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The relative natural resistance of rats and mice to experimental pulmonary tuberculosis*

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INTRODUCTION

Confirmation of the theory that rats infected with pulmonary tuberculosis should exhibit a syndrome resembling that in mice, not only in the early course of the disease and the onset of the immune phase, but also in the development of a detectable allergic response to footpad inoculation of tuberculin, brought the pathology of the rat response *qualitatively* into line with other animal species (Gray, Noble & O'Hara, 1961).

Some years ago Gray & Mattinson (1952) indicated that any difference in the tuberculous response of mice and guinea-pigs lay outside the first few weeks of the disease by showing that both these animals responded equally to very small intranasal infecting doses. Subsequently, Ratcliffe & Palladino (1953) similarly failed to detect any early difference in the response of rats, mice, hamsters and guinea-pigs to inhaled tubercle bacilli.

More recently, in discussing the results of their experiments showing that differences between 'susceptible', 'moderately resistant' and 'resistant' strains of mice measured as death rates were governed by the *rapidity of the immune response*, Gray, Graham-Smith & Noble (1960) suggested the possibility of a similar basis for observed differences in species resistance to tuberculosis. In those experiments the three strains of mice were compared at frequent intervals after intranasal inoculation with the same dose of tubercle bacilli to determine the progress of bacillary multiplication and lung consolidation. At the same time footpad tests were used to determine the rate of conversion to the allergic state as an index of the onset of the immune phase, when the disease either remained quiescent, or actually retrogressed. There was found to be as much as ten days difference in the average conversion time of the susceptible C57 strain and the resistant MUA strain of mice, while the moderately resistant HI strain fell in time, between these two extremes.

The similarity of the disease studied in rats (Gray *et al.* 1961) to that now so well characterized in mice, appeared sufficiently striking to warrant a detailed study of the response of comparable animals from each species. Accordingly, a comparison was undertaken of intranasally infected mice and rats with particular reference to multiplication of bacilli, progress of lung lesions and allergic response,

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as well as pre-allergic and terminal anergic deaths. The object of this study was to provide further evidence bearing on the reality of 'natural resistance' to tuberculosis and the results reported here favour the hypothesis that there is no appreciable species difference in the natural resistance of rats and mice to tuberculosis.

MATERIALS AND METHODS

The experimental methods employed in this study, of which the salient features follow, were the same as those reported in comparing the resistance of the three mouse strains referred to above (Gray *et al.* 1960).

Experimental animals

Rats and mice

The rat strain selected for this study was a Wistar-albino cross, maintained as a closed, randomly mated line for many years, the same as that on which the recently reported observations on the pathogenesis and allergic response of the rat were made (Gray *et al.* 1961). The mice were of the Melbourne University albino (MUA) strain which, although resistant to tuberculosis in terms of death rate, showed no greater ability during the first 2 weeks of the disease to curb the progress of tuberculosis, than the highly susceptible C57 black strain that has been used here in routine tuberculosis studies for many years.

Both mice and rats were maintained after weaning on a vitamin-supplemented, complete pelleted ration, with adequate water and no change in diet was made during the experiments.

Infection

Under a bacteriological hood, mice and rats of the same age and sex were infected intranasally (Gray & Mattinson, 1952) from the same culture suspension. The infecting dose of tubercle bacilli of strain H37Rv that had been grown for 10 days in dispersed culture was suspended in 0·1% albumin water. The mouse dose volume was the usual 0·06 ml. but that for the rats was increased to 0·08 ml. in view of their larger lung volume; i.e. the animals received respectively 3 and 4 drops from a 50-drop pipette.

Assessment of disease

A pair each of rats and mice was killed immediately after infection to determine the efficiency of dosing, and then twice each week throughout the pre-allergic phase of the disease. Allergic animals, earmarked at the time of the first positive footpad reaction, were killed in pairs at selected times after conversion. After weighing to assess the disease in terms of consolidation, the lungs were ground in an MSE Micro-Homogenizer (Gray, 1959) and culturable counts carried out in the usual manner on Lowenstein–Jensen medium.

Allergic reaction to tuberculin

Allergy was assessed by measuring the reaction to approximately 0·03 ml. of Old Tuberculin diluted 1/25 (mice) or 1/3·5 (rats), injected into a footpad of alternate hind feet. The tests were repeated every 3–4 days until conversion occurred, and

then at weekly intervals. The reaction was recorded in mm. of increased thickness of the test foot over the control foot in each animal and a further control was provided by the routine testing at the same intervals, of a group of normal animals. It was arbitrarily decided to record any reaction of more than 0·1 mm. at 48 hr., as a positive reaction; though it is emphasized that individual swellings were often more than ten times the threshold reading.

RESULTS

The pathogenesis of tuberculosis in mice and rats related to their tuberculin reaction, using a moderate infecting dose of 43,000 *culturable units*

Under an ether-chloroform anaesthesia thirty male mice and thirty male rats 6 weeks of age were infected intranasally with an estimated 30,000 bacilli, but the culturable count proved the dose to be rather higher at about 43,000 units. One pair of each group was killed 1 hr. later and a pair each of pre-allergic rats and mice was killed twice weekly. However as soon as allergic animals became available, a pair of these was also killed in addition to the negative reactors. For convenience in interpretation, the pre-allergic and allergic animals of each species have been separated in Table 1. It will be noted that while the pre-allergic group is arranged in order of time elapsing since infection, the order of the allergic group is also related to the time of conversion.

The results presented here both for the pre-allergic and allergic phases, may be compared for each species in terms of four observations: (*a*) multiplication rate of tubercle bacilli; (*b*) percentage increase in lung weight over an arbitrarily selected normal weight at the time of infection. This figure arbitrarily makes no allowance for normal lung weight increments over the 6 weeks of the experiment; (*c*) deaths, and (*d*) rate of conversion to the allergic state. As both groups were numerically reduced at the same rate by animals sacrificed twice weekly, it is considered that the conversion rate of the remainder in each case, as shown, is a comparable figure. The experiment was terminated after 6 weeks.

(*a*) *Multiplication of tubercle bacilli in the lungs*

The pre-allergic multiplication rate in the two species is perhaps most easily followed by referring to Fig. 1. Within the limits of the small number of animals killed at each interval and the inherent errors of the experimental procedures used, these differences are no greater than are found between two groups of the same species. Therefore, it is safe to conclude that the rats and mice used here showed no significant difference in this phase of the disease. The rate of multiplication in fact, did not differ from what we have come to regard as typical of the logarithmic growth of tubercle bacilli in the temporary absence of any really effective opposition from the host.

The allergic phase also failed to produce any surprises in either species, as an effective arrest of bacilli first detected during the 3rd week and fully established by the 5th week of the disease, was uniformly maintained by both species over the next 4 weeks of observation.

Table 1. *Progress of pulmonary tuberculosis and bacillary counts in rats and mice related to tuberculin reaction*

(Infecting dose = 43,000 units intranasally.)

	Mice					Rats				
			Culturable lung counts tubercle bacilli					Culturable lung counts tubercle bacilli		
Weeks since infection	Mouse no.	Footpad reaction 1/25 O.T. (duration)	Individual count $\times 10^6$	Average log count (Fig. 1)	Average lung weight (g.)	Rat no.	Footpad reaction 1/3·5 O.T (duration)	Individual count $\times 10^6$	Average log count (Fig. 1)	Average lung weight (g.)
					Pre-allergic					
0	1	–	0·045	4·78	0·26	1	–	0·065	4·81	0·70
	2	–	0·75			2	–	Contam.		
½	3	–	1·0	5·76	0·25	3	–	Contam.	5·85	0·94
	4	–	0·14			4	–	0·7		
1	5	–	1·15	5·99	0·27	5	–	0·2	5·29	0·95
	6	–	0·8			6	–	0·18		
1½	7	–	5·0	6·70	0·38	7	–	0·8	6·41	0·88
	8	–	5·0			8	–	4·5		
2	9	–	5·5	6·68	0·35	9	–	2·5	6·72	1·2
	10	–	4·0			10	–	8·0		
3	11	–	28·0	7·44	0·46	11	–	50·0	7·70	1·62
3½	12	–	500·0*	8·70	0·50	12	–	90·0*	7·95	2·72
4	13	–	112·0 sick	8·05	0·48	13	–	85·0	7·93	3·14
4½	.†					14	–	106·0*	8·03	3·4
5	.†					15	–	750·0 sick	8·88	3·8
					Allergic					
2½	14	+	7·0	6·80	0·43	16	+	2·0	6·60	1·21
	15	+	5·5			17	+	6·0		
3	16	+ (1)	4·0	6·83	0·46	18	+ (1)	3·5	6·54	1·06
	17	+ (1)	9·5			19	+ (2)	Contam.		
4	18	+ (2)	4·5	6·60	0·6	20	+ (2)	6·4	6·82	2·4
	19	+ (2)	3·5			21	+ (2)	6·95		
5	20	+ (3)	6·5	6·63	0·55	22	+ (3)	7·5	6·94	2·23
	21	+ (3)	2·0			23	+ (3)	10·0		
6	22	+ (4)	7·5	6·92	0·47	24	+ (4)	11·5	7·09	1·98
	23	+ (4)	9·0			25	+ (5)	13·0		

* Died.

† All the mice had converted by the 4th week of the test.

Footpad reaction. A swelling of > 1 mm. in foot inoculated with c. 0·03 ml. of tuberculin diluted 1/25 (mice) or 1/3·5 (rats).

In this respect then, the responses of the rat and the 'resistant' MUA mouse were inseparable. By inference (Gray *et al.* 1960), the pre-allergic rat response was therefore identical with that of 'moderately resistant' H1 mouse and the 'highly susceptible' C57 black mouse, for these strains were shown to be the same as the MUA strain of mice. It will be also remembered that the C57 mouse was shown to be as susceptible to intranasal infection as the guinea-pig (Gray & Mattinson, 1952).

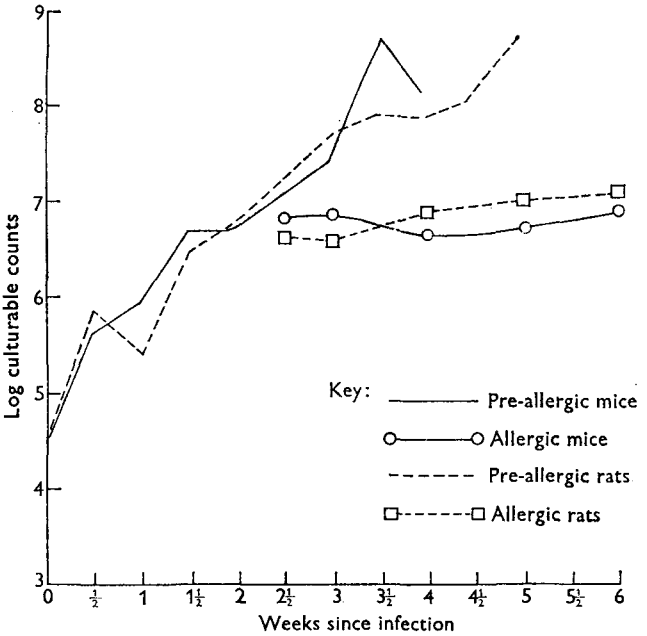

Fig. 1. Mean culturable lung counts on pairs of rats and mice infected intranasally with 43,000 tubercle bacilli.

(*b*) *Percentage increase in rat and mouse lung weights*

These figures, recorded as an index of the rate of lung consolidation under the influence of the disease have always followed closely the pre-allergic and allergic multiplication rates in mouse experiments. In the present case there was little difference in rat and mouse increments for 3 weeks but thereafter it was surprising to note that progress of the lesions in the rats seemed to be less well controlled than in the mice, which in the allergic groups, stabilized at an average of 100% weight increase, compared with 155% in the reputedly resistant rats with a milder allergic response.

(*c*) *Deaths*

At this dose level, there were only three pre-allergic deaths altogether. This was rather fewer than would have been expected from previous experience with these strains of rats and mice receiving 43,000 infective units, but it must be remembered that more than half of each group was killed in the pre-allergic phase. One death occurred in the mice and two in the rats, though it seems likely that at least one

more of each, killed while sick, would have died shortly. There would appear to be no significant difference in pre-allergic death rates in this experiment; there were naturally no deaths in the allergic phase, and the experiment was terminated before any terminal anergic deaths were encountered. Thus, observed death rates also failed to suggest any species difference under these conditions.

(*d*) *Conversion rates*

Reference to the time and number of conversions (see Table 1), similarly reveals no significant difference between the two species in this respect. Ten of twenty-three mice and ten of twenty-five rats developed positive tuberculin reactions between 2 and 4 weeks after infection, the pre-allergic phase passing, if anything, a little more rapidly in the mice (4 weeks) than in the rats (5 weeks). There were no reversions to the anergic state in either group in this experiment, but it should be noted that no allergic animal was permitted to live more than 4 weeks after conversion. In short, the differences between the rats and the mice were no greater than would be expected if two similar groups of mice had been compared with each other.

DISCUSSION

It seems clear from the results outlined above, that neither in multiplication of bacilli, in death rates during the period of observation nor in conversion rates to tuberculin hypersensitivity was there any observable difference under these conditions in the response of rats and mice to intranasal infection with a moderate dose of tubercle bacilli. It might be argued that if there was, in fact, a slightly greater progress of lung consolidation in one group than in another, then this occurred in the rats. But, although rats develop a less readily detectable allergic response to tuberculosis than mice, there was no evidence obtained in the present studies that rats possessed any greater degree of natural resistance, nor did they exhibit a quicker or stronger specific immune reaction than mice. On the contrary, lung involvement tended to be greater and conversion perhaps a little slower in the rats. The response then, of these Wistar-albino rats to tuberculosis did not differ appreciably from that of the 'resistant' MUA strain of mice under these experimental conditions. Because it has already been shown that MUA mice possess no greater natural resistance to pulmonary tuberculosis than C57 mice, and that C57 mice are no more resistant to early lung infection than guinea pigs, it seems safe to conclude that natural resistance to tuberculosis if it does exist in any of these species, is not of a high order.

It must be pointed out that this conclusion is based solely on the observed response of each species to the introduction of tubercle bacilli into the lungs. For reasons that are not yet clear, certain animal species, particularly those with a low natural hypersensitivity potential, are much less susceptible than others to infection by routes that avoid the lodgment of bacilli in the lungs. The importance of the antituberculous substances that have been reported in certain organs of several animal species is hard to assess and must await further species comparisons. The latent period of inoculated tubercle bacilli lasts about 5 days in the mouse

lung, (Gray, 1959), but as far as the writer is aware there is no information about its duration in other organs. If it were prolonged, or, alternatively, if there were qualitative differences between organs in macrophage efficiency, the onset of active immunity could conceivably anticipate extensive multiplication in some organs.

As a conclusion from the evidence presented here it seems justified to reiterate the suggestion put forward tentatively in a recent publication, that the adjectives 'natural', 'innate' or 'inherent' are no longer permissible when we describe variations in the response of different animals to natural or experimental tuberculosis. It would seem to be preferable to refer to species differences in 'immunity' to the disease.

SUMMARY

1. Wistar-albino rats showed no significant difference from albino mice in their response to pulmonary tuberculosis. This conclusion was reached after a detailed comparison of bacillary multiplication, lung involvement, death rates and the rate of conversion to the immune state as determined by footpad reaction to tuberculin in the two species.

2. These findings support the theory that the key to *species* differences in resistance to tuberculosis is the rate of onset of *acquired immunity* and it is therefore unrelated to *natural resistance*.

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