ON THE RESPONSE OF GENETICALLY RESISTANT AND SUSCEPTIBLE RABBITS TO THE QUANTITATIVE INHALATION OF HUMAN TYPE TUBERCLE BACILLI AND THE NATURE OF RESISTANCE TO TUBERCULOSIS*

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Plates 2 and 3

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The fundamental variant in the tuberculosis caused by virulent tubercle bacilli of the bovine type in highly inbred rabbit strains of different genetic resistance to the disease is the degree of localization of the infection at the portal of entry and the chronicity of the ensuing disease (1). Rarely, if ever, is a primary bovine infection completely overcome even by the most resistant rabbit. On the other hand, the great majority of human beings infected with tubercle bacilli completely arrest their disease. Therefore, to understand the nature of human resistance to tuberculosis, a disease must be produced in animals which is but rarely fatal. While the progress of the disease induced by the quantitative inhalation of bovine tubercle bacilli differs profoundly in degree in susceptible and resistant rabbits, it does not differ in kind. It was demonstrated by Heppleston in this laboratory (2) that primary pulmonary tuberculosis in unselected rabbits induced by quantitative inhalation of human type tubercle bacilli may retrogress or progress. It is the purpose of this report to show that if genetically susceptible and resistant rabbits simultaneously inhale virulent human type tubercle bacilli, the resistant animals, in the great majority of instances, overcome the infection completely in a given time, while in the susceptible animals there is usually a disease of varying extent which often involves the greater part of the lung parenchyma. The allergic response, the antibody production, the rate of growth and destruction of tubercle bacilli in the lungs, and the associated histological responses have been analyzed in these resistant and susceptible animals. It is further shown that the number of

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primary pulmonary foci induced by natural quantitative inhalation of human type tubercle bacilli is a function of the native resistance of the animal exposed. A critique of these findings has yielded certain concepts on the nature of resistance to tuberculosis.

Materials and Methods

The following inbred strains were used: The resistant race III (3) together with a subgroup thereof, III R, and the susceptible strains, FC, C, and Ca.

Race III is a group of normal New Zealand white rabbits that had been inbred for 15 generations by Dr. Paul B. Sawin of the Roscoe B. Jackson Memorial Laboratory, chiefly by parent and offspring mating. Since 1945 the inbreeding of this strain has been extended for four more generations at the Henry Phipps Institute. All these last four generations have shown great resistance to virulent bovine tuberculosis irrespective of the mode of infection. On natural inhalation of virulent bovine type tubercle bacilli of the Ravenel strain, this race develops a slowly progressive localized ulcerative pulmonary phthisis with little or no hematogenous or lymphogenous dissemination beyond the pulmonary portal of entry.

Race III R are normal race III rabbits of the seventeenth to nineteenth inbred generation. They differ from other race III rabbits in that one male, race III ancestor of this subgroup, had survived a virulent bovine infection after preliminary treatment with heat-killed tubercle bacilli.[1]

Animals of race C are normal susceptible rabbits that have been inbred by brother and sister mating and recently by parent and offspring mating for nine to eleven generations.

Race FC are susceptible rabbits resulting from a cross between the highly and uniformly susceptible strain F, which had been inbred by brother and sister mating for seven to eight generations, and the susceptible strain C, which had been inbred in the same manner for six to seven generations. The FC rabbits in this study were of the second to the fourth inbred generations of the original FC crosses.

The Ca rabbits were obtained from the Carworth Farms in 1947 and have been inbred by brother and sister mating for five generations. This is also a susceptible strain.

All these three susceptible strains carry the Dutch quality. They all respond to natural inhalation of Ravenel by a rapidly progressive caseous pneumonia with extensive hematogenous and lymphogenous dissemination of the disease.

The method of quantitative air-borne infection has been described in detail in a previous publication (4). Suffice it to say that rabbits were made to inhale for a known time an aerosol containing a known number of bacillary units of viable, virulent human type tubercle bacilli, H37Rv. The aerosol was derived from the atomization of a suspension in which the vast majority of the microorganisms were present singly; only occasionally were small groups present, none of which contained more than 3 to 6 bacteria. For the exact procedures involved and the manner of estimating the number of bacillary units inhaled, the reader is referred to the original paper. It is pertinent to state that the quantitative aspects of the methods used have led to conclusions which were substantiated by a number of independent observers (5). The development of allergic sensitivity was measured, at weekly intervals following inhalation, with a 10 per cent solution of O. T. of constant strength (1). The antibody production was measured by the Middlebrook and Dubos hemagglutination test (6). The growth and destruction of the bacilli in the lungs and draining lymph nodes of the exposed animals were determined by culture as previously described (7). The results of these estimates accorded well with the histological observations on aliquots of the tissues cultured.

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[1] We are indebted to Dr. Esmond R. Long for suggesting the use of this method of natural selection for the development of rabbit strains of very high native resistance.

RESULTS

Pulmonary Tuberculosis in Genetically Susceptible and Resistant Rabbits.—Before evaluating the effect of quantitative inhalation of human type tubercle bacilli on different inbred rabbit families, it is essential to ascertain the resistance of these races to a standard dose of virulent, bovine tubercle bacilli. For this reason, a group of race III and FC rabbits were inoculated intracutaneously with 0.1 mg. of the same suspension of the Ravenel strain of tubercle bacilli. Both rabbit groups had received the same number of intracutaneous injections of heat-killed Ravenel bacilli[2] at the same intervals prior to their infection with living bovine bacilli. Table I presents the results.

The average survival of the race III rabbits was 422 ±154 days while that of the FC rabbits was 132 ±42 days following the injection of virulent bovine organisms. Not only is this survival difference highly significant statistically, but the type of tuberculosis developed by these two races was quite distinct. The FC rabbits died of an extensive generalized tuberculosis. In the race III animals, on the other hand, one rabbit is still living more than 3 years after inoculation, without any evidence of disease, another had tuberculosis limited to the ileocecal junction, and the rest showed at death either minimal pulmonary disease or ulcerative phthisis. It is plain therefore that race III rabbits are genetically much more resistant than the FC rabbits.

In a second experiment the action of human type tubercle bacilli was studied. Table II shows that of 10 susceptible FC rabbits, which were estimated to have inhaled 100 to 2000 units of human type tubercle bacilli, 9 showed numerous tuberculous lesions in the lungs 2 to 8 months following infection. Of 12 resistant race III rabbits exposed to the same aerosols of human type tubercle bacilli, 10 showed no gross evidence of disease whatsoever when killed at the same time. This all-or-none response difference is illustrated in Fig. 1. Furthermore, where there was no gross evidence of tuberculosis, the bacilli had almost completely disappeared from the lungs of the resistant rabbits as revealed by culture and guinea pig inoculation (Fig. 2). Thus, in 90 per cent of the susceptible rabbits there was a varying degree of pulmonary tuberculosis; by contrast, in 80 per cent of the resistant animals there was no disease at all. It is noteworthy that the single FC animal that failed to develop gross tuberculosis, FC 2-14, was estimated to have inhaled 127 units of tubercle bacilli. As will be seen below, this is close to the average minimum number of units of the human type bacilli necessary to generate a single gross primary pulmonary focus in this family.

The Development of Allergy.—Since allergy plays a significant role in the pathogenesis of human tuberculosis, it was important to determine the rate of development of this sensitivity in resistant and susceptible rabbits after a single

[2] This was done in order to determine the allergic irritability of these two families. This procedure does not affect the relative resistance of different rabbit groups (1).

TABLE I

*The Relative Resistance of Race III and FC Rabbits to the Intracutaneous Inoculation of 0.1 Mg. of Virulent Bovine Tubercle Bacilli, Ravenel Strain**

Race III			Race FC		
Rabbit No.	Length of survival after infection	Type and extent of tuberculosis	Rabbit No.	Length of survival after infection	Type and extent of tuberculosis
	days			*days*	
III V 819	243	Minimal pulmonary tuberculosis; nodes draining inoculation site tuberculous	FC 2 — 6	78	Moderate generalized tuberculosis
III V 821	275	Massive ulcerative fibrocaseous pulmonary tuberculosis	FC 2 — 20	84	Extensive generalized tuberculosis
III V 880B	283	Minimal pulmonary and pleural tuberculosis; nodes draining inoculation site and one eye tuberculous	FC 2 — 24	101	Extensive generalized tuberculosis
III Y 385	391	Tuberculosis of mammary gland and of nodes draining inoculation site	FC 2 — 15	114	Extensive generalized tuberculosis
III U 57	444	Ulcerative pulmonary tuberculosis and tuberculous laryngitis	FC 2 — 18	151	Extensive generalized tuberculosis
III V 880A	>467‡	Still living, 3 years and 3 months after infection	FC 1 — 7	154	Extensive generalized tuberculosis
III V 368	535	Ulcerative pulmonary tuberculosis and pyelonephritis	FC 2 — 19	162	Massive generalized tuberculosis
III V 685	741	Minimal tuberculosis of ileocecal junction and pyelonephritis	FC 2 — 22	208	Massive generalized tuberculosis
Mean.....	422 ± 154		Mean....	132 ± 42	

Critical ratio of difference in survival following infection between races III and FC = 5.1; P = 0.00

* All rabbits were given 6 intracutaneous injections of 1 mg. heat-killed Ravenel bacilli at weekly intervals. Virulent infection followed 70 days after last treatment with heat-killed tubercle bacilli.

‡ Tuberculous lymph nodes were removed from this rabbit 467 days after infection.

simultaneous inhalation of human type tubercle bacilli. Table III presents the observations. It is clear that, with doses of inhaled bacilli ranging from 100

TABLE II

Pulmonary Tuberculosis in Genetically Susceptible and Resistant Rabbits at Varying Intervals Following the Quantitative Inhalation of Virulent Human Type Tubercle Bacilli, H37Rv

Experiment No.	No. of bacilli estimated to be inhaled	Interval between exposure and death	Name of rabbit		Tuberculosis in lungs of:	
			Susceptible	Resistant	Susceptible	Resistant
		days				
1	607–640	36	FC 2 – 29	III 2 – 6	2 large tubercles	2 small tubercles
2	1750–2250	47	FC 2 – 7	III 3 – 13	28 tubercles with slight caseation	0
3	1952–2038	98–99	FC 3 = 1	III 3 – 15	4 clusters of tubercles in left and 3 isolated in right, some caseous	0
4	3759–4187	108	FC 3 = 8	III 3 – 25	Numerous clusters of caseous bronchogenic tubercles in both lungs	2 bronchogenic clusters, and 1 nodule in left lung
5	528–541	112	FC 1 – 15	III 3 – 9	8 tubercles, discrete and in clusters, with caseation	0
6	106–113	113	FC 2 – 4	III 3 – 7	Extensive bronchogenic tuberculosis in right lung and limited lesions in left	0
7	127	150	FC 2 – 14	—	0	—
8	1699	166	—	III 3 – 14	—	0*
9	1864–2263	165–167	FC 3 = 2	III 3 – 6	Numerous caseous and non-caseous tubercles in both lungs	1 isolated, excavated, 3 mm. tubercle in left lung
10	1953–2038	166–167	FC 2 – 30	III 3 – 21	Numerous tubercles throughout both lungs, many caseous, some not	0
11	2044–2191	166–168	FC 3 = 3	III 3 – 18	7 discrete caseous, and many non-caseous tubercles	0‡
12	575–588	192–264	FC 1 – 13	III 3 – 4	Clusters of bronchogenic tubercles in right	0
13	112–122	217–264	—	III 3 – 8 III V880C	—	0 0

* Culture and guinea pig negative.

‡ 1 colony cultured, guinea pig inoculation negative.

to about 2000, the tuberculin reaction appeared more quickly in the resistant than in the susceptible animals. This difference is statistically significant. With larger inhaled doses, while there is still a tendency for allergy to develop

TABLE III

Rapidity of Development of Allergic Sensitivity in Resistant Races, III and III R, and in Susceptible Races, FC, C and Ca, after Quantitative Inhalation of Virulent Human Type (H37Rv) Tubercle Bacilli

Date of experiment	No. of tubercle bacilli inhaled	Rabbit No.		Duration of preallergic period	
		Resistant	Susceptible	Resistant	Susceptible
				days	*days*
Feb. 27, '48	106–127	III 3 – 7	FC 2 – 4	17	45
		III 3 – 8	FC 2 – 14	24	No allergy
Feb. 27, '48	541–640	III 3 – 4	FC 1 – 13	No allergy	24
		III 3 – 9	FC 1 – 15	17	24
		III 2 – 6	FC 2 – 29	9	17
Nov. 15, '48	584–750	III 3 = 1	FC 3 = 14	16	23
		III 3 = 2	Ca 4 – 15	16	16
		—	Ca 4 – 11	—	23
Feb. 10, '49	1080–1152	III R2 – 7	C 7 – 43	12	20
Mar. 22, '49	1230–2214	III 3 – 30	Ca 4 – 23	13	11
		III 3 – 31	Ca 4 – 26	9	20
		III 3 – 33	Ca 4 – 25	Intercurrent illness	18
June 25, '48	1699–2263	III 3 – 14	FC 3 = 2	18	26
		III 3 – 13	FC 3 = 1	18	18
		III 3 – 6	FC 2 – 7	18	26
Mean				16 ± 1.2*	22 ± 2*
June 24, '48	1953–2344	III 3 – 15	FC 2 – 30	19	19
		III 3 – 21	FC 3 = 13	19	27
		III 3 – 18	—	19	—
Nov. 8, '48	3147–4327	III 3 – 25	FC 3 = 8	16	16
		III 3 – 26	FC 2 – 9	16	16
		—	Ca 4 – 1	—	16
Dec. 29, '50	3906–5868	III R3 = 7	C 10 = 4	11	Negative in 18 days
		III R3 = 13	C 11 = 18	18	Negative in 18 days
Jan. 11, '50	4238–5868	III R2 – 2	C 11 = 7	18	29
		III R3 = 10	C 9 = 1	29	29
Mean				18	>21

*"*P*" value of difference in the rate of development of allergy = 0.009.

more rapidly in the resistant animals, this was not the case in each instance. This is understood on the basis of the well known fact that the greater the infecting dose the more rapid the development of allergy. With these larger doses, the more rapid response to stimulation characteristic of the resistant animal can be masked by the great intensity of the stimulus. Furthermore, since the tuberculin tests were done only at weekly intervals, it is possible that differences in the rate of development of allergy were missed through failure to test at more frequent intervals.

Antibody Production.—While the role of antibodies in immunity to tuberculosis has not yet been established, their importance in the host's responses to penetration of foreign agents in general cannot be overlooked. The hemagglutination

TABLE IV

Hemagglutinin Titer at Different Intervals Following Quantitative Inhalation of Human Type Tubercle Bacilli

Rabbit No.		1 day before infection		6 days after infection		19 days after infection		32 days after infection	
Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
III R 3 = 9	C 11 = 9	0	2	4	4				
III R 3 = 5	C 10 = 8	2	2	16	2				
III R 3 = 7	C 10 = 4	2	0	4	8	4	8		
III R 3 = 13	C 11 = 18	4	0	32	2	32	2		
III R 2 = 2	C 9 = 1	4	2	4	2	8	2	8	4
III R 3 = 10	C 11 = 7	4	0	8	0	8	0	8	16
Geometric mean.....		2.5	1.4	8 ± 1.4*	2.5 ± 1.2*	9.8	2.5	8	8

* The "*P*" value of the difference in the titer 1 day before infection and 6 days after infection in the III R rabbits is 0.000; the "*P*" value of this difference in the C rabbits is 0.051.

procedure of Middlebrook and Dubos (6) was used to assess the antibody production of resistant and susceptible animals at different intervals following a single inhalation of 3000 to 6000 viable H37Rv bacilli, Table IV. In this test, the antibody response of the susceptible strain, C, was compared to that of the resistant strain, III R. That the C rabbits used in this experiment are much more susceptible than the race III R rabbits to bovine tuberculosis was shown by the following: 4 C and 4 III R rabbits were vaccinated with BCG simultaneously; 161 days thereafter, these 8 rabbits were infected with 28,000,000 viable, virulent bovine bacilli intracutaneously. The C rabbits died of tuberculosis 205 to 322 days after this virulent infection, with an average survival of 265 days. Of the race III R rabbits, one died 340 days after the infection from amyloid degeneration and minimal pulmonary tuberculosis; the remaining 3 rabbits are still living 736 days after the reinfection.

It is obvious from Table IV that the hemagglutinins generated by this infection were of low titer in both groups. Nevertheless, there is evidence of a clear tendency for a more rapid antibody response in the resistant rabbits. 6 days after infection the geometric mean of the titer in the resistant rabbits was 8 ±1.4; in the susceptible rabbits, it was 2.5 ±1.2. Of greater import is the observation that the rise of antibodies at this time over the preinfection level is statistically highly significant in the resistant rabbits while not quite in this category in the susceptible animals. These results confirm previous observations on other resistant and susceptible rabbit families (1).

Rate of Growth and Destruction of Human Type Tubercle Bacilli.—The fate of the bacilli in the lungs of susceptible and resistant rabbits at varying intervals following inhalation of this microorganism is illustrated in Table V.

It is clear that in the susceptible races, C and FC, the bacilli multiplied at a rapid rate for at least 5 weeks following inhalation. On the other hand, in the resistant animals the bacilli barely maintained their original numbers without significant increase at the end of 2 to 3 weeks. 7 weeks after inhalation, the bacilli in the lungs of the resistant rabbits had been almost completely destroyed, whereas in the susceptible animals they were still increasing in numbers. It is noteworthy that even in the grossly unaffected lung tissue of the susceptible animals, numerous living bacilli were recovered 5 weeks after infection, whereas aliquots of the entire lung of some of the resistant rabbits were free of tubercle bacilli as early as 3 weeks following inhalation. It is clear therefore that resistant rabbits rapidly destroy inhaled human type tubercle bacilli after a preliminary, limited, and evanescent multiplication. In the susceptible rabbits, on the other hand, the bacilli multiply at a high rate and persist in large numbers for a long time even in grossly unaffected lung parenchyma.

Histological Response.—The histological response to inhaled human type tubercle bacilli in the susceptible and resistant animals, 3 weeks after infection, is illustrated in Figs. 4 and 5. It will be seen that in the susceptible rabbit, C 10 = 4, (Fig. 4), the lesion consists of young epithelioid cell centers swarming with tubercle bacilli. These are surrounded by a great profusion of rapidly growing mononuclear cells infiltrating the surrounding alveolar septa and consolidating a considerable portion of the lung parenchyma. It is noteworthy that over five million tubercle bacilli were present in its lungs, as estimated by culture. On the other hand, Fig. 5 illustrates the response of the resistant rabbit, III R 3 = 13, at the same time following the same infection. All that could be seen was an extremely infrequent collection of epithelioid cells, some of which were quite mature and in which tubercle bacilli were rarely found. It is noteworthy that no tubercle bacilli could be cultured from aliquots of the contralateral lung of this rabbit. Another example illustrates the same striking difference. 5 weeks after infection, in the lung of C 9 = 1 (Fig. 6), there were typical tuberculous granulomas with caseous centers and numerous bacilli

TABLE V

Rate of Multiplication of Inhaled Human Type Tubercle Bacilli in the Lungs of Genetically Susceptible and Resistant Rabbits

Interval after infection	No. of rabbit		No. of bacilli cultured from lungs of		Ratio between No. of bacilli found in lungs and No. estimated to be inhaled	
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
days						
0	C 7 – 51	III R2 – 7	1,080*	1,152*		
13	C 7 – 51	III R2 – 7	32,418‡	1,300‡	30	1.1
21	C 11 = 18	III R3 = 7	590,000	5,800	150	1.1
	C 10 = 4	III R3 = 13	5,459,000	0	1,400	Complete destruction
33	C 7 – 43	—	29,760,000‡	—	29,000	—
34	C 11 = 7	III R2 – 2	4,305,000	0	921	Complete destruction
	C 9 = 1	III R3 = 10	2,640,000	296	622	0.05
36	FC 2 – 29	III 2 – 6	43,225§	0‖	71	Complete destruction
47	FC 2–7	III 3–13	1,861,000	45	827	0.08
166	—	III 3–14	—	0	—	Complete destruction
168	—	III 3–18	—	1 colony isolated from 12 cultures¶	—	Almost complete destruction

* Estimated as inhaled on basis of number of tubercle bacilli in respired air. No bacilli were cultured from treated specimens of lungs of susceptible and resistant rabbits, C 9=2 and III R 2–9, respectively, which were simultaneously exposed and had inhaled 936 and 1152 bacilli, by estimation.

‡ Bacilli recovered from treated specimens of lung.

§ From grossly unaffected lung after treatment.

‖ From grossly unaffected lung after treatment and guinea pig inoculation.

¶ Guinea pig inoculation—negative.

surrounded by an extensive perifocal inflammation. Over two million tubercle bacilli were estimated to be present in the lungs of this susceptible rabbit. The tracheobronchial lymph nodes of this rabbit showed extensive tubercle forma-

tion, slight caseation, and small numbers of tubercle bacilli. By contrast, in III R 2 = 2 (Fig. 7), both the lungs and the tracheobronchial lymph nodes were normal except for a rare slightly thickened septum in the former. No tubercle bacilli were cultured from these lungs.

The Number of Tubercle Bacilli Required to Generate a Single Primary Pulmonary Focus.—Since resistant rabbits destroy inhaled human type tubercle

TABLE VI

*The Relative Resistance of Race III and Ca Rabbits to the Intracutaneous Inoculation of 28,000,000 Viable Bovine Tubercle Bacilli, Ravenel**

Race III				Race Ca			
Rabbit No.	Survival in days after infection	Type and extent of tuberculosis	Cause of death	Rabbit No.	Survival in days after infection	Type and extent of tuberculosis	Cause of death
III 3 — 17	659	Still living		Ca 4 — 4	192	Massive caseous pneumonia with generalization	Tuberculosis
III 3 — 20	659	Still living		Ca 4 — 13	131	Minimal pulmonary tuberculosis and generalization	? plus tuberculosis
III 3 — 27	659	Still living		Ca 4 — 20	156	Minimal pulmonary tuberculosis, extensive intestinal and joint tuberculosis	Tuberculosis
III 3 — 28	659	Still living		Ca 4 — 24	462	Extensive generalized tuberculosis	Tuberculosis
III 3 — 29	263	Slight local tuberculosis	Enterocolitis and tuberculosis				

* All these rabbits were given a single intracutaneous inoculation of 1,240,000 viable BCG 61 days before the virulent bovine infection.

bacilli so much more rapidly than susceptible animals, it is clear why, at the end of several months, there is usually no pulmonary tuberculosis in the resistant race, whereas in the susceptible rabbits there is, as a rule, a variable and often extensive disease. It became of interest, therefore, to determine whether the varying resistance of rabbits to human type bacilli is of sufficient degree very early in the course of the infection to affect the genesis of the initial, primary, grossly visible pulmonary foci. For this purpose, the resistant race III rabbits were compared to the susceptible strain FC. The response of the

race III rabbits was also compared to that of the susceptible strain, Ca. Table VI shows that the Ca rabbits are much more susceptible to a standard dose of virulent bovine tubercle bacilli than race III.

Table VII shows that in race III over 600 viable H37Rv bacillary units had to be inhaled in order to generate a single, pulmonary focus, visible in the gross, 5 weeks after infection, whereas in the susceptible families Ca and FC, 49 and 107 units, respectively, estimated to be inhaled, were sufficient to generate such a primary pulmonary focus at this time. Notwithstanding the great individual variations in this ratio in the different experiments done at

TABLE VII

Estimated No. of Inhaled Units of Human Type Tubercle Bacilli Yielding One Primary Pulmonary Tubercle in Rabbits of Different Genetic Resistance

Rabbit No.	No. of bacillary units inhaled	No. of tubercles generated	No. of tubercle bacilli yielding one tubercle	Rabbit No.	No. of bacillary units inhaled	No. of tubercles generated	No. of tubercle bacilli yielding one tubercle	Rabbit No.	No. of bacillary units inhaled	No. of tubercles generated	No. of tubercle bacilli yielding one tubercle
FC 2 — 9	4215	107	39	Ca 4 — 23	1476	43	34	III 3 — 26	4327	4	1082
FC 3 = 14	700	14	50	Ca 4 — 25	1230	93	13	III 3 = 2	855	0	>855
FC 2 — 46	4470	55	81	Ca 4 — 26	1353	65	21	III 3 — 30	1968	2	984
FC 2 — 47	5960	63	95	Ca 4 — 1	3147	22	143	III 3 — 31	1722	7	246
FC 3 — 18	5960	201	30	Ca 4 — 11	515	3	172	III 3 — 33	2214	11	201
FC 2 — 50	6647	72	92	Ca 4 — 18	1944	194	10	III 4 = 8	597	0	>597
FC 3 = 29	7233	32	226	Ca 5 — 8	2592	436	6	III 4 = 11	600	0	>600
FC 3 = 31	1796	17	106	Ca 5 — 10	2376	307	8	III 4 = 13	570	0	>570
FC 4 = 3	2120	8	265	Ca 4 — 29	1665	44	38				
FC 3 = 37	1590	36	44								
FC 3 — 36	1440	9	160								
FC 2 — 56	1080	11	98								
Mean.....			107 ± 20				49 ± 19				642 ± 107

The "*P*" value of the difference between race III on the one hand, and races FC and Ca, respectively, on the other hand, equals 0.000.

different times, a statistical analysis of these results shows them to be highly significant. Thus 6 to 15 times as many units of human type bacilli must be inhaled by a genetically resistant rabbit as by a susceptible animal to yield the same number of primary foci. Hence the number of primary pulmonary foci caused by the inhalation of a given quantity of human type tubercle bacilli is a function of the native resistance of the rabbit exposed. This is illustrated in Fig. 3.

DISCUSSION

The practically all-or-none response of susceptible and resistant rabbits to the quantitative inhalation of human type tubercle bacilli illustrates sharply the role of genetic factors in resistance to tuberculosis. This difference is due

to the fact that in the resistant rabbit tubercle bacilli of the human type multiply for but a short time and are soon destroyed. On the other hand, in the susceptible animal the bacilli multiply profusely and for a long time. It is important to emphasize that even in the most resistant rabbit thus far studied, destruction does not take place before some preliminary multiplication. This is in accord with previous observations on the fate of human type tubercle bacilli injected intravenously into rabbits (8). Native resistance is apparently merely a tendency for a rapid development of acquired resistance. Native susceptibility is essentially a tendency for a tardy or ineffective development of acquired resistance. It is noteworthy that the response of the rabbit to the much more virulent bovine bacillus differs only in tempo, at least in certain organs, by comparison with its response to the less virulent human type bacillus. As was shown previously (8), bovine bacilli focalized in the liver, spleen, and bone marrow are destroyed just like human type bacilli similarly seeded. The only difference is the rapidity of this destruction. Human type bacilli are more rapidly annihilated than the bovine microorganism. Thus the greater resistance of rabbits, as a species, to human as opposed to bovine type bacilli is of the same nature as the greater resistance of certain rabbit families to the human bacillus, there being, in each case, a difference in the rate of acquiring an increased capacity to destroy tubercle bacilli.

Since genetically resistant rabbits destroy inhaled human type tubercle bacilli more rapidly than susceptible animals, and since allergy and antibodies develop more rapidly in the former, one may assume that the varying rates of destruction of the microorganism in the tissues of resistant and susceptible animals are the result of the varying rates of development of allergy and antibodies in these two types of animals. This would involve the hypothesis that the tissues of the resistant animal are more quickly and effectively altered, *i.e.*, sensitized and immunized, by a given amount of tubercle bacilli and their products than the more slowly responding tissues of the susceptible animal. Evidence for such a concept may be obtained by the use of soluble antigens administered in the same amounts to these two types of animals, and by determining the rate of sensitization and antibody formation in the resistant and susceptible animals. This remains to be done. In the light of present knowledge, it is clear that, irrespective of the cause of the destruction of the bacilli, the more rapidly the bacilli are disintegrated the more are the tissues exposed to the antigens released by their dissolution.

It is true that a factor related to virulence has recently been suggested to reside in a lipid on the surface of virulent tubercle bacilli, the "cord factor" of Middlebrook, Dubos, and Pierce (9) and the petroleum ether-soluble substance of Bloch (10). Nevertheless, these surface substances have not been demonstrated to cause the development of allergic sensitivity or the acquisition of antibodies against the tubercle bacillus. The former is the response to a

wax-protein complex (11) in the tubercle bacillus; the latter is conditioned by carbohydrates (6). All these substances, together with other antigens which may yet be demonstrated to play a role in the development of immunity to tuberculosis, are released on the destruction of the bacilli. Hence, the more rapid release of these antigens in the resistant animal will further enhance the development of allergic sensitivity, the production of antibodies, and the development of increased resistance to the infection.

It has been demonstrated in this study that the number of primary pulmonary foci, visible in the gross, initially generated by the inhalation of the same number of units of human type tubercle bacilli in different animals is a function of the genetic resistance of the animal exposed. The greater the resistance, the fewer these foci.

It is important to emphasize at this point that the estimate of the number of tubercle bacilli inhaled by the exposed animals was based on the culture of the air respired by the rabbits. Each colony cultured from this air may have been derived from an isolated organism or from a group of them. Despite the fact that the suspension atomized consisted very largely of isolated microorganisms, one cannot say whether a given droplet nucleus resulted from the evaporation of a droplet containing one or more isolated microorganisms. For this reason the term bacillary unit is used in referring to the infectious particles. Furthermore, it is impossible to state whether a given gross primary pulmonary tubercle resulted from a single microorganism or from a group of microorganisms, small enough to penetrate into the alveoli. With these provisions in mind, we may now consider the significance of the number of primary tubercles generated in the susceptible and resistant rabbits.

It is essential to state that even in susceptible rabbits only a fraction of the human type bacilli that reach the terminal air passages of the lung induces the formation of visible tubercles. With bovine bacilli, on the other hand, it has been shown that, irrespective of the genetic resistance of the animal exposed (4), the ratio between the number of bacillary units calculated as inhaled and the number of gross primary pulmonary tubercles developed within 5 weeks after infection is constant, namely, about three units of highly virulent bovine bacilli of the Ravenel strain for each tubercle. It has been shown by Brown *et al.* in man inhaling clay particles, as well as by Goldberg and Leif who studied respiratory infection with *Bacillus pestis* in mice (5), that only one-third of the inhaled particles of the size of tubercle bacilli reach the alveoli of the lung. It follows, therefore, that each viable unit of highly virulent bovine bacilli that reaches the terminal pulmonary air passages yields a visible pulmonary tubercle, irrespective of the resistance of the host, or the number of tubercle bacilli contained in each particle. From this one may say that neither the possible variations in virulence of the individual Ravenel bacillary units, nor the variations in the resistance of the different alveolar phagocytes that ingest

these inhaled bacilli are of sufficient range to prevent the multiplication of each of these bacillary units to form a visible tubercle. When human bacilli are inhaled, on the other hand, only a fraction of the bacillary units that reach the terminal air passages give rise to a tubercle. This may be due primarily to one of three variables: (*a*) the number of individual organisms present in each bacillary unit, (*b*) the different virulence of the individual particles inhaled, and (*c*) the difference in the rate of development of sufficient resistance by the individual phagocytes to inhibit rapidly the growth of the bacilli in their cytoplasm. There is no evidence at present for the first two alternatives. On the other hand, if the last supposition is true, it would follow that any measure which reduces the resistance of the phagocytes to the growth of the bacilli in their cytoplasm should increase the number of primary tubercles that originate from the inhalation of the same number of tubercle bacilli. Conversely, any measure which increases the resistance of these phagocytes to the growth of the bacilli in their substance should reduce the number of tubercles that will result from the inhalation of a given number of human type tubercle bacilli. The first condition has already been demonstrated (12). If litter mates of the susceptible strain, FC, are divided into two groups, and if one of them is given pharmacologic doses of cortisone, the latter will develop three to four times as many tubercles as the untreated rabbits. In the above report it has been shown by histological and cultural studies that cortisone enhances the growth of tubercle bacilli within the alveolar phagocytes. On the other hand, in a forthcoming paper (13) it will be reported that if rabbits of the same genetic resistance are immunized with BCG the number of initial tubercles generated from the inhalation of a given number of human type tubercle bacilli is greatly reduced. It has been demonstrated by the eye chamber technique (14) that immunization decreases the growth of the bacilli within the phagocytes. Therefore, the varying genetic resistance of rabbits to the inhalation of human type tubercle bacilli may depend upon the prevalence of alveolar phagocytes capable of developing an adequate power to destroy or inhibit the growth of the bacilli ingested in their cytoplasm. The greater the incidence of such phagocytes, the greater the resistance of the animal. The prevalence of such phagocytes in a given animal is subject to hormonal and immunological influences.

The importance of this method in assaying genetic resistance and in studying the effect of different constitutional and other forces on host resistance is obvious.

SUMMARY AND CONCLUSIONS

If genetically resistant and susceptible rabbits inhale a certain number of human type tubercle bacilli, no tuberculosis in the lungs of the resistant animals is seen, as a rule several months after infection, while there is a variable and often extensive disease in the susceptible rabbits. The analogy to the presence

or absence of active tuberculosis in man infected with the tubercle bacillus is evident.

The inhaled tubercle bacilli multiply for but a short time in the resistant rabbits and are usually rapidly and completely destroyed. In the susceptible rabbits, the bacilli multiply profusely for a much longer time and persist in large numbers even months after inhalation.

Whatever be the cause of the more rapid destruction of tubercle bacilli in the resistant animal, the resulting more rapid release of the contained antigens enhances the development of allergic sensitivity and antibodies in these animals.

The development of an acquired resistance against tubercle bacilli of the human type is sufficiently rapid to affect the genesis of the initial gross primary pulmonary foci that result from the inhalation of a given number of bacilli. The greater the genetic resistance, the fewer the initial primary foci.

Variations in genetic resistance are essentially variations in the rate of development of acquired resistance.

It is suggested that variations in genetic resistance to inhaled human type tubercle bacilli may affect the prevalence of alveolar phagocytes capable of acquiring adequate resistance to the growth of the bacilli in their cytoplasm. The prevalence of such cells is subject to hormonal and immunological influences.

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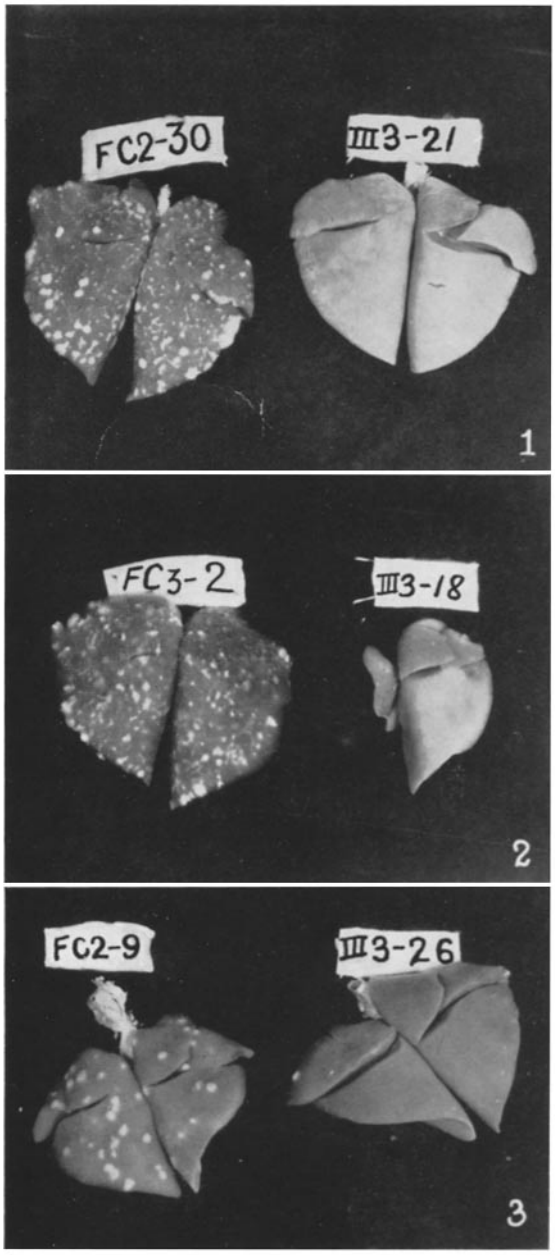
EXPLANATION OF PLATES

PLATE 2

FIG. 1. The lungs of susceptible rabbit FC 2-30 and of resistant rabbit III 3-21, 166 to 167 days after the simultaneous inhalation of about 2000 human type tubercle bacilli. The lungs of the susceptible rabbit contain numerous tubercles. The lungs of the resistant rabbit are free from tuberculosis visible in the gross.

FIG. 2. The lungs of susceptible rabbit FC 3-2 and of resistant rabbit III 3-18, 165 to 168 days after the inhalation of 1900 to 2000 human type tubercle bacilli. No microorganisms were recovered from the contralateral lung of III 3-18 by guinea pig inoculation.

FIG. 3. The lungs of susceptible rabbit FC 2-9 and of resistant rabbit III 3-26, 32 days after the simultaneous inhalation of 4200 to 4300 human type tubercle bacilli. One of 39 inhaled human type bacillary units generated a tubercle in the susceptible animal, while only one of 1000 inhaled bacillary units gave rise to a primary pulmonary focus in the resistant animal.



(Lurie *et al.*: Resistance to tuberculosis)

PLATE 3

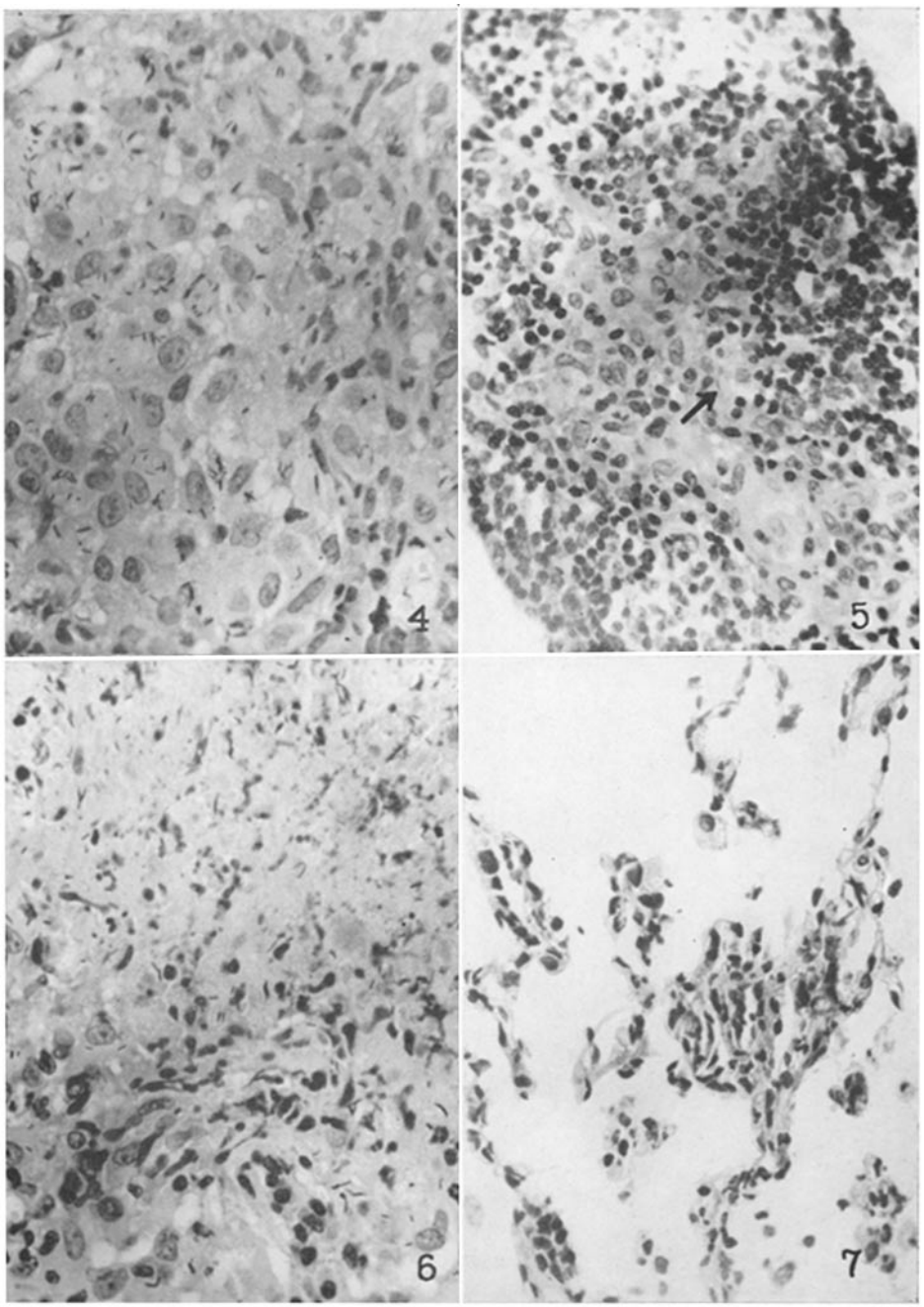
All photomicrographs were prepared from tissues stained by the Ziehl-Neelsen method and counterstained with hematoxylin. The magnifications are: Figs. 4 and 6 about × 550; Figs. 5 and 7 about × 440.

FIG. 4. A primary tubercle in the lung of susceptible rabbit C 10-4, 21 days after inhalation of 3900 human type tubercle bacilli; 5,400,000 tubercle bacilli were estimated, by culture, to be present in these lungs. Young epithelioid cells swarming with tubercle bacilli may be seen.

FIG. 5. A minute tubercle in a peribronchial lymph follicle in the lung of the resistant rabbit III R 3-13, 21 days after the inhalation of 5800 human type tubercle bacilli. No tubercle bacilli were cultured from the contralateral lung of this rabbit. One of the very rare collections of mature epithelioid cells is seen. Rare, faintly stained tubercle bacilli are found. One of these is indicated by the arrow.

FIG. 6. A well advanced tubercle with central caseation and numerous bacilli in the surrounding tuberculous granulation tissue in the lung of susceptible rabbit C 9-1, 34 days after the inhalation of 3600 human type tubercle bacilli; 2,600,000 tubercle bacilli were estimated, by culture, to be present in the lungs of this rabbit.

FIG. 7. The remains of an almost completely resorbed subpleural tubercle in the lung of resistant rabbit III R 2-2, exposed simultaneously with, and killed at the same time as susceptible rabbit C 9-1 illustrated in Fig. 6. This rabbit inhaled 4700 human type tubercle bacilli. Slightly thickened alveolar septa and a few residual exudative cells are all that remain from the healing tubercle. No tubercle bacilli were cultured from this lung.



(Lurie *et al.*: Resistance to tuberculosis)