J. Hyg., Camb. (1960), **58**, 215
Printed in Great Britain

Variations in natural resistance to tuberculosis

By DAVID F. GRAY, HEATHER GRAHAM-SMITH*, and JOHN L. NOBLE†
From the School of Bacteriology, University of Melbourne, Parkville N. 2, Victoria, Australia

(*Received* 20 *February* 1960)

INTRODUCTION

The present uncertainty concerning the mechanisms of natural or inherent resistance to tuberculosis was expressed by Rich in his closing remarks as Chairman of the 1955 Ciba Symposium, and a reluctance on the part of some workers to distinguish between natural resistance and acquired immunity has not simplified the problem. Therefore, it should be clearly understood in the present discussion that 'natural resistance' refers only to antituberculous mechanisms present at the time of infection in a previously unexposed and unvaccinated individual. There has also been a tendency in discussing the interplay of the host and the tubercle bacillus to confuse the mechanisms underlying the response (*a*) of different animal species to a particular strain of tubercle bacillus, and (*b*) of a particular animal species to different strains of the organism. The view taken here is that these two interactions should be considered separately and no further reference will be made to variations in the infecting organism, but attention will be directed to so-called natural species and strain variations upon exposure to a particular mammalian tubercle bacillus.

Species resistance

Ratcliffe & Palladino (1953) suggested that observed differences in the response of rats, mice, hamsters and guinea-pigs to inhaled tubercle bacilli, because they were delayed for several weeks, were attributable to acquired immunity and not to natural resistance; observations that are in accord with the view that there is no significant variation in the ability of normal monocytes derived from different species to destroy tubercle bacilli *in vitro* (Rich, 1951; Mackaness, 1952, 1954).

On the other hand, the reports of Dubos (1952), Hirsch & Dubos (1952) and Hirsch (1954) on the presence of humoral antituberculous substances, such as spermine in kidney extracts from rabbits and cows, and peptides in the thymus of calves, suggest that these represent true variations in natural species resistance. Soltys too (1953) isolated an antituberculous substance from lymph nodes of tuberculous bovines, and Tsuji & Ito (1955), who implanted semi-permeable capsules containing tubercle bacilli into normal rabbits, reported growth inhibition by low molecular weight humoral fractions of the peritoneal fluid. As this inhibitory effect was neutralized by the high molecular weight fractions, the total

* Supported by grant from National Health and Medical Research Council.
† Supported by grant from Melbourne University Medical Research Funds.

body fluids of rabbits actually promoted intracapsular growth. Kumashiro (1958) extending these observations to rats, found intracapsular growth to be inhibited by both high and low molecular weight fractions of the peritoneal fluid and concluded that normal rat body fluids were more unsuited to the *in vivo* growth of tubercle bacilli than those of the rabbit.

Thus while it is difficult to assess the importance of these findings, in the present state of our knowledge it seems possible that natural humoral factors could influence *species* resistance to some extent. However, the role of acquired immunity is apparently a major one.

Strain resistance

The problem of variations in resistance within a species at the *familial* level has been the subject of extensive studies by Lurie (1944, 1950, and Lurie, Zapposodi & Tickner, 1955). Working with susceptible and resistant rabbit families derived by selection from the same stock, the results obtained by Lurie and his colleagues led them to the conclusion that differences in rabbits at this level were attributable solely to the speed of development of acquired immunity.

During work on the mechanisms of pathogenesis and acquired immunity carried on in the Tuberculosis Research Unit of this School in recent years, a number of similar observations were made with an apparent bearing on species and strain resistance. In 1952 Gray & Mattinson found that the C57 black mouse—a susceptible strain of an allegedly resistant species—could be regularly infected with the smallest dose of H37 Rv capable of inducing tubercle formation in guinea-pigs, provided the organisms were introduced directly into the lung. Following an initial lag phase lasting 5 or 6 days (Gray, 1959) the bacilli in the lung entered an active logarithmic phase lasting at least 3 weeks, but occasionally longer. This phase and the accompanying pneumonia ended, either in the pre-allergic death of the animal when insufficient normal lung remained to support life, or in a simultaneous arrest of bacillary multiplication and of pneumonia, coinciding approximately with the appearance of foot-pad sensitivity to tuberculin. The impression gained was that as no obvious natural defences were brought into play, the result of the disease hinged solely on whether the animal survived long enough for acquired immunity to arrest the logarithmic phase of the bacilli in the lungs (Gray & Jennings, 1955; Gray & Affleck, 1958; Gray, 1958).

In 1956 an opportunity occurred to perform intranasal inoculations on other mouse strains, viz. those used by Dr Dubos and his colleagues at the Rockefeller Institute. It was found there that the reputedly resistant Rockefeller Institute and Rockefeller Swiss Strains could also be infected with small doses of H 37 Rv by this route. When it transpired that the subsequent early course of the disease resembled that previously observed in the reputedly more susceptible C 57 black strain, Dubos suggested that the strain differences involved might be determined by the acquired immune response rather than by natural differences.

The present paper reports a detailed study of the behaviour of three different strains of mice infected intranasally with *Mycobacterium tuberculosis* H37 Rv. It is probable that the differences studied in the present paper were racial rather than

strain differences and should be interpreted as such. But, as it cannot be determined whether or not all laboratory mice originated from a common genetic group, it is perhaps more correct to refer in the present context to *strain*, rather than *racial* variations.

MATERIALS AND METHODS

The previous descriptions of most of the materials and methods should be referred to for precise details omitted here (Gray & Mattinson, 1952; Gray & Jennings, 1955; Gray, 1958).

Mouse strains

The three strains of mice used have been maintained as closed, random-mated colonies for 12 years or longer. The C57 black strain originated from the Roscoe Jackson Memorial Laboratories, while the Hall Institute multi-coloured mice (HI) and the Melbourne University albino (MUA) strains are local strains of uncertain origin. Conditions of age, sex and diet were the same in each experimental group.

Cultures

Mycobacterium tuberculosis (H37 Rv) grown for 10 days as a dispersed liquid culture was homogenized, filtered twice through cotton-wool and suitable dilutions used for infecting mice intranasally.

Resistance of mouse strains to intranasal infection

Previous experience had shown that tuberculous mice will die either in the pre-allergic (early) or post-allergic (late) phase of the infection (Gray & Affleck, 1958) and that these two phases may be separated by an immune phase of variable duration, depending partly on the size of the infecting dose. In the present experiment the dose of tubercle bacilli was adjusted to about 30,000 viable units to cause a high proportion of pre-allergic deaths in the susceptible C57 strain and smaller numbers in the other two groups.

Progress of pulmonary disease

A smaller infecting dose (150 viable units) was selected here to avoid pre-allergic deaths as far as possible, while causing extensive lung involvement. At 2- or 3-day intervals when one mouse from each group was killed with coal gas, the rate of progress of pulmonary disease was assessed in terms of progressive lung-weight increments. These were compared with the normal lung weight for mice of that age, because this method provided a clearer picture than either a description or numerical assessment of visible changes in organ. After weighing, the lobes of the lung were homogenized for 5 min. at 10,000 r.p.m. in 2 ml. of 0·1 % albumin water in an MSE micro-homogenizer* (Gray, 1959). Culturable counts were made from serial dilutions of the resulting suspension on Löwenstein's medium, rinsed immediately before sowing with an antibiotic mixture containing 10 units of penicillin and 10 μg. chloramphenicol per ml.

* Thomas Optical Co., Melbourne.

https://doi.org/10.1017/S0022172400038304 Published online by Cambridge University Press

Rate of conversion of mouse strains to tuberculin hypersensitivity

It is known that tuberculous mice invariably develop a positive foot-pad reaction to 1/25 Old Tuberculin. With larger infecting doses, for example, in C57 mice, the conversion rate of the group is rapid and fairly uniform so that all mice still alive after 4 or 5 weeks would be expected to give a positive reaction. With smaller doses, for example ten to twenty bacilli, individual conversions may be delayed up to the ninth week with maximal numbers in the 4–6 week period. Predictable variation in the conversion rate due to age occurring within a strain (Gray & Jennings, 1955), were avoided in the present experiment. All experimental animals, together with normal controls, were tested each week by inoculating approximately 0·03 ml. of 1/25 O.T. in saline into a foot-pad of alternate hind feet. Results were read in mm. of antero-posterior thickening of the foot as determined by micrometer measurements* 24 and 48 hr. later. Increases in foot thickness ranging from 0·2 to 1·5 mm. above the average of the controls (2 mm.) were recorded as positive tuberculin reactions.

RESULTS

Resistance of mouse strains to Mycobacterium tuberculosis *H 37 Rv*

Three groups each of thirty-three mice of the C57, HI and MUA strains infected intranasally with a dose later shown to contain 31,500 culturable units, were observed daily and the death rates recorded. Lungs removed aseptically from the dead mice were weighed and the visible consolidation recorded as a percentage

Table 1. *Gross lung changes as determined by increase in lung weights in mice infected intranasally with* 31,500 *bacilli* (*H 37 Rv*) (*referred to in Fig.* 1)

		Gross lung changes observed			
		Lung weight			
Mouse strain	Classification	Average normal mice (no. of mice)	Average tuberculous mice (no. of mice)	Average weight increase	Estimated average lung consolidation (%)
C57 strain	Highly susceptible	0·27 (10)	1·06 g. (28)	× 3·9	90
HI strain	Moderately susceptible	0·28 (10)	1·28 g. (14)	× 4·6	93
MUA strain	Resistant	0·29 (10)	1·32 g. (5)	× 4·6	95

involvement of the lung, while smears and cultures ruled out deaths from other causes. Foot-pad tests for tuberculin hypersensitivity were performed from the end of the second week and the experiment was terminated when pre-allergic deaths ceased.

* Schnelltaster (0–10 mm in 0·05 mm.) donated by Dr S. Boyden, State Serum Institute, Copenhagen.

https://doi.org/10.1017/S0022172400038304 Published online by Cambridge University Press

From the results set out in Fig. 1, it will be seen that the three strains were satisfactorily separated at this dose level. The C57 strain emerged as highly susceptible with 84% of the group dead by the thirty-fifth day and the MUA strain as resistant (15% dead), while the HI strain occupied an intermediate position with 42% dead. Table 1 shows the average fourfold increase in lung weights of the dead mice to be in keeping with observed virtually complete consolidation of the lungs in all strains.

It is significant that no indication of the level of mouse-strain resistance was to be gained by studying the disease in individual dead mice and these findings support the belief that pre-allergic deaths, being independent of strain resistance, occurred in mice of any strain as soon as insufficient normal lung remained to supply the physiological needs of the animal.

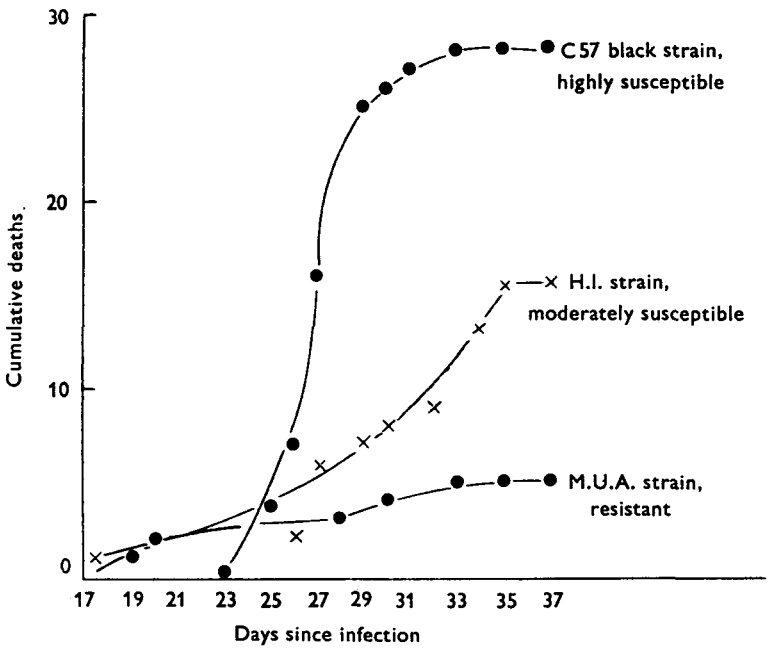

Fig. 1. Cumulative pre-allergic tuberculosis death rates in groups of thirty-three C57, HI and MUA mice infected intranasally with 31,500 bacilli (H37 Rv).

Lung consolidation and growth of tubercle bacilli in the lungs of three mouse strains

As it may be argued that gross lung changes, in the dead animals (Table 1), prove only that a proportion of the animals in each mouse strain possessed no natural resistance at all, an experiment was designed to follow the course of the disease by repeated examination of the three strains at frequent intervals. The infecting dose was small enough to have caused few pre-allergic deaths had no animals been sacrificed (see Table 4). Therefore, there was reasonable certainty that the picture derived from the mice examined at these regular intervals represented accurately the course of the disease as it occurs in animals surviving to enter the immune

phase. This is important in establishing the concept that there does occur, not only an arrest of the disease with immunity, but that lung lesions and bacilli both actually decrease at this time.

Three groups each of thirty-three mice of the same age and sex were infected intranasally with 150 culturable units of H37 Rv, the inoculum showing the usual moderate clumping with some units of five to ten bacilli. At intervals of approximately three days one mouse from each strain was killed and lung weights and culturable bacilli were determined. Table 2 sets out the comparative progress of gross lung consolidation. An examination of cumulative lung weights shows a

Table 2. *Comparative progress of lung disease in C57, HI and MUA mice infected intranasally with* 150 *bacilli* (*H37 Rv*)

	Lung weights								
Days since infection	C57 strain highly susceptible (g.)		HI strain moderately susceptible (g.)		MUA strain resistant (g.)	
1	0·23		0·26		0·27	
3	0·28		0·25		0·26	
6	0·28		0·22		0·22	
8	0·29		0·23		0·25	
10	0·27		0·24		0·25	
13	0·28		0·34		0·23	
14	0·30		0·35		0·32	
15	0·34		0·36		0·30	
17	0·32		0·29		0·29	
20	0·25		0·29		0·36	
22	0·36		0·29		0·29	
24	0·4		0·30		0·32	
27	0·47		0·29		0·40	
29	0·52		0·32		0·54	
30	0·65		0·56		0·90*	
31	0·60		0·88*		0·98	
34	1·29		1·11*		0·96	
			1·05*			
36	0·87		1·27		0·50 (+)	
38	0·95		1·79		0·54 (+)	
			1·46*			
41	0·83		0·41 (+)		0·42 (+)	
43	1·07		0·43 (+)		0·60 (+)	
45	0·43 (+)		0·36 (+)		0·43 (+)	Av.† 0·61
48	0·69 (+)		0·45 (+)		0·33 (+)	
50	0·80 (+)		0·91 (+)	Av.†= 0·55	0·69 (+)	
52	0·46 (+)	Av.†= 0·58	0·36 (+)		0·46 (+)	
55	0·47 (+)		0·84 (+)		0·64 (+)	
57	0·60 (+)		0·61 (+)		1·17 (+)	
59	0·55 (+)		0·37 (+)		0·73 (+)	
62	0·60 (+)		0·80 (+)		0·71 (+)	

* = died naturally.

(+) = foot-pad reaction positive when killed: all others negative at weekly test prior to sacrifice.

† = Average lung weights of tuberculin positive mice: highly susceptible = 0·58 g.; moderately susceptible = 0·55 g.; resistant = 0·61 g.

https://doi.org/10.1017/S0022172400038304 Published online by Cambridge University Press

slightly greater initial rate of increase in the susceptible C57 strain, with a tendency for the resistant MUA strain to lag behind, but the three groups were not separable at this stage and dramatic changes in the progress of lung lesions occurred only when positive tuberculin reactors were killed. In these animals, regardless of strain, the lungs were lighter in weight than in those that had not yet converted. As this had been anticipated (Gray & Affleck, 1958), animals were earmarked on converson and held over until there were no more negative reactors to be killed, viz.: after 36 days (MUA) 41 days (HI) and 45 days (C57). It should be noted,

Table 3. *Comparative culturable lung counts in three mouse strains infected intranasally with* 150 *bacilli, showing bacillary numbers in relation to duration of infection and foot-pad sensitivity to tuberculin*

	Culturable lung counts*					
	C57 strain		HI strain		MAU strain	
Days since infection	Bacilli ($\times 10^4$)	Foot-pad reaction	Bacilli ($\times 10^4$)	Foot-pad reaction	Bacilli ($\times 10^4$)	Foot-pad reaction
1	0·083	–	0·108	–	0·091	–
3	0·114	–	0·094	–	0·125	–
6	0·048	–	0·125	–	0·245	–
8	0·185	–	0·420	–	0·615	–
10	0·575	–	0·765	–	1·0	–
13	7·5	–	6·5	–	70·0	–
14	15·0	–	12·0	–	.	.
15	23·3	–	83·3	–	192·0	–
17	45·0	–	170·0	–	83·3	–
20	933·0	–	1,200·0	–	683·0	–
22	1,550·0	–	1,250·0	–	1,117·0	–
24	1,580·0	–	2,700·0	–	7,330·0	–
27	5,170·0	–	3,000·0	–	28,300·0	–
29	20,000·0	–	3,000·0	–	200,000·0	–
31	4,700·0	–	3,600·0	–	233,000·0	–
			250,000·0†	–	400,000·0†	–
34	283,000·0	–	550,000·0	–	300,000·0	–
			1,700,000·0†	–	.	.
			1,500,000·0†	.	.	.
36	258,000·0	–	.	.	3,050·0	+
38	55,000·0	–	28,300·0	–	1,500·0	+
			2,500,000·0†	–	.	.
41	67,000·0	–	3,500·0	+	1,830·0	+
43	1,700,000·0	–	3,830·0	+	450·0	+
45	170·0	+	1,300·0	+	1,700·0	+
48	8,300·0	+	660·0	+	1,500·0	+
50	8,500·0	+	170·0	+	5,000·0	+
52	1,700·0	+	830·0	+	2,300·0	+
55	3,700·0	+	2,500·0	+	830·0	+
57	2,300·0	+	1,500·0	+	4,200·0	+
59	1,830·0	+	1,080·0	+	4,000·0	+
62	1,830·0	+	2,170·0	+	1,700·0	+
64	2,000·0	+	.	.	.	.

* = counts made on Löwenstein's medium treated with penicillin and chloramphenicol.
† = pre-allergic deaths.

https://doi.org/10.1017/S0022172400038304 Published online by Cambridge University Press

therefore, that positive reactors were present in all groups before the times indicated in Table 2, and that actual conversion rates of the three strains at this age and dose level are shown later (Table 4).

If the progress of lung consolidation was not notably different between strains in the pre-allergic period, was this also true of the rate of bacillary multiplication?

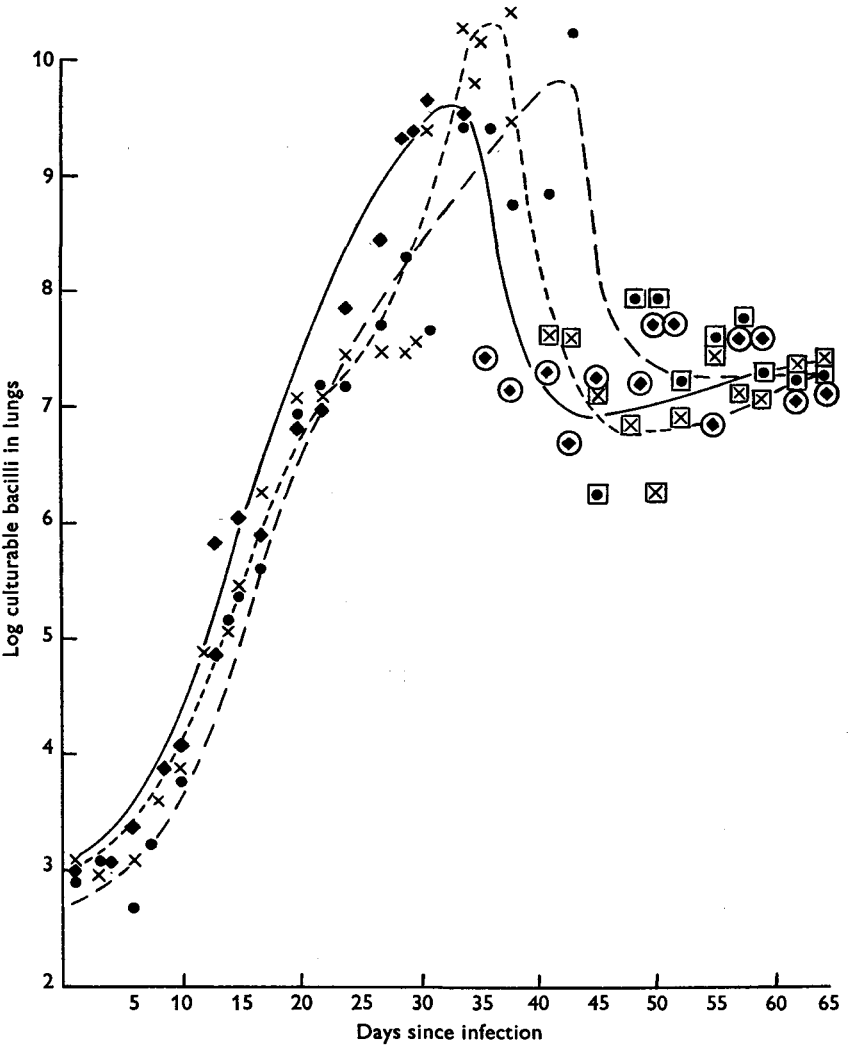

Fig. 2. Comparison of culturable lung counts in relation to duration of infection and foot-pad sensitivity to tuberculin in three strains of mice. ●——● C57 strain—highly susceptible; × – – – ×, H.I. strain—moderately susceptible; ◆—◆, M.U.A. strain—resistant; ⊡ ⊠ ⊕, animals showing positive tuberculin reaction.

Table 3 gives a complete account of the lung cultures, while in Fig. 2 are presented superimposed graphs in which the lung counts have been plotted against time. It will be seen here, in contrast to lung consolidation, that the rate of multiplication appears to have been slightly faster in the resistant strains. The arbitrarily

https://doi.org/10.1017/S0022172400038304 Published online by Cambridge University Press

arranged end of the logarithmic phase (see above), occurred 10 days earlier in the resistant MUA strain than in the susceptible C57 mice, while the HI strain occupied an intermediate position. The bacteriological status and the average lung weights of allergic mice in the three groups (Tables 2 and 3, Fig. 2) both indicate that once achieved, the level of acquired immunity was about equal in the three strains. This equality persisted for at least 3 months after conversion, but because the experiment was then terminated, it is not known whether strain differences in the duration of the immune phase might eventually have become evident.

In order to determine whether the information arrived at by inference (Tables 2 and 3) was a true indication of the relative conversion rates of the three strains to foot-pad positivity, further mouse groups of the same age were inoculated with 150 units of H37 Rv intranasally and tested at weekly intervals. The cumulative totals of animals showing a positive reaction were plotted against time (Table 4). The conversion rates of the three mouse strains fell into the pattern suggested

Table 4. *Rate of conversion of mouse strains to tuberculin sensitivity.* (*Infecting dose* 150 *bacilli*)

Time since infection (weeks)	Mice responding with positive foot-pad reactions to 1/25 O.T. (cumulative totals). No. of animals per group = 33		
	C57 strain susceptible	HI strain moderately susceptible	MUA strain resistant
2	.	.	.
3	.	.	3
4	.	1	9
5	3	8	14
6	15	18	24
7	24	29	32
8	32*	33	32*

* One pre-allergic death in each of these groups.

by the results of the large infecting dose (Table 2), being highest in the resistant strain and lowest in the susceptible one. Interestingly enough this experiment also showed that if the infecting dose used was small enough to minimize *pre-allergic* deaths, the differences in strain resistance as shown by mortality rates (Fig. 1), disappeared.

As all the present results point to conversion rate as the key to differences in resistance between mouse strains, it would appear (in the absence of detectable participation of natural humoral or cellular factors), that strain-resistance stems from genetically based differences in the speed, but probably not in the quality of the immune response. The difference in rate is not great, but occurring as it does towards the peak of lung involvement and therefore, of pre-allergic deaths, it accounts for the observed difference in death rates.

DISCUSSION

The observations reported here permit certain conclusions:

(1) Dramatic mouse-strain differences in resistance to tuberculosis observed at moderate to high intranasal dose levels, diminish and even disappear as the infecting dose is lowered.

(2) These differences are apparently confined largely to pre-allergic deaths.

(3) There is no significant strain difference during the first few weeks in the rate of bacillary multiplication or of lung consolidation, and mice of all strains die as soon as functional lung tissue is reduced below an effective level.

(4) Bacillary multiplication and lung consolidation are arrested with the onset of acquired immunity. The bacilli then appear actually to diminish in numbers and the lung consolidation to be reduced.

(5) It is the speed of onset of immunity rather than natural resistance *per se* that determines the level of mouse *strain* resistance to tuberculosis.

The question now arises whether *strain* resistance and *species* resistance are in fact separate phenomena as Rich suggested (1951), with natural antituberculous activity playing an important role only at the species level. At the same time it is profitable to ask, how real the accepted species differences in resistance are, and to what extent they depend on natural resistance on the one hand, and on acquired immunity on the other.

Strain resistance

The results obtained in the present experiments are based on a comparison of three mouse strains showing a marked difference in mortality rates when infected intranasally with about 30,000 human bacilli. It was found that the early progress of lung lesions in susceptible and resistant mice was quite unrelated to the observed differences in mortality. The only detectable strain difference, apart from the proportion of pre-allergic deaths in each group, was in the rate at which conversion took place to tuberculin sensitivity. This in turn was the most convenient index of the relative speed of onset of acquired immunity. The surviving immune mice of all three strains showed uniformly less lung involvement and lower bacillary counts than animals which, although infected for the same time, had not yet entered the immune phase. Once established, the quality and the duration of immunity up to 3 months did not seem to differ from one strain to another (Fig. 2).

These findings confirm Lurie's results with susceptible and resistant strains, or families of rabbits selected from the same original stock Therefore mouse strains, representing genetic differences that are probably best described as 'racial' and rabbit strains that were genetically speaking even more closely related, both showed a remarkable degree of tolerance to early multiplication of tubercle bacilli. It seems justified to conclude on this evidence that *natural strain resistance* is not a variable; it simply does not exist. The observed differences between strains of these two animal species are apparently due solely to the speed, quality and duration of the acquired immune response.

This hypothesis, while accounting for the lack of early lung reaction to lung

invasion, still leaves to be explained the apparently less tolerant reaction of other organs towards tubercle bacilli. The fact that the spleen of the mouse appears to be less susceptible than the lung, could be due to natural or acquired mechanisms, or to both. To explain it on a natural basis would require a determinative role for antituberculous substances, or acceptance of Canetti's suggestion (1955) of a variation in the bactericidal power of monocytes according to organ. The available evidence does not appear to justify either of these views. First, it would have to be shown that the organ distribution of antituberculous substances and of effective monocytes corresponds to organ resistance; secondly, it is pointed out that the highly susceptible rabbit kidney is a recognized source of the antituberculous substance, spermine. Finally, it seems fairly certain that these organ differences, being delayed in appearance are therefore more the result of acquired immunity than of natural resistance.

Species resistance

Before considering the possible mechanisms of natural species resistance to tuberculosis it is pertinent to re-examine the accepted standards of species resistance. The guinea-pig is normally classified as highly susceptible, man as moderately so, and the mouse and rat as highly resistant (Rich, 1951). Francis (1958) brackets the mouse with urban man as moderately resistant, but agrees that the guinea-pig is very susceptible and the rat resistant. These generalizations may puzzle the worker in this field using intravenous or intranasal inoculation, unless he realizes that Rich's figures, based on *subcutaneous* inoculation bear little relationship to the manner in which these species would be grouped according to resistance to pulmonary infection.

Gray & Jennings reported in this respect (1955) and confirmed subsequently that guinea-pigs infected intranasally with 2000 viable units of H37 Rv survived significantly longer than C57 mice infected by the same route with only one-twentieth of that dose. It is also pointed out that C57 mice (in contrast to man, who tends to recover from pulmonary infection) have never recovered in our experience, even with small infecting doses. On this basis, the C57 mouse would be regarded as highly susceptible, the guinea-pig moderately so and man as highly resistant.

Our experience with rats too, though less extensive than with mice, supports Wessels' observations (1941) and the more recent observations of Kumashiro (1958), that rapid early multiplication occurs in the lungs of this species 'without effective opposition'. As the present writers have found that intranasal infection with small doses of H37 Rv produces progressive tuberculosis in rats (to be published), it is suggested that their *natural* species resistance is not as high as is commonly supposed.

It will be recalled, moreover, that Ratcliffe & Palladino failed to detect any species variation between resistant and susceptible experimental animals in the initial response to inhaled tubercle bacilli and attributed ultimate species differences to acquired immunity. In view of the tolerance shown by most, if not all experimental animal species, to early multiplication of bacilli in the lung, it now

seems pertinent to question whether *natural species* resistance actually exists as such, any more than natural strain resistance does.

Obviously we cannot ignore the possible role of known or unknown natural antituberculous substances and other biochemical differences between species that may be unfavourable to the multiplication of tubercle bacilli. Nevertheless, it seems to the present writers that most of the observed species differences can be satisfactorily accounted for by acquired phenomena, including both the immune reaction and the degree of species sensitivity to allergic damage.

In conclusion it may be asked whether the adjectives 'natural', 'innate', or 'inherent' should be retained any longer to describe observed variations in the response to tuberculosis of either species or strains of animals.

SUMMARY

1. 'Resistant' and 'susceptible' *species* of animals appear to be more or less equally susceptible to *lung infection* with equal doses of tubercle bacilli. Therefore it is pertinent to ask whether recognized *natural* differences in species resistant are in fact significant. For example, in terms of *death rates* (i.e. of overall resistance) the C57 mouse is at least as susceptible to tuberculosis as the guinea-pig and much more so than man.

2. Resistant, moderately susceptible and susceptible *strains* of mice as determined by death rates when exposed to large infecting doses, were equally susceptible to intranasal infection with small numbers of tubercle bacilli.

3. A state of tolerance of the parasite by the host lasting for about 3 weeks was observed in all mouse strains, regardless of ultimate strain resistance.

4. Pre-allergic deaths commenced in all groups when the tuberculous processes left insufficient normal lung to support life, but the deaths stopped first in the resistant strain and last in the susceptible strain, coinciding approximately in each strain with the onset of allergy.

5. Acquired immunity, once established, appeared not to vary in quality from one mouse strain to another, at least during 3 months' observation.

6. Racial or strain variations in the resistance of mice to tuberculosis are therefore natural, only in the sense that speed of onset of acquired immunity is probably genetically determined for each strain.

7. It is suggested that both species and racial variations in natural resistance to *pulmonary infection* are insignificant. Differences in the *subsequent course* of the disease appear to be explainable by the rapidity, efficiency and duration of the acquired immune response.

REFERENCES

CANETTI, G. (1955). *The tubercle Bacillus in the Pulmonary Lesions of Man.* (revised ed.). New York: Springer.

DUBOS, R. J. (1952). A tuberculostatic agent present in animal tissues. *Amer. Rev. Tuberc.* **63**, 119.

FRANCIS, J. (1958). *Tuberculosis in Animals and Man.* London: Cassell.

GRAY, D. F. & MATTINSON, M. W. (1952). Detection of small numbers of tubercle bacilli from dispersed cultures. *Amer. Rev. Tuberc.* **65**, 572.

GRAY, D. F. & JENNINGS, P. A. (1955). Allergy in experimental mouse tuberculosis. *Amer. Rev. Tuberc.* **72**, 171.

GRAY, D. F. & AFFLECK, M. N. (1958). Relationship of allergy to gross lung disease and culturable bacilli in tuberculous mice. *Amer. Rev. Tuberc.* **78**, 226.

GRAY, D. F. (1958). Immunity, natural anergy and artificial desensitization in experimental tuberculosis. *Amer. Rev. Tuberc.* **78**, 235.

GRAY, D. F. (1959). Fate of tubercle bacilli in early experimental infection of the mouse. *J. Hyg., Camb.*, **57**, 473.

HIRSCH, J. G. & DUBOS, R. J. (1952). The effect of spermine on tubercle bacilli. *J. Exp. Med.* **95**, 191.

HIRSCH, J. G. (1954). Mechanisms involved in the antimycobacterial activity of certain basic peptides. *J. Exp. Med.* **99**, 79.

KUMASHIRO, A. (1958). Studies on the susceptibility of rats to various strains of Mycobacteria, Parts I, II and III. *Acta Tuberc. Japon.* **8**, 1.

LURIE, M. B. (1944). Experimental epidemiology of tuberculosis. *J. Exp. Med.* **79**, 573.

LURIE, M. B. (1950). Native and acquired resistance to tuberculosis. *Amer. J. Med.* **9**, 591.

LURIE, M. B., ZAPPOSODI, P. & TICKNER, C. (1955). On the nature of genetic resistance to tuberculosis in the light of the host parasite relationships in natively resistant and susceptible rabbits. *Amer. Rev. Tuberc.* **72**, 297.

MACKANESS, G. B. (1952). The action of drugs on intracellular tubercle bacilli. *J. Path. Bact.* **64**, 429.

MACKANESS, G. B. (1954). The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. *Amer. Rev. Tuberc.* **69**, 495.

RATCLIFFE, H. L. & PALLADINO, V. S. (1953). Tuberculosis induced by droplet nuclei infection. *J. Exp. Med.* **97**, 61.

RICH, A. R. (1951). *The Pathogenesis of Tuberculosis.* 2nd edition. Oxford: Blackwell.

RICH, A. R. (1955). *Experimental Tuberculosis.* Ciba Found. Symp. p. 337. London: S. & A. Churchill Ltd.

SOLTYS, M. A. (1953). An antituberculous substance in tuberculous organs. *J. comp. Path.* **63**, 147.

TSUJI, S. & ITO, K. (1955). An *in vivo* method of culturing tubercle bacilli: the chamber method. *Amer. Rev. Tuberc.* **72**, 393.

WESSELS, C. C. (1941). Tuberculosis in the rat. Parts I, II and III. *Amer. Rev. Tuberc.* **43**, 449, 459, 637.