INFECTION OF MICE WITH MAMMALIAN TUBERCLE BACILLI GROWN IN TWEEN-ALBUMIN LIQUID MEDIUM

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Cultures of pathogenic mycobacteria growing rapidly and diffusely in liquid media containing serum albumin and a synthetic water-dispersible ester of oleic acid are extremely virulent for chick embryos, mice, guinea pigs, and rabbits (1, 2). The present paper deals with some of the factors which affect the course and outcome of the experimental disease produced in mice by the injection of mammalian tubercle bacilli cultivated in this manner.

Materials and Methods

Cultures.—Several strains of human and bovine tubercle bacilli of various degrees of virulence were used in the course of the present study. For the sake of brevity, the results will be illustrated chiefly with data obtained with two cultures derived from the same strain, H37Rv (virulent) and H37Ra (avirulent).[1]

Medium.—Cultures of mammalian tubercle bacilli growing diffusely in aqueous solution and exhibiting a high degree of pathogenicity were obtained in a variety of synthetic media to which were added small amounts of the water-dispersible ester of oleic acid (Tween 80)[2] (0.05 to 0.1 per cent) and of bovine serum albumin, fraction V[3] (0.1 to 0.5 per cent). Most experiments were carried out with a medium of the following composition:

	gm.
KH_2PO_4	1.0
$Na_2HPO_4 \cdot 12\,H_2O$	6.5
$CaCl_2$	0.0005
$MgSO_4 \cdot 7\,H_2O$	0.001
$ZnSO_4$	0.0001
$CuSO_4$	0.0001
Ferric ammonium citrate	0.05
Asparagine	2.0
Enzymatic digest of casein	2.0
Tween 80	0.5
H_2O	1000.0

[1] These cultures were obtained through the courtesy of Mr. William Steenken, Jr., from the National Tuberculosis Association Standard Culture Depot at Trudeau, in the spring of 1946.

[2] Tween 80 was generously supplied by the Atlas Powder Company, Wilmington, Delaware.

[3] Bovine plasma fraction V (serum albumin) was obtained from the Armour Laboratories, Chicago, Illinois.

The medium, adjusted to pH 6.8, was distributed in 5 cc. amounts into Pyrex test tubes (25 mm. diameter) and autoclaved at 17 pounds pressure for 15 minutes. To each tube was then added 0.2 cc. of 5 per cent bovine albumin (plasma fraction V) and 0.05 cc. of a 50 per cent glucose solution (autoclaved in distilled water). Details concerning the preparation and properties of the medium are described elsewhere (3, 4).

Maintenance of Cultures.—Stock cultures were maintained by inoculating 0.1 cc. of a 7 day old culture into 5 cc. of medium, and incubating at 37.5°C. for 7 days. At the end of the incubation period, the growth appeared as a dense deposit in the bottom of the tube. Gentle agitation gave rise to a fine and fairly stable bacterial suspension which seemed homogeneous on macroscopic examination although microscopic study showed it to consist chiefly of small clumps of bacilli. These cultures contained 0.15 to 0.2 mg. of bacilli (dry weight) per cc. of medium (corresponding to 10^8 to 10^9 organisms). More detailed description of their characteristics will be presented in the following publication (5).

The virulent cultures were frequently passed through mice and reisolated from infected lung, heart, or spleen tissue. The organ utilized was removed aseptically, and ground with sand in a sterile mortar in the presence of an equal volume of 0.5 per cent bovine albumin solution in distilled water. Serial dilutions of this suspension were inoculated in 0.5 cc. amounts into tubes containing 5 cc. of liquid medium. In the case of H37Rv, heavy growth was usually obtained in the tubes inoculated with the 10^{-4} and 10^{-5} dilution of infected tissue suspension after 10 to 14 days' incubation at 37.5°C. The culture was then transferred to new medium for use as an infective inoculum. Virulent cultures can also be readily recovered by inoculation of 5 cc. of medium with a loopful of mouse brain tissue removed from animals infected by the intracerebral route. In general, cultures were discarded after 8 to 10 transfers in liquid medium, and isolated again from infected mice.

Preparation of Bacterial Suspension in Egg Yolk.—Fresh yolk was removed aseptically from a hen's egg and diluted with an equal volume of 0.85 per cent saline. One part of the suspension was added to an equal part of the bacterial culture and the mixture emulsified by vigorous pipetting just prior to inoculation. It is interesting to note that the presence of Tween 80 (0.05 per cent) greatly facilitates the preparation of a fine emulsion of egg yolk, thus preventing the formation of emboli as a result of intravenous injection.

Maintenance of Mice.—Mice 3 to 5 weeks old of a number of strains were obtained from the breeding colonies of the Rockefeller Institute and from some commercial farms. They were raised and kept in a room free from tuberculous animals until the time of infection. Immediately after this they were transferred to glass jars bedded with cedar shavings and covered with wire mesh tops, 4 or 6 mice per jar, in a room continuously irradiated with ultraviolet light. They were fed once daily a diet of white bread and milk. There was no obvious evidence of mouse typhoid, or of any infectious disease (other than tuberculous infection in the inoculated animals) during the course of the experiments to be reported.

The mice were weighed once a week, the 4 or 6 animals in each jar (or their survivors) being weighed as a unit. They were transferred to clean jars with new bedding at that time.

Records were made of dead animals every morning, and autopsies performed on them as well as on all surviving animals at the end of the experiments (3 to 6 weeks after infection).

RESULTS

1. Infection of Mice by the Intravenous Route

The finely dispersed state of tubercle bacilli growing diffusely in Tween 80-albumin liquid medium permits direct introduction of these cultures into the blood stream of experimental animals. This eliminates the necessity

of mechanical trituration of the culture, the usual practice with surface growths obtained on solid egg media or as pellicles on liquid media. The following experiment was instituted to determine the pathogenicity of mammalian tubercle bacilli for Swiss albino and for line 1 dba mice.

Swiss albino mice, 4 weeks of age (average weight 17 gm.), and line 1 dba strain mice, 4 weeks of age (average weight 14 gm.), were inoculated by the intravenous route with amounts of H37Rv culture in Tween-albumin medium ranging from 0.02 to 0.00003 cc., diluted to a final volume of 0.1 cc. with sterile medium. All animals surviving for 39 days after infection were sacrificed then and observations were made as to the presence, extent, and character of pulmonary disease. The results are presented in Table I.

TABLE I

Response of Swiss Albino and Line 1 dba Mice to Infection with Mammalian Tubercle Bacilli via the Intravenous Route

Mouse strain	Culture H37Rv	No. mice	Death and survival	Macroscopic appearance of lungs of surviving animals
	cc.			
Swiss albino	0.02	6	D25, D33, S, S, S, S	Small, discrete, non-elevated gray areas Entire surface of lungs flat
	0.003	6	S, S, S, S, S, S	
	0.0003	6	S, S, S, S, S, S,	
	0.00003	6	S, S, S, S, S, S	
Line 1 dba	0.02	6	D5, D27, D27, S,* S,* S*	Large, confluent, elevated glistening, grayish-white nodules, occasionally involving whole lobes
	0.003	6	D30, D32, D36, D36, S,* S*	
	0.0003	6	S, S, S, S, S, S	
	0.00003	6	S, S, S, S, S, S	

D= death; the numeral indicates the number of days after infection at which death occurred.

S= survival for a period of 39 days at which time all surviving animals were sacrificed.

* These animals were extremely emaciated at the end of the experiment and would have probably died within a few days; in all subsequent experiments dba mice infected with 0.01 cc. of culture died within 3 weeks after infection.

As indicated in Table I, intravenous injection of tubercle bacilli resulted in detectable pulmonary lesions in all animals, however small the infective dose. It is obvious, however, that the disease was much more severe in the line 1 dba than in the Swiss albino mice. Only two of the albino animals died of infection with the largest infective dose (0.02 cc.) and the others appeared healthy and were gaining weight when the experiment was terminated. A much smaller infective dose (0.003 cc.) caused higher mortality of the line 1 dba mice; moreover, the surviving animals of this strain were so emaciated when sacrificed that it is unlikely that they would have survived for many more days. Subsequent experience with larger numbers of experimental

animals has repeatedly confirmed the trend of the observations reported in Table I. In general, line 1 dba mice infected intravenously with 0.1 cc. of culture die within 1 week, too soon to show gross pulmonary lesions; infection with 0.01 cc. of culture usually results in death within 3 weeks, with extensive involvement of lung tissue.

In all of the strains of mice tested, 22 in all, the lungs of animals dying within the first 2 weeks after infection showed as a rule many hemorrhagic areas 1 to 2 mm. in diameter whereas pearly gray lesions characterized the later stages of the disease. Although the histopathology of experimental tuberculous infections in mice will be described elsewhere, it may be useful to present at this time a short statement of some of our preliminary observations:—

Histological sections of the lungs of mice dying 2 or more weeks after infection with mammalian tubercle bacilli revealed the presence of numerous lesions when stained by hematoxylin-eosin technique. These lesions varied in size from small collections of polymorphonuclear leucocytes and round cells filling an alveolus, to lesions involving many alveoli, characterized by a central area of necrosis and massive infiltration with inflammatory cells. Shadowy outlines of the alveolar septae were always visible even in the central area of necrosis. Distension of the alveoli at the periphery of these lesions indicated the presence of edema fluid. Parallel histological sections stained by the Ziehl-Neelsen technique revealed the presence of enormous numbers of acid-fast bacilli located both intracellularly and extracellularly. Although bacilli were usually found only in lesions in the pulmonary parenchyma, they could also be seen in some cases in the lumen of the bronchi.

Figs. 1 to 4 illustrate the type of lesions obtained in Swiss albino and line 1 dba mice.

The progress of tuberculous infection in mice can be followed not only by the development of pulmonary lesions, but also by weight losses of the infected animals.

Albino mice of the Rockefeller Institute strain 4 weeks of age and weighing 17 gm. were inoculated intravenously with 0.1 cc. or 0.01 cc. of the virulent culture, H37Rv, or with 0.2 cc. of the avirulent variant of the same strain, H37Ra. Body weights were recorded (as the average of six animals or their survivors) at weekly intervals. The experiment was terminated at the end of 4 weeks.

As appears from the results presented in Table II, weight loss became evident 2 weeks after injection of 0.1 cc. and 3 weeks after injection of 0.01 cc. of virulent H37Rv bacilli. This weight loss was not due merely to a non-specific toxic effect of the bacillary material which was unrelated to virulence, since no similar effect was observed when even larger amounts of culture of the avirulent variant H37Ra were injected, also by the intravenous route. No gross pulmonary lesions were observed in animals inoculated with the avirulent culture; further-

more, impression smears of the lungs stained by the Ziehl-Neelsen technique failed to reveal the presence of acid-fast bacilli.

TABLE II

Response of Albino Mice of Rockefeller Institute Strain to Intravenous Inoculation with H37Rv (Virulent) and H37Ra (Avirulent) Mammalian Tubercle Bacilli

Culture	Egg yolk suspension	Weekly weight after inoculation					No. dead/Total
		Initial	1 wk.	2 wks.	3 wks.	4 wks.	
cc.	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
H37Rv 0.1	0	18.3	20.0	17.9	15.8	Dead	6/6
H37Rv 0.01	0	18.3	21.3	22.2	19.7	19.2	5/6
H37Ra 0.2	0	18.3	21.6	24.2	25.9	26.7	0/6
H37Ra 0.2	0.1	18.3	20.9	23.3	24.6	25.3	0/6
0 0	0.1	18.4	19.8	23.6	24.2	25.3	0/6

2. Infection of Mice by the Intracerebral Route

The following experiment was set up to determine whether virulent tubercle bacilli injected into mice by the intracerebral route multiply in the brain tissue and can be disseminated thence to other tissues.

Swiss albino mice, 4 weeks of age and 17 gm. in weight, and line 1 dba mice, 4 weeks of age and 14 gm. in weight, were inoculated into the brain, while under ether anesthesia, with graded amounts of bacilli resuspended in a final volume of 0.03 cc. of sterile medium. At weekly intervals some of the animals were sacrificed, and examined for the presence of gross pulmonary lesions; at the same time, impression smears of the brain tissue were stained by the Ziehl-Neelsen technique and examined for the presence of acid-fast bacilli. Four strains of tubercle bacilli were used for infection: H37Rv, a classical laboratory strain of mammalian origin, Ravenel, a classical laboratory strain of bovine origin, Waller, a human strain recently isolated from sputum, and Number 3817, a bovine strain recently isolated from human pathological material. The results with all four strains were identical. They are summarized in Table III.

The results presented in Table III reveal the surprising fact that evidence of marked multiplication of bacilli could be detected within 1 week in the cerebral hemisphere of the brains infected with even a very small dose (10 to 100 organisms). But, on the other hand, it should be remarked that invasion of the other hemisphere and gross pulmonary infection were detectable only after 2 to 3 weeks. As in the case of infection by the intravenous route the extent of the pulmonary lesions resulting from intracerebral inoculation was dependent upon the strain of mouse used; the dba mice exhibited much more extensive lesions than Swiss albino animals.

Experiments are now in progress to follow more accurately by histological and quantitative bacteriological techniques the process of bacterial proliferation

within the brain tissue. Preliminary results indicate that an increase in the number of bacilli becomes evident 2 to 3 days after infection and that, at this early time, most of the bacilli appear packed within large mononuclear cells. Finally, it may be worth mentioning at the present time that tuberculous infection has been established by injecting intracerebrally 0.01 cc. of sputum obtained from tuberculous patients.[4]

TABLE III

Response of Swiss Albino and Line 1 dba Mice to Intracerebral Infection with Mammalian Tubercle Bacilli

Culture H37Rv	Presence of bacilli in brain tissue 1 wk. after infection		Pulmonary lesions 3 wks. after infection	
	Swiss mice	dba mice	Swiss mice	dba mice
cc.				
0.01	+	+	++++	++++
0.0015	+	+	+++	+++
0.00015	+	+	++	++
0.00003	+	+	++	++
0.000003	+	+	+	+
0.0000003	−	+	−	+

3. Intraperitoneal Infection of Mice; The Effect of Egg Yolk

It was soon observed that the minimal infective doses of mammalian tubercle bacilli required for the establishment of a fatal infection are much larger (five- to ten-fold) by intraperitoneal than by intravenous injection. The results of the infection are also less regular and in particular, selective localization of the lesions in the lung tissue is a less prominent feature.

Many efforts were made to increase the virulence of the cultures by passage through mice and through chick embryos. No convincing quantitative data are as yet available concerning the effect of mouse passage on virulence. It was found, however, that direct injection of some of the contents of the yolk sac of infected chick embryos into the peritoneal cavity of mice gave rise to a rapidly fatal infection with striking pulmonary localization. This phenomenon is illustrated and analyzed in the following experiments.

Embryonated hen eggs 7 days old were inoculated with 0.1 cc. of the virulent culture H37Rv, introduced into the yolk sac. Ten days after infection, multiple lesions were present on the chorioallantoic membrane (1). The yolk sac and its contents were collected at that time and together emulsified with an equal volume of physiological saline; stained preparations

[4] We wish to acknowledge with thanks the cooperation of Dr. Walsh McDermott and Dr. Susan Hadley of the New York Hospital, who supplied us with selected samples of human tuberculous material.

and cultivation of this material in Tween-albumin media revealed the presence of enormous numbers of acid-fast bacilli (approximately 10^9 per cc.). Within 2 hours after recovery from the infected embryo, graded amounts of the diluted yolk material were injected intraperitoneally into albino mice; for the purpose of comparison, other mice of the same breed and age were inoculated with comparable numbers of bacilli grown in Tween-albumin liquid medium.

Identical results—not to be detailed here—were obtained in a large number of experiments. The bacilli in the yolk sac exhibited much higher infectivity than those recovered from culture media as measured in terms of death rate or of the extent of the gross pulmonary lesions. However, bacilli recovered from infected yolk sacs and cultivated in Tween-albumin media did not appear more virulent than the stock culture when injected into mice by any route. Further analysis of the findings was therefore undertaken in order to determine the possible influence of the egg yolk material as such upon the infectivity of tubercle bacilli.

Albino mice of the Rockefeller Institute strain, 4 weeks of age and 18 to 20 gm. in average weight, were inoculated *via* the intraperitoneal route with graded amounts of cultures grown in Tween-albumin medium. Sterile egg yolk emulsion was added to some of the inocula—as indicated in Table IV—according to the method described earlier in this report. The results of the injections are presented in Table IV in terms of weekly weight changes of the animals, numbers of deaths within a period of 3 weeks, presence and extent of gross pulmonary lesions, and enlargement of the spleen and lymph nodes.

The results presented in Table IV show that the addition of normal egg yolk to the culture of H37Rv prior to intraperitoneal injection markedly enhances the infectious process, as measured either in terms of weight loss or by death of the animals. The longer survival time was correlated with extreme splenic enlargement and small pulmonary lesions, whereas more acute disease resulted in less pronounced enlargement of the spleen, but much more pronounced pulmonary lesions. All attempts to reproduce the enhancing effect of egg yolk by injecting the material into mice either before or after introduction of the bacilli failed to modify the course of the disease. This fact is illustrated in the last line of Table IV where it is shown that addition of 0.25 cc. of diluted egg yolk 48 hours after infection resulted in extreme enlargement of the spleen, minimal pulmonary lesions, and no death within 3 weeks, this being a disease picture which could not be differentiated from that obtained with bacilli alone.

Further evidence of the increase in infectivity obtained by addition of egg yolk to the inoculum is illustrated in Table V. This enhancing effect is not due to a non-specific toxic action of the egg yolk since addition of this material to larger doses of the avirulent variant of H37 (H37Ra) failed to affect the weight curves of the inoculated animals, or to cause in them any evidence of disease.

The enhancement of infectivity of virulent tubercle bacilli by means of egg

TABLE IV

Effect of Egg Yolk upon Infectivity of Mammalian Tubercle Bacilli for Rockefeller Institute Albino Mice

Culture H37Rv (i.p.)	Egg yolk suspension	Weekly weight changes				Average spleen weight	Gross pathological findings Macroscopic appearance of tissues	No. dead/Total
		Initial	1 wk.	2 wks.	3 wks.			
cc.	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg.*		
0	0	18.8	19.4	20.8	21.8	97	Normal	0/6
0	0.25	20.0	19.9	20.5	22.5	75	Normal	0/6
1.0	0	21.0	20.3	21.7	22.6	553	Enlarged infected lymph nodes, pinpoint pulmonary lesions, numerous scattered abscess-like lesions in other tissues	0/6
1.0	0.25	21.4	All dead					6/6
0.25	0	20.0	20.2	22.6	24.2	740	Enlarged infected lymph nodes, pinpoint pulmonary lesions, occasional scattered abscess-like lesions throughout other tissues	0/6
0.25	0.25	20.5	20.7	18.0	All dead			6/6
0.25	0.12	20.2	20.1	20.3	20.3	360	Enlarged infected lymph nodes, extensive pulmonary lesions, few other lesions	1/6
0.25	0.25*	20.3	20.3	21.3	22.8	765	Enlarged infected lymph nodes, pinpoint pulmonary lesions, occasional scattered abscess-like lesions throughout other tissues	0/6

i.p. = intraperitoneal injection.

*Egg yolk suspension administered intraperitoneally 48 hours after intraperitoneal injection of the bacilli.

yolk proved to be a readily reproducible phenomenon when the mixture was introduced intraperitoneally. Experiments based on the chemical fractionation of egg yolk (6) have revealed that lipovitellin and lipovitellenin, in amounts comparable to those present in it do not reproduce the effect of the whole material. However, a suspension of egg oil from the evaporated ether

extract of the whole yolk together with an alcohol-insoluble fraction of the ether extract after removal of the oil was as active as the whole egg yolk when mixed with the culture of tubercle bacilli. Furthermore, a similar effect could be obtained when egg oil was replaced by a light paraffin oil (Bayol F). Oil and the alcohol-insoluble fraction were separately ineffective. When examined microscopically, the bacilli in the mixture of culture and egg yolk suspension appeared to be located in the small globules of oil. The same finding was obtained when the culture was mixed with the suspension mixture of egg oil or paraffin oil and the alcohol-insoluble fraction of egg yolk.

TABLE V

Response of Albino Mice of Rockefeller Institute Strain to Intraperitoneal Inoculation with H37Rv (Virulent) and H37Ra (Avirulent) Mammalian Tubercle Bacilli

Culture	Egg yolk suspension	Weekly weight after inoculation					No. dead/Total
		Initial	1 wk.	2 wks.	3 wks.	4 wks.	
cc.	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
H37Rv 0.25	0	20.0	20.2	22.6	24.2	Killed	0/6
" 0.25	0.25	20.5	20.7	18.0	Dead		6/6
" 0.5	0	20.0	23.3	24.6	25.3	22.8	0/6
" 0.5	0.25	21.0	19.8	16.4	Dead		6/6
H37Ra 1.0	0	17.6	21.9	25.3	26.8	28.0	0/6
H37Ra 1.0	0.25	20.2	23.5	26.8	28.2	30.1	0/6
0	0	21.0	26.2	28.0	29.3	31.4	0/6

4. Infection of Mice by Feeding

The following experiment was devised to test the possibility of infecting mice by feeding them once with food contaminated with tubercle bacilli cultivated in the Tween-albumin medium.

Swiss albino mice (4 weeks of age and 15 gm. in weight) and line 1 dba mice (4 weeks of age and 11 gm. in weight) were fasted for 1 day. Each mouse was then fed a piece of dry white bread (approximately 1 c.cm.) soaked immediately prior to feeding with 0.5 cc. of a 7 day old culture of H37Rv. The mice were then placed and maintained on a diet consisting of cornmeal, gelatin, butter, and a salt mixture (to be described more fully in a later publication) and sacrificed 4 weeks after the feeding with bacilli.

Autopsies revealed tuberculous pulmonary disease in all the fed animals. In this case again, the lesions were much more extensive in the dba than in the Swiss albino mice.

5. Comparative Susceptibilities of Different Breeds of Mice to Experimental Tuberculous Infection

The results presented in Table I reveal a striking difference in behavior toward infection with the virulent culture H37Rv between line 1 dba and Swiss

albino mice. Whether measured in terms of minimal lethal dose of tubercle bacilli, or of rate of progression of pulmonary lesions, the susceptibility of the dba mice was much greater than that of the Swiss albino. The age (between 3 and 7 weeks) and the weight (between 12 and 20 gm.) of the animals appeared to be of little significance in determining the susceptibility to infection.

In the course of the present study, extensive comparisons have been made of the susceptibility of 22 different strains of mice obtained from commerical breeding farms or from genetical laboratories. It appears unnecessary to describe the details of these tests since their results are not entirely comparable because they could not be carried out under identical conditions.[5] Nevertheless, records of the results of those carried out within the last year with animals of approximately the same age (4 to 6 weeks) fed on a bread and milk diet and inoculated with cultures grown in Tween-albumin liquid medium, are sufficiently consistent to warrant the drawing of certain comparisons. In terms of minimal lethal dose of culture H37Rv and of extent of pulmonary lesions, 18 different strains of mice can be arranged approximately according to the following order of increasing susceptibility to tuberculous infection.

Mouse strain	Source from which obtained
Swiss albino	Brought by Dr. Clara Lynch to the Rockefeller Institute from Switzerland.
" "	Obtained from Dr. Lynch and raised by R. G. Hahn at the Rockefeller Institute.
" "	Viktor Schwentker, Tumblebrook Farm, Brant Lake, New York.
" "	Rockefeller Institute stock, raised by J. Pomarico.
Swiss albino (CF_w)	Procured from the Rockefeller Institute stock and raised by Carworth Farms, New City, New York[6]
Swiss albino (CF_{ow})	Mutant derived from CF_w. Raised by Carworth Farms.
Strong A	Procured from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and raised by J. Pomarico.
" C	" " "
Buffalo	Procured from the State Institute for the Study of Malignant Diseases, 663 North Oak Street, Buffalo, New York, raised by J. Pomarico.
Rockefeller Institute strain	Heterozygous albino strain maintained at the Rockefeller Institute and to be distinguished from the so called "Swiss Albino" strains.
Chocolate	Carworth Farms
Brown spot	" "
C_3H	Roscoe B. Jackson Memorial Laboratory

[5] It is probable that unrecognized variations in the culture, the season of the year, and other uncontrolled factors have an appreciable influence on the course and outcome of the experimental infection.

[6] The strains obtained from Carworth Farms were generously supplied by Mr. C. N. Wentworth Cumming.

Mouse strain	Source from which obtained
C_3H	Roscoe B. Jackson Memorial Laboratory and raised by J. Pomarico.
Strong I	Procured from Dr. L. C. Strong and raised by J. Pomarico.
Mus musculus domesticus	Stock of Dr. Howard Schneider, Rockefeller Institute.
C57 black	Roscoe B. Jackson Memorial Laboratory–
dba line 1	" " " " "

In general, but not uniformly, pigmented animals were much more susceptible than the albino strains. A suggestion of this difference is to be found in the results of earlier experiments involving the infection of albino mice and of the C57 black strain with bovine tubercle bacilli by the intraperitoneal route (7). Difference in susceptibility was manifested in the time of survival following injection of a given amount of culture, in the minimal infective dose required to cause death within a given period of time, and in the extent and character of the pulmonary lesions. It is less certain, however, that differences in susceptibility affected the initial phases of multiplication of the injected organisms. Multiplication of the bacilli was observed to take place within a few days, even when the infective inoculum was very small, in the most resistant as well as in the most susceptible strains of mice. Similarly, intravenous injection of small amounts of bacilli resulted within 2 weeks in the production of pulmonary lesions in all animals. It was in the subsequent course of events that susceptibility and resistance became manifest. The lesions generally remained small in albino mice, with a tendency to regress when few in number. In animals of the susceptible line 1 dba and C57 black strains, on the other hand, rapid progression of the disease took place, and the presence of a few lesions was sufficient to cause the destruction of a whole pulmonary lobe within a few weeks.

The difference in susceptibility between different mouse strains is illustrated by the following experiment in which are compared three strains of mice, Swiss albino, C_3H, and C57 black, which exhibit respectively high, intermediate, and low resistance to experimental tuberculous infection.

The mice used were of the following age and average weight: Swiss albino 6 weeks, 22 gm.; C_3H 6 weeks, 17 gm.; C57 black 6 weeks, 17 gm. They were infected with varying amounts of H37Rv cultures grown in Tween-albumin liquid medium introduced by the intraperitoneal or intravenous route. The number of deaths occurring at weekly intervals (up to 6 weeks) is recorded in Table VI.

It is obvious that the order of increasing susceptibility of the three strains of mice, Swiss albino, C_3H, and C57 black was the same, whether the infective dose was introduced intravenously or intraperitoneally, with or without egg yolk, and irrespective of whether susceptibility was measured in terms of the number of deaths occurring within a given period, or by the rapidity with which death occurred after a given infective dose. Pulmonary lesions were

TABLE VI

Comparative Susceptibility of Three Mouse Strains to Infection with Mammalian Tubercle Bacilli via Intraperitoneal and Intravenous Routes

Culture H37Rv	Egg yolk suspension	No. mice of each strain	Swiss albino							C3H							C57 black						
			No. deaths weekly						Total dead	No. deaths weekly						Total dead	No. deaths weekly						Total dead
			1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.		1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.		1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	
Intraperitoneal																							
cc.	*cc.*																						
0.25	0	6							0							0	2	2		1			5
0.25	0.25	6							0			2				2	1	1		1			3
0.05	0	6							0							0							0
0.05	0.25	6							0							0	2		1	1			4
Intravenous																							
cc.	*cc.*																						
0.05	0	6							0			2	4			6	1	2		3			6
0.05	0.1	6				5			5			6				6	4	2					6
0.01	0	6							0				1	2		3	2	2					4
0.01	0.1	6							0			3	2			5	3	3					6

found in all infected animals, but as indicated earlier in this report, the rate of progression of these lesions was highly characteristic for each mouse strain. The lesions observed in resistant (Swiss albino) and susceptible (line 1 dba) mice are illustrated in Figs. 1 to 4. Mice of the C57 black strain gave results similar to those obtained with line 1 dba.

DISCUSSION

Recent studies have established that mice are more susceptible to experimental infections with mammalian tubercle bacilli than was formerly believed (7–16). Thus, intravenous injection of 0.1 to 0.2 mg. human or bovine bacilli has been found by several workers to cause death of a large percentage of albino mice within 3 to 4 weeks (8, 10, 14, 15, 16). Of special interest is the finding that intravenous inoculation with 10 to 100 living bacilli can lead to a mild chronic disease, during which the organisms proliferate although they may fail to produce visible tubercles (9). Chronic infection can also be produced by causing mice to inhale small numbers of bovine bacilli in the form of an aerosol mist (13). The results described in the present paper confirm and extend these findings. They establish in particular that one can modify almost at will the rate of progression and the outcome of experimental tuberculous infection of mice by modifying a number of factors which influence the infective organism and as will be shown later, the infected host.

It has long been known that variants of a given culture of tubercle bacillus can differ in virulence; this is illustrated in Tables II and V and will be discussed further in an accompanying communication (5). The physiological state of the culture used for inoculation is also worth consideration. Cultures grown for several weeks according to the classical techniques (on egg yolk slants, or as surface pellicles on synthetic liquid media) contain a large percentage of dead cells and of cells of differing age and physiological activity (17). It is not surprising, therefore, that these cultures have often been found ineffective in producing disease in an animal somewhat resistant to tuberculous infection, as in the albino mouse. The cultures used in the present study were young (1 week old) and homogeneous (diffuse growth); comparison of the number of cells determined by direct microscopic examination and of the number capable of initiating growth in liquid and on agar media revealed in repeated tests that most of those in our cultures were viable and physiologically active. Moreover, the diffuse character of the cultures growing in liquid Tween-albumin medium made unnecessary mechanical trituration for the preparation of homogeneous bacterial suspensions for infection. The youth and viability of Tween-albumin cultures as well as elimination of trauma in the preparation of the bacterial suspension probably accounted for its high infectivity for mice.

The mode of injection of the bacilli had a marked influence on the type of disease produced. Intravenous inoculation of the bacilli resulted in a localiza-

tion of the disease that was predominantly pulmonary; the minimal infective dose capable of causing death within a month ranged from 0.0005 to 0.02 mg. of bacilli, depending upon the strain of mice used. Introduction of the bacilli by the intraperitoneal route was much less effective and resulted in a less predictable localization of the infection. Admixture of the bacilli with egg yolk (or with a mixture of oil and the phosphatide fraction of the yolk) prior to injection markedly increased the severity of the pulmonary disease and the death rate. It is very likely, however, that this enhancement of infection was not due to an effect on the host, or on the virulence of the bacilli, but rather to some modification by the yolk material of the surface of the bacteria which caused the latter to aggregate within oil droplets. It appears probable that these small droplets containing bacilli were not phagocytized locally in the peritoneal cavity but were transported to the lung where they were deposited as infective foci. Bacilli introduced into the brain multiplied very rapidly, even when the infective dose was very small (10 to 100 bacilli); preliminary evidence indicates that in this organ bacterial proliferation takes place within the larger monocytes, at least to begin with. Surprisingly enough, the animals manifested little evidence of disease during the intracerebral phase of bacterial multiplication. Within 2 to 3 weeks after infection, however, invasion of lung tissue became manifest and the disease process took the form observed after infection by the intravenous route.

Nothing is known as yet of the factors—immunological or physiological—which hold in check the progress of the lesions in the more resistant animals. In the course of the present study, however, many observations have been made which indicate that this resistance can be decreased by a number of nonspecific circumstances. Thus, as will be shown in a forthcoming publication, changes in diet can greatly shorten the survival time of mice infected with mammalian tubercle bacilli.

SUMMARY

Introduction of the bacilli by the intravenous route or by feeding gives rise to a disease predominantly localized in the lungs. Following intracerebral infection, the bacilli first multiply rapidly in the brain tissue, and then invade other organs, producing lesions especially in the lungs. Injection of the bacilli by the intraperitoneal route is less effective than by either the intravenous or intracerebral routes; however, admixture of the bacilli with some of the components of egg yolk increases both the infectivity and the pulmonary localization.

Different strains of mice differ markedly in their susceptibility to experimental tuberculous infection; the highest susceptibility was observed among the pigmented strains (line 1 dba and C57 black). Greater resistance does not

appear to depend on the ability to prevent the establishment of infection, but rather corresponds to a slower rate of progression of the infectious process.

It is possible to produce in mice tuberculosis presenting any desired degree of acuteness or chronicity by controlling certain factors which condition the initiation and the progression of the infection.

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EXPLANATION OF PLATE 18

Lung sections stained with hematoxylin-eosin. × 8.5

The photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Mouse strain: Swiss albino.
Inoculum: 0.003 cc. H37Rv (intravenous).
Autopsy: 3 weeks after infection.

Note many small areas of parenchymal and subpleural lesions. Occasional epithelioid cells are visible in these lesions at higher magnification.

FIG. 2. Mouse strain: dba.
Inoculum: 0.003 cc. H37Rv (intravenous).
Autopsy: 3 weeks after infection.

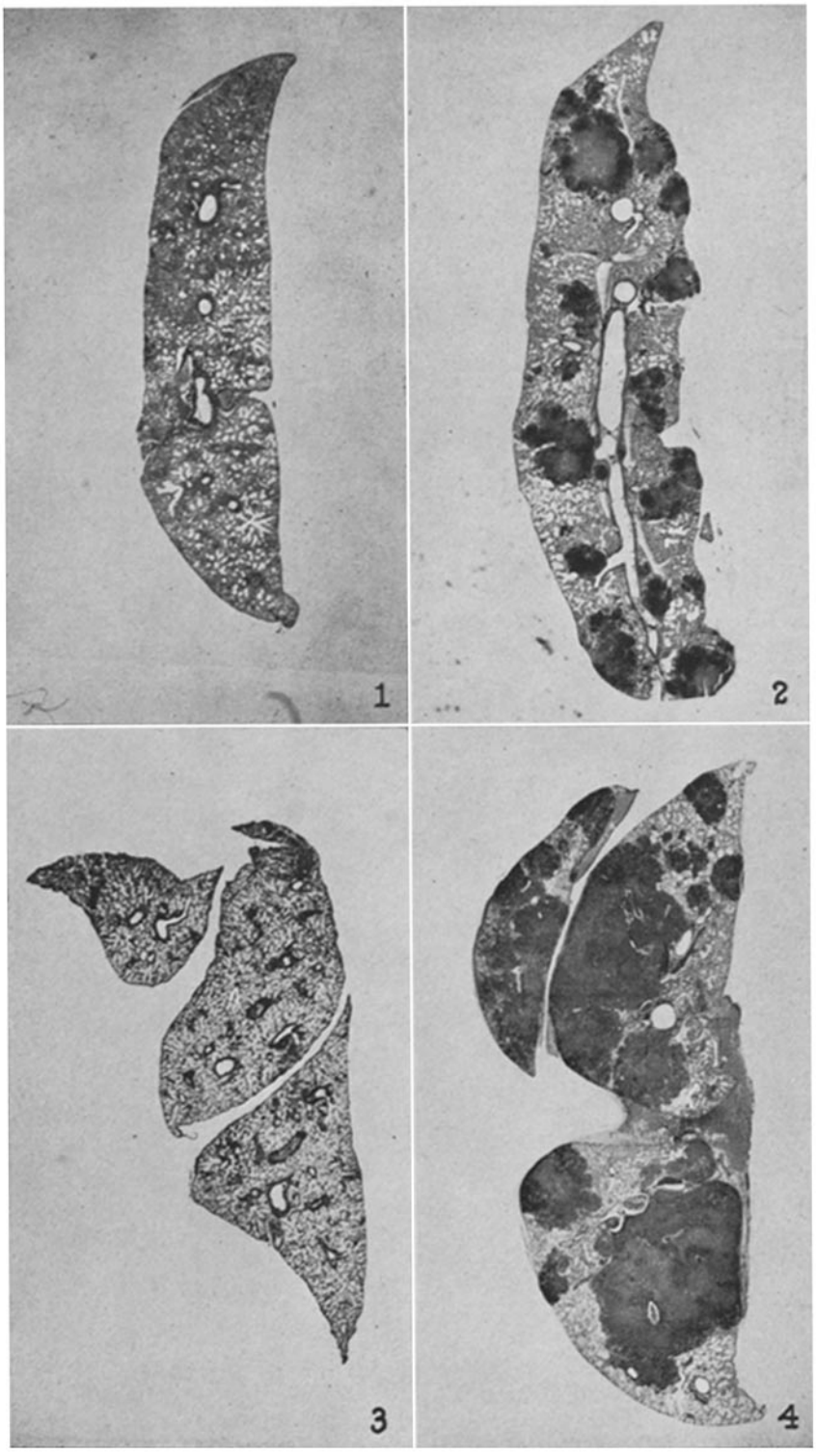
Note large, sometimes confluent parenchymal, peribronchial, and subpleural lesions. Central necrosis is evident. Many epithelioid cells are visible in these lesions at higher magnification.

FIG. 3. Mouse strain: Swiss albino.
Inoculum: 0.01 cc. H37Rv (intravenous).
Autopsy: 3 weeks after infection.

Note several small, dark areas in the parenchyma. Higher magnification reveals these to consist of inflammatory lesions without necrosis, which are usually perivascular.

FIG. 4. Mouse strain: dba.
Inoculum: 0.01 cc. H37Rv (intravenous).
Autopsy: 3 weeks after infection.

Note massive consolidation of most of the lung with large areas of necrosis.



(Pierce *et al.*: Experimental tuberculous infection of mice)