dilution of *C. kutscheri*.

‡ SPF colony derived from Rockefeller Swiss strain (20).

of disease when sacrificed 4 to 6 weeks after injection. Mice of the CF_1 strain were generally resistant to infection, whereas the SPF animals derived from the CF_1 strain were extremely susceptible. Further comparative studies of these two groups could not be made, because the breeding of CF_1 SPF mice unfortunately was discontinued during the present investigation. No essential difference was found between another pair of mouse strains, the Rockefeller Swiss

TABLE VI

Number of Colonies Isolated from Organ Homogenates*

Time of sacrifice postinjection	Swiss Lynch				C57Bl/6 Lynch			
	Spleen	Kidneys	Liver	Lungs	Spleen	Kidneys	Liver	Lungs
min.								
15	21	17	800	10	20	17	550	10
	37	48	500	2	14	8	360 (12,000)‡	20
day								
1	2	$>10^5$	C	2700	1	3.0×10^4	5 (300)	10 (140)
	7	$>10^5$	3.4×10^4	800	0	3.5×10^4	14 (600)	30 (160)
2	130	$>10^6$	4.5×10^5	$>10^6$	3	3.7×10^5	50	C
	120	$>10^6$	3.7×10^5	3.0×10^5	0	4.0×10^5	100 (1400)	C
3	50×10^3	$>10^7$	$>10^7$	$>10^6$	5	$>10^6$	15	50
	89×10^3	$>10^7$	$>10^7$	$>10^6$	2	$>10^6$	4	C
	0.7×10^3	$>10^7$	6.8×10^6	$>10^6$	Dead	—	—	—
	52×10^3	$>10^7$	C	C				
	Dead	—	—	—				
	"	—	—	—				
6	None survived				0	3.7×10^6	2	0
					0	89×10^6	5	0
					Dead	—	—	—
					"	—	—	—
9					0	2×10^4	6	C (2200)
					2×10^3	$>10^7$	7×10^4 (30,000)	C (1600)
					0	2.8×10^4	40	C (400)
					Dead	—	—	—

All mice were inoculated intravenously with 0.2 ml of 10^{-8} culture dilution of *C. kutscheri*.

2 to 4 mice from each strain were sacrificed on the days indicated.

C, contaminated.

* The figures represent the viable units of *C. kutscheri* present in 0.025 ml of homogenate. Multiply (x 200) to obtain total population per organ.

‡ Figures in parentheses indicate atypical small colonies (non-identified). See text.

and its derived NCS strain (20). Animals from both colonies were relatively resistant to infection.

It is apparent from this survey that only few mouse strains were as susceptible to infection as the Swiss Lynch; the majority exhibited the high degree of resistance shown by C57Bl/6 mice. The effect of cortisone administration to

normal, non-infected, mice from the various strains was determined by experiments run in parallel with the infection tests; the results are presented in the following paper (8).

Multiplication of C. kutscheri in Susceptible and Resistant Mouse Strains.—

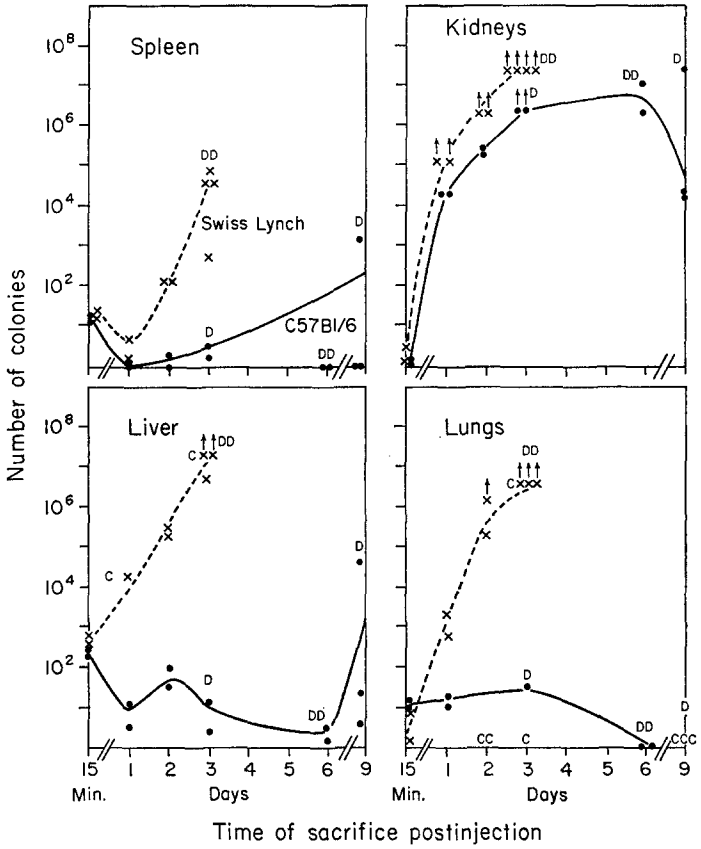

FIG. 1. Number of colonies of *C. kutscheri* isolated from organ homogenates of mice inoculated with 10^{-3} culture dilution. (Schematic representation of data presented in Table VI.)

xxx, Swiss Lynch; • • •, C57Bl/6; D, dead mouse, organs not cultured; C, contaminated culture; ↑, actual figure undetermined but greater than plotted.

Repeated infection tests with *C. kutscheri* in Swiss and C57Bl/6 mouse strains raised at The Rockefeller Institute gave uniformly reproducible results throughout a 5 year period. These 2 strains were therefore used as prototypes to study in further detail the contrast in host response to corynebacterial infection of susceptible and resistant mice.

The extent of bacterial multiplication in various organs was determined by enumerating viable units of corynebacteria recovered at various intervals after intravenous inoculation. Two separate experiments were conducted, one using as inoculum 0.2 ml of 10^{-4} culture dilution, the other, 10^{-3}. In the majority of instances 4 animals per group were sacrificed for each comparative series of bacterial quantitation.

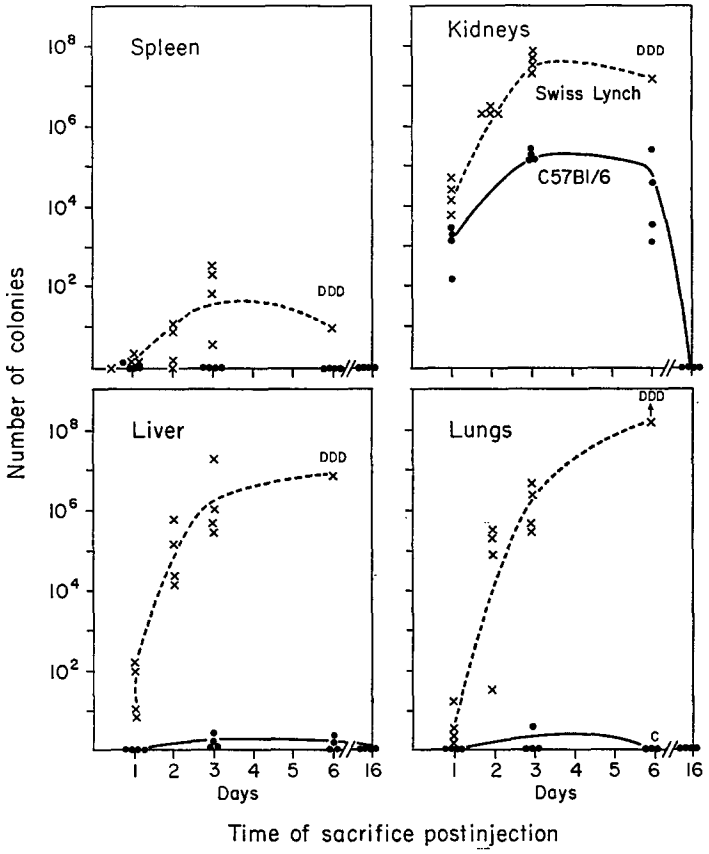

FIG. 2. Number of colonies of *C. kutscheri* isolated from organ homogenates of mice inoculated with 10^{-4} culture dilution. (Schematic representation of data presented in Table VII.)

xxx, Swiss Lynch; • • •, C57Bl/6; D, dead mouse, organs not cultured; C, contaminated culture; ↑, actual figure undetermined but greater than plotted.

It can be seen in Tables VI and VII (also in Figs. 1 and 2) that the difference in susceptibility between mouse strains is indeed reflected in the number of corynebacteria recovered from the organs subsequent to inoculation. In Swiss mice given an inoculum of 10^{-3} culture dilution (Table VI and Fig. 1), rapid bacterial multiplication took place in all organs (though of a limited degree in

TABLE VII

Number of Colonies Isolated from Organ Homogenates*

Time of sacrifice postinjection	Swiss Lynch				C57Bl/6 Lynch			
	Spleen	Kidneys	Liver	Lungs	Spleen	Kidneys	Liver	Lungs
day								
1	0	0.8×10^4	9	2	0	0.2×10^3	0	0 (250) ‡
	1	4.1×10^4	100	3	0	5.7×10^3	0	0 (60)
	1	2.1×10^4	10	1	0	1.7×10^3	0 (5500)	0 (350)
	3	6.5×10^4	210	20	0	2.9×10^3	0 (2300)	0
2	11	3.5×10^6	80×10^4	2.6×10^5	No test	—	—	—
	2	3.5×10^6	11×10^4	0.8×10^5	—	—	—	—
	9	3.4×10^6	2.0×10^4	3.1×10^5	—	—	—	—
	0	4.7×10^6	3.0×10^4	50	—	—	—	—
3	18	4.6×10^7	1.0×10^6	0.6×10^6	1	1.7×10^5	2 (5)	0 (120)
	6	3.5×10^7	0.6×10^6	6.0×10^6	0	2.7×10^5	1	0 (80)
	260	7.5×10^7	0.5×10^6	2.5×10^6	0	3.2×10^5	1	5
	430	5.8×10^7	32×10^6	0.4×10^6	0	1.8×10^5	4 (40)	0 (60)
6	10	1.3×10^7	8.0×10^6	$>10^8$	0	42×10^4	2	C
	Dead	—	—	—	0	1.6×10^3	0	0 (400)
	"	—	—	—	0	5.0×10^3	0	0 (150)
	"	—	—	—	0	60×10^3	3 (4000)	0 (150)
16	None sur- vived				0	0	0	0
					0	0	0 (1)	0
					0	0	0	0
					0	0	0 (5)	0

All mice were inoculated intravenously with 0.2 ml of 10^{-4} culture dilution of *C. kutscheri*.

4 mice from each strain were sacrificed on the days indicated.

C, contaminated.

* The figures represent the viable units of *C. kutscheri* present in 0.025 ml. of homogenate. Multiply (x 200) to obtain total population per organ.

‡ Figures in parentheses indicate atypical small colonies (non-identified). See text.

the spleen), and all animals had died by the 4th day postinfection. In comparison, the multiplication of corynebacteria was retarded in C57Bl/6 mice. Since this infective dose causes death of a certain proportion of animals of this latter strain, it was not surprising that large numbers of organisms could be recovered from individual mice. The bacterial population was highest in the kidneys; this was to be expected since renal abscesses are a predominant feature of the disease.

A more striking difference between the 2 mouse strains was observed with an inoculum of 10^{-4} culture dilution (Table VII and Fig. 2), a dose which results

in 100 per cent mortality among Swiss mice, and survival of all C57Bl/6. Rapid multiplication of corynebacteria occurred in all organs of Swiss mice with the exception of the spleen, and resulted in death of all animals by the 6th day after inoculation. In striking contrast, viable organisms could not be recovered from the spleens of C57Bl/6 mice, and only very few from the livers and lungs. That infection became established but was overcome is shown by the bacterial multiplication in the kidney during the first few days; however, the microbial population never reached as high a level as in Swiss mice, and by the 16th day postinjection, all tissues of C57Bl/6 animals were free of culturable corynebacteria. These findings are in keeping with the fact that although gross renal abscesses are found during the early phase of infection in animals of this strain, they do not leave any residual pathology, as proven by autopsy of mice sacrificed at 1 to 6 days and then later at 2 to 4 weeks' postinfection.

Whereas corynebacteria were recovered only occasionally and only in small numbers from the lungs and livers of C57Bl/6 mice, very small translucent colonies appeared on the plates in numbers sufficiently large to suggest that they might have significance. However, these colonies did not develop into typical corynebacteria upon further incubation nor upon repeated subcultures, and they proved to be avirulent in Swiss mice even when large inocula were injected. The implication of these findings, and their relevance to infection immunity and activation of latent infection, will be considered in the general discussion at the end of the following paper (8).

SUMMARY

The susceptibility of mice to experimental infection with *Corynebacterium kutscheri* was studied by comparing the host response to this organism of mice obtained from 31 different colonies, representing 15 different genetic types.

A standardized infective dose, administered intravenously, made it possible to separate the animals into two sharply differentiated groups. All the animals of the following colonies died: Swiss Lynch, Swiss R/J, A/Jax, Princeton, RFVL, and CF_1 (SPF). All the animals of the following colonies survived: CFW, ICR, Balb/C, BSVS, BRVR, RIII, YBR/He, DBA/2 (from 3 different colonies), and C57Bl/6 (from 12 different colonies).

The two highly inbred strains, Swiss Lynch and C57Bl/6, were selected as prototypes of susceptible and resistant animals respectively, for more detailed studies.

Following injection of an infective dose of 0.2×10^{-4} ml of culture of *C. kutscheri*, all Swiss Lynch animals died within 3 to 11 days (the majority within 4 to 7 days); whereas all C57Bl/6 animals survived. The outcome of the infection in each strain was independent of age and sex of the animals.

In Swiss Lynch animals, the corynebacteria multiplied rapidly in lungs, liver, kidneys, and to some extent in the spleen. In C57Bl/6 mice, there was no in-

crease of the corynebacterial population in the lungs, liver, or spleen, but multiplication occurred in the kidneys during the early phase of the infectious process with resultant abscess formation. However, the renal infection soon subsided leaving no residual pathology. *C. kutscheri* could not be recovered from any organs of C57Bl/6 mice sacrificed 16 days after infection.

Homogenates of organs from Swiss Lynch mice obtained while the infection was progressing contained only typical *C. kutscheri.* In contrast, the lungs and livers of similarly infected C57Bl/6 animals occasionally yielded large numbers of small translucent colonies distinctly different from those of typical corynebacteria.

The use of mouse strains differing markedly in response to experimental infection with *C. kutscheri* is presented as a biologic model lending itself to further studies concerning factors which condition resistance to corynebacterial pseudotuberculosis, a disease of practical importance for investigators conducting experiments with murine species.

The authors gratefully acknowledge the technical assistance of Mrs. Jean D. Wolff and Mrs. Junia G. Hedberg and express appreciation to Dr. Clara J. Lynch for generously supplying mice from her inbred strains.

BIBLIOGRAPHY

1. Meyer, K. L., Latent infections, *J. Bact.*, 1936, **31,** 109.
2. Walker, D. L., Hanson, R. P., and Evans, A. S., editors, Symposium on Latency and Masking in Viral and Rickettsial Infections, Minneapolis, Burgess Publishing Co., 1958.
3. Dubos, R. J., The evolution and the ecology of microbial diseases, *in* Bacterial and Mycotic Infections of Man, (R. J. Dubos, editor), Philadelphia, J. B. Lippincott Co., 3rd edition, 1958, 14.
4. McDermott, W., Microbial persistence, *Yale J. Biol. Med.*, 1958, **30,** 257.
5. McDermott, W., Inapparent infection, *Public Health Rep.*, 1959, **74,** 485.
6. Kutscher, Ein Beitrag zu Kenntniss der Bacillären Pseudotuberculose der Nagethiere, *Z. Hyg. u. Infectionskrankh.*, 1894, **18,** 327.
7. Bongert, R., *Corynethrix pseudotuberculosis murium*, ein neuer pathogener Bacillus für Maüse, *Z. Hyg. u. Infectionskrankh.*, 1901, **37,** 449.
8. 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.
9. Lynch, C. J., Pierce-Chase, C. H., and Dubos, R., A genetic study of susceptibility to experimental tuberculosis in mice infected with mammalian tubercle bacilli, to be published.
10. Pierce, C., 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.
11. Dubos, R. J., and Middlebrook, G., Media for tubercle bacilli, *Am. Rev. Tuberc.*, 1947, **56,** 334.

12. Iossifides, I. A., Gutzait, L., Brand, M., and Tocantins, L. M., Enhanced survival of skin homografts in mice with a bacterial infection, *Ann. New York Acad. Sc.*, 1964, **114,** 487.
13. Iossifides, I. A. data to be published.
14. Wittler, R. G., Cary, S. G., and Lindberg, R. B., Reversion of a pleuropneumonia-like organism to a corynebacterium during tissue culture passage, *J. Gen. Microbiol.*, 1956, **14,** 763.
15. Standardized nomenclature for inbred strains of mice: Second Listing, Prepared by the Committee on Standardized Genetic Nomenclature for Mice, *Cancer Research*, 1960, **20,** 145.
16. Laboratory Animals. II. Animals for Research, *Nat. Acad. Sc.—Nat. Research Council, Publ.* 1094, 1963.
17. Wilson, G. S., and Miles, A. A., Topley and Wilson's Principles of Bacteriology and Immunity, Baltimore, Williams and Wilkins Co., 1946, **1,** 461, 470, 473; **2,** 1404.
18. Dingle, J. H., Infectious diseases of mice, *in* Biology of The Laboratory Mouse, (G. D. Snell, editor), New York, Dover Publications, Inc., 1956, 380–474.
19. 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.
20. Nelson, J. B., and Collins, G. R., The establishment and maintenance of a specific pathogen-free colony of Swiss mice, *Proc. Animal Care Panel*, 1961, **11,** 65.