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Allergy in experimental rat tuberculosis

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INTRODUCTION

Early workers described the rat as highly resistant to human, bovine and avian strains of tubercle bacilli, regardless of whether they were introduced orally, subcutaneously or intraperitoneally (Gloyne & Page, 1923; Ornstein & Steinbach, 1925). However, it became evident from later studies that when the bacilli had an opportunity to lodge in certain organs, notably the lungs, rats could develop a serious and sometimes fatal disease (Smith, 1925–26; Perla, 1936; Wessels, 1941; Kumashiro, 1958*a*). In recent years Gray & Mattinson (1952) reported that mice and guinea-pigs were equally susceptible to intranasal inoculation while Ratcliffe & Palladino (1953) found rats exposed to airborne tubercle bacilli to be as susceptible as guinea-pigs to initial infection.

Reports on the response of tuberculous rats to local or systemic contact with tuberculin are sufficiently contradictory to be difficult to assess and interpret. In an attempt to do so, Rich (1951) remarked that hypersensitivity to tuberculin and to intact tubercle bacilli developed feebly or not at all in infected rats on a balanced diet.

Analysis of these early reports on the behaviour of infected rats reveals that Hehre & Freund (1939), as well as Wessels, Ratcliffe & Palladino and Kumashiro all observed, after several weeks, a dramatic arrest in the progress of the disease that followed an early uninhibited multiplication of the bacilli within rapidly developing lesions. It was this observation that directed the attention of the present writers to the similarity between rat and mouse tuberculosis. Such an arrest in the multiplication of bacilli in lung lesions, coinciding as it did with the onset of the immune phase in mice, led to the discovery of tuberculin allergy in that species. In 1952 (Gray & Mattinson) it was shown that the reputedly *resistant* and *anergic* mouse could be infected with about one viable unit, given intranasally, and that temporarily unrestricted multiplication and lung involvement was replaced by an immune phase of the disease. This in turn was characterized by a positive footpad reaction to 1/25 tuberculin (Gray & Jennings, 1955) and it was also shown that, as the immune phase progressed the mice became susceptible to fatal shock occurring 24–48 hr. after intraperitoneal inoculation of concentrated tuberculin. Subsequent work has elaborated these findings.

Studies begun in 1955 confirmed the supposition that rats, like mice, could be

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infected with small intranasal doses of the H37Rv strain of tubercle bacilli but, while the pathogenesis of the disease was very close to that seen in the mouse, initial attempts to elicit a footpad response to tuberculin at 1/25 or 1/10 dilution gave inconclusive results. Working on the assumption that if mice are approximately 800 times as resistant to histamine as guinea-pigs (Trethewie, 1947), rats might be even more resistant than mice to both immediate and delayed allergies (Dougherty, 1952), further tests with more concentrated tuberculin produced the results presented here.

Except for the minor differences that the histological changes in rats included giant cells lacking in mouse tuberculosis and that their tuberculin sensitivity was less readily detected, it is reported here that the pattern of tuberculosis in the rat was remarkably similar to that which is now so well known in the mouse. This means that one more species, regarded for many years as atypical in its response to tuberculosis, has been shown to follow the conventional pattern except for merely minor, quantitative variations in those aspects of the disease related to hypersensitivity. A later report will consider the question of the alleged high natural resistance of the rat as a species to tuberculosis.

MATERIALS AND METHODS

For a detailed description of most of the materials and methods employed here, reference should be made to reports by Gray & Mattinson (1952), Gray & Jennings (1955), Gray (1958) and Gray, Graham-Smith & Noble (1960).

Rats

Rats derived from a Wistar-albino cross and bred as a randomly mated closed line for more than 15 years in the Melbourne University Medical School Small Animal Unit, were infected intranasally at the age of 4 or 6 weeks, when weighing from 100–150 g. They were fed on a complete maintenance diet in pelleted form both before and during the experiments reported here.

Cultures

Mycobacterium tuberculosis (H37Rv) recently passaged for virulence and grown for 10–12 days as a dispersed liquid culture in Tween-albumin medium was twice filtered through cotton wool to reduce clumps and diluted suitably to give the required intranasal infecting dose in a volume of 0·1 ml., delivered on to the anterior nares with a 50-drop pipette. The rats were anaesthetized in groups of three in a suitable cylindrical museum jar with a two to one mixture of ether and chloroform placed under a layer of dry cotton wool in the bottom of the jar.

Manipulations

All dilution procedures and the inoculation of tubercle bacilli into animals were performed under slight negative pressure in a suitable bacteriologial hood by masked operators and the animals subsequently were transferred to an ultra-violet-

irradiated isolation unit 1 hr. after completing the inoculation when return of organisms from the respiratory tract to the environment became minimal (Gray & Mattinson, 1952).

Footpad tuberculin tests

Following the initial, inconclusive results in rats obtained with the 1/25 tuberculin dilution used successfully in mice and with a 1/10 dilution, the concentration was increased progressively. Eventually we adopted the highest dose giving either no, or minimal, response when inoculated at weekly intervals into the footpad of uninfected rats, namely, approximately 0·03 ml. of a 1/3·5 dilution of Old Tuberculin in physiological saline. The allergic reaction was measured by a Schnell-taster micrometer in mm. of antero-posterior thickening of the test foot compared with the normal foot, 24 and 48 hr. after inoculation (Gray *et al.* 1960).

Tuberculin shock

The systemic effects of tuberculin hypersensitivity were induced by inoculating undiluted tuberculin intraperitoneally into allergic rats in the eighth or subsequent weeks of the disease. Deaths occurring 24–48 hr. later with a characteristic haemorrhagic flare about the lung lesions were regarded as specific.

Assessment of disease

Infected animals were killed with coal gas at suitable intervals and the lungs, after inspection and weighing, were homogenized in an MSE micro-homogenizer (Gray, 1959) with 0·1 % albumin water and then diluted for culturable counts in the usual manner. Loewenstein's medium slopes in screw-capped McCartney bottles rinsed with penicillin, 10 units, and chloramphenicol, 10 μg./ml. were used for this purpose.

The rapid weight increments of the infected rat lungs, compared with the relatively slow increments of those of normal rats of the same age, afforded a reliable index of the rate of lung consolidation, in the same manner as has been reported in mouse studies (Gray & Affleck, 1958).

RESULTS

Pulmonary tuberculosis in rats infected intranasally with a small dose (500 units) of tubercle bacilli

Male rats 4½ weeks old were infected intranasally with about 500 viable units of H37Rv. One week later and thereafter every 3–4 days a rat was sacrificed for examination. Each week all the remaining animals, together with uninfected controls, were footpad-inoculated in alternate hind feet with *c.* 0·3 ml. of 1/3·5 tuberculin and, as each rat developed a measurable allergic response (> 0·1 mm.), it was earmarked with an appropriate symbol, using a chicken punch. Killing of positive reactors was deferred until no more negative reactors remained in the group and the order of killing was then arranged according to the duration of the allergic state. The results of this experiment, grouped for ease of assessment of each phase of the disease, are set out in Table 1.

The results obtained were remarkably close to what had been anticipated, in that a pre-allergic phase of relatively unrestricted growth occurred which resulted in one death at $3\frac{1}{2}$ weeks with extensive lung consolidation and a high bacterial count. This phase was followed by the allergic (immune) phase when the lung weights remained reasonably stable and so, within limits, did the numbers of tubercle bacilli recovered from them; certainly the rapid multiplication and consolidation observed earlier did not continue. This pattern, together with the terminal anergic flare noted in two of the rats, was quite reminiscent of the well-established mouse response to tuberculosis similarly induced in that species (Gray & Affleck, 1958).

Table 1. *Pathogenesis of pulmonary tuberculosis in intranasally infected rats related to duration of disease and to allergic response*

(Small infecting dose = 500 culturable units.)

Rat no.	Weeks since infection	Fate	Footpad reaction to 1/3·5 tuberculin (duration in weeks)	Body weight at death (g.)	Examination of lungs		
					Weight (g.)	Culturable count $\times 10^6$	Log of count
			Pre-allergic phase				
1	1	Killed	–	95	0·98	0·012	4·07
2	$1\frac{1}{2}$	Killed	–	100	1·01	0·017	4·23
3	2	Killed	– (1)	148	1·41	0·18	4·25
4	$2\frac{1}{2}$	Killed	– ($1\frac{1}{2}$)	135	1·27	0·25	5·40
5	$3\frac{1}{2}$	Died	– ($2\frac{1}{2}$)	85	3·25	1000	9·0
			Allergic phase				
6	$3\frac{1}{2}$	Killed	+ (< 1)	120	1·45	5·0	6·70
7	$3\frac{1}{2}$	Killed	+ (< 1)	182	1·08	8·3	6·92
8	4	Killed	+ (1)	120	1·28	50·0	7·70
9	$4\frac{1}{2}$	Killed	+ (2)	159	1·72	3·5	6·54
10	5	Killed	+ ($2\frac{1}{2}$)	232	2·10	13·3	7·12
11	$5\frac{1}{2}$	Killed	+ (3)	190	1·8	0·2	5·30
12	$6\frac{1}{2}$	Killed	+ ($4\frac{1}{2}$)	240	2·3	35·0	7·54
13	7	Killed	+ (5)	220	1·1	5·5	6·74
14	8	Killed	+ (6)	205	3·6	0·83	5·92
15	9	Killed	+ (7)	210	2·97	1·0	6·0
16	10	Killed	+ (8)	210	2·60	8·3	6·92
			Terminal anergic phase				
17	6	Died	+ (3) → – (1)	90	4·4	117	8·07
18	$6\frac{1}{2}$	Killed (Sick)	+ (3) → – ($1\frac{1}{2}$)	191	3·5	283	8·45

Pulmonary tuberculosis in rats receiving a moderate dose (50,000 units) of tubercle bacilli

Conditions were much the same in this experiment except that the twenty-five male rats used were older by $1\frac{1}{2}$ weeks and received a hundredfold larger infecting dose. The results are presented in Table 2.

The larger dose emphasized the pre-allergic phase in this group, which once more

conformed to the mouse pattern. Two rats died in this experiment and another (no. 10) probably would have done so. Curiously enough, and as has also been observed in mice (to be published), the bacillary content of the lungs as well as the lung weights stabilized in the allergic phase at much the same level as in the previous experiment despite the hundredfold larger infecting dose that was given in this experiment.

Table 2. *Pathogenesis of pulmonary tuberculosis in intranasally infected rats related to duration of disease and to allergic response*

(Moderate infecting dose = 50,000 culturable units.)

Rat no.	Weeks since infection	Fate	Footpad reaction to 1/3·5 tuberculin (duration in weeks)	Examination of lungs		
				Weight (g.)	Culturable count $\times 10^6$	Log of count
			Pre-allergic phase			
1	1	Killed	–	1·13	0·18	5·25
2	1	Killed	–	0·78	0·2	5·30
3	1½	Killed	–	1·11	0·8	5·90
4	1½	Killed	–	0·66	4·5	6·65
5	2	Killed	– (1)	1·25	2·5	6·40
6	2	Killed	– (1)	1·14	8·0	6·90
7	3	Killed	– (2)	1·62	50·0	7·70
8	3½	Died	– (2)	3·73	90·0	7·95
9	4	Killed	– (3)	3·14	85·0	7·93
10	4½	Killed (sick)	– (3½)	3·40	106·0	8·03
11	5	Died	– (4)	3·80	750·0	8·88
			Allergic phase			
12	2½	Killed	+ (½)	1·01	2·0	6·30
13	2½	Killed	+ (½)	1·42	6·0	6·78
14	3	Killed	+ (1)	1·06	3·5	6·54
15	3½	Killed	+ (1½)	2·07	2·0	6·30
16	3½	Killed	+ (1½)	1·43	40·0	7·60
17	4	Killed	+ (2)	2·06	2·5	6·40
18	4	Killed	+ (2)	2·75	9·0	6·95
19	4½	Killed	+ (2½)	2·56	1·5	6·18
20	4½	Killed	+ (2½)	2·80	12·0	7·08
21	4½	Killed	+ (1½) – (1)	3·73	15·0	7·18
22	5½	Killed	+ (3½)	1·76	7·5	6·88
23	5½	Killed	+ (3½)	3·10	10·0	7·00
24	5½	Killed	+ (3½)	1·86	13·0	7·11

Analysis of the allergic response

Tuberculin dilution in the footpad reaction

As stated above, early attempts to demonstrate a footpad reaction in rats with 1/25 or 1/10 dilutions of tuberculin were inconclusive, even though these animals could be fatally shocked with intraperitoneal tuberculin. Published reports indicated that other workers in this field had failed to test tuberculous rats with

stronger concentrations, so, being convinced that an allergic phase could be demonstrated given optimal conditions and keeping in mind the fact that routine tuberculin testing in cattle employs synthetic medium tuberculin equivalent in strength to undiluted O.T., it was decided to try stronger concentrations on infected and control rats. It seemed that undiluted and 1/2·5 O.T. were too strong, both producing a moderate swelling in normal rats at 24 hr., which still persisted slightly at 48 hr. On the other hand, 1/5 O.T. produced no visible reaction in normal rats, while a 1/3·5 dilution rarely produced a transient swelling not exceeding 0·1 mm. in depth. This dilution (1/3·5), which produced substantial swellings in infected rats, was therefore used for subsequent footpad tests, and uninfected controls were always inoculated in parallel with the infected animals.

Macroscopic and microscopic appearance of the footpad reaction

A few hours after injection a diffuse, visible and palpable swelling appeared at the site, increasing up to 24 hr. and then decreasing to a smaller 48 hr. reading. Induration was usually more marked than oedema or erythema, though these were always present and the foot was correspondingly painful. As was observed in the mouse, the swelling seldom remained apparent beyond 72 hr.

Sections of the reacting tissue taken at 48 hr (see Pl. 1) showed a diffuse, focal inflammatory reaction, mainly perivascular in distribution and extending through the dermis into the muscular layers of the foot. There was a marked infiltration of large mononuclear cells in the oedema surrounding the capillaries of the dermis and the arterioles of the muscular layer. This strong focal accumulation of mononuclear leucocytes, which was completely lacking in comparable sections from healthy rats inoculated with tuberculin, removes any doubt regarding the specificity of the observed reaction and it would appear that the hypersensitive response of the tuberculous rat differs from that observed in other animals only in the amount of tuberculin needed to invoke it in a species with a low hypersensitive threshold.

Conversion rates

The results listed in Tables 1 and 2 under the heading of 'footpad reaction' are based upon any swelling of not less than 0·1 mm. This means an increase of that order in the antero–posterior thickness of the inoculated foot, compared with the opposite, uninoculated foot. It will be noted that first reactions were obtained between the 2nd and 3rd weeks but that some rats did not become reactors until the 5th week of the disease.

However, as these were both selected groups likely to give an atypical picture, in that non-reacting animals were being sacrificed twice a week, another group of forty rats was infected intranasally with approximately 7000 viable units. The rat numbers during this experiment were influenced only by the occurrence of five pre-allergic and three terminal anergic deaths.

As seen in Table 3, conversion began 2 weeks after infection and the average foot swelling week by week is recorded, a figure obtained by averaging the reaction

of all mice showing 0·1 mm. or more of swelling. The control animals, six in number, seldom showed more than one or two transient 24 hr. reactions, no individual exceeding 0·1 mm. at any time during the tests.

Table 3. *Incidence and amplitude of footpad reaction to 1/3·5 Old Tuberculin in a group of forty tuberculous rats infected intranasally with 7000 viable units*

Weeks since infection	Percentage of surviving animals showing swelling of 0·1 mm.* or more	Deaths		Average swelling of foot* in mm.			
				Infected Rats (40)		Normal Rats (6)	
		Pre-allergic	Terminal anergic	24 hr.	48 hr.	24 hr.	48 hr.
1	0	—	—	< 0·1	0	< 0·1†	0
2	7·5	—	—	0·35	0·25	0	0
$2\frac{1}{2}$	20	—	—	0·6	0·32	< 0·1	0
3	67·5	1	—	0·83	0·46	< 0·1	0
$3\frac{1}{2}$	75	—	—	0·78	0·5	0	0
4	82·5	2	—	0·55	0·35	0	0
5	100	2	—	0·74	0·47	0	0
6	100	—	—	0·47	0·33	< 0·1	0
7	95	—	—	0·52	0·34	0	0
8	100	—	2	0·6	0·16	0	0
9	92·5	—	—	0·5	0·23	< 0·1	0
10	92·5	—	—	0·36	0·16	< 0·1	0
11	90	—	1	0·43	0·25	0	0

* Swelling of foot = difference in antero–posterior thickness between test and normal foot, as measured with Schnelltaster micrometer.

† Swelling of feet observed occasionally in controls did not exceed 0·1 mm. in any one instance at the 24 hr. reading.

Size and persistence of reaction

Two observations are worth comment. As in mice (to be published), the size of the 24 hr. rection was greater than the 48 hr. reading. When considered in relation to the control reactions this could be due, in part at least, to non-specific components of the inoculum, but the difference between the 24 and 48 hr. readings is believed not to be entirely non-specific. Secondly, with repeated testing the average size of the response decreased, suggesting that tuberculin inoculation at this concentration and frequency may induce partial desensitization. No attempt has yet been made to compare the average response after a selected interval, of two groups of rats infected together with only one group being regularly footpad tested.

Systemic response to tuberculin

The results in the present study confirm the reports of Hehre & Freund (1939) and Wessels (1941) that a fatal systemic reaction may be induced under optimal conditions in rats. Rats which had developed a strongly positive footpad reaction (1 mm. or more) could, in our experience, usually be killed by an intraperitoneal inoculation of 0·5–1·0 ml. of concentrated tuberculin. As in mice and guinea-pigs, death occurred 24 to 48 hr. later and the lung lesions were found to be surrounded by a pronounced haemorrhagic inflammatory reaction.

DISCUSSION

It has been widely accepted that the rat as a species is highly resistant to natural and experimental infection with tuberculosis and that when infected, deaths, or even progressive disease, are relatively uncommon. Moreover the rat is reputed to develop little or no tuberculin hypersensitivity during the course of its disease.

It would be surprising, however, if a disease aginst which little natural resistance appears to be exhibited by any of the commonly infected species (Ratcliffe & Palladino, 1953; Gray *et al.* 1952, 1960) should fail to run an initially similar course in all species; and also if the subsequent progress of the disease should fail to be governed by the speed of onset and efficiency of the acquired immune response and by the degree of allergic damage to which the host species' potential for hypersensitive responses subjects it.

On the basis of experiments arising from this hypothesis, the present paper reports that the rat shows a very similar reaction to the mouse when exposed to pulmonary tuberculosis. It is now evident that the rat's reputation for resistance to infection originated from experiments using unsatisfactory routes of infection and that its reputation for anergy was related to a lower threshold of general allergic responsiveness than is present in certain other animal species. Its reported low death rate is related to the speed of onset and perhaps to the efficiency and duration of the immune response. In any case, the death rate in rats is probably no lower than in 'resistant' strains of mice tested under identical conditions (Gray, 1961).

Present results report that the rat shows no detectable early resistance to infection even with small numbers of tubercle bacilli, but that as in other species, there is a dramatic change in the character of the disease with the onset of the immune phase. The rat then develops its low grade allergic response, and this occurs at about the same time as other species, i.e. 2–5 weeks after infection. This allergic response persists during the immune phase of the disease, as it does in mice and guinea pigs (Gray, 1958). The intensity of the footpad response tends to diminish with repeated testing, but it seems not unlikely that this may be referable to a partial desensitization induced by the cumulative effects of the high tuberculin concentrations required to elicit a reaction.

The systemic effects of large doses of tuberculin were observed in rats which had been allergic for 2 weeks or more. It was shown that these animals may be killed by inoculating 0·5–1 ml. of undiluted tuberculin intraperitoneally, thus confirming the reports of Hehre & Freund (1939) and Wessels (1941).

It seems reasonable in the light of present knowledge to postulate that all susceptible animal species will show some degree of allergic responsivenesss to tuberculosis if suitably tested. At one end of the scale may be placed highly responsive species such as guinea-pigs, man, pigs, monkeys, and at the other mink, ferrets, hamsters, mice and rats. Other species might well be arranged in some sort of order in between these two extremes.

SUMMARY

1. This paper confirms and extends several observations during the past 20 years that, despite many reports to the contrary, the rat is not unduly resistant to initial infection with tubercle bacilli provided they lodge in the lungs.

2. The pattern of pathogenesis in the rat is probably closest to the now classical picture in the mouse, i.e. the response of a species with a low hypersensitivity potential. The pathology of the lesions agreed closely with the descriptions of Wessels (1941) and Kumashiro (1958*b*) resembling the mouse in most respects but, unlike the mouse, including the production of giant cells.

3. When tested by footpad inoculation with 1/3·5 Old Tuberculin a positive reaction was demonstrated, commencing between 2 and 5 weeks after infection and persisting for several weeks. A fatal systemic reaction could often be induced with large doses of tuberculin given intraperitoneally.

4. In a few cases loss of allergy was shown to be associated with a terminal anergic flare of the type observed previously in mice and guinea-pigs.

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EXPLANATION OF PLATE

Sagittal section through the foot of a tuberculous rat, showing the histology of the tuberculin reaction 48 hr. after injecting 0·03 ml. of 1/3·5 O.T. (H. & E. × 300).

Note the diffuse focal inflammatory reaction with the perivascular aggregation of large mononuclear cells typical of the classical delayed response to tuberculin of the hypersensitive animal. No such aggregation occurred in healthy animals inoculated with O.T.

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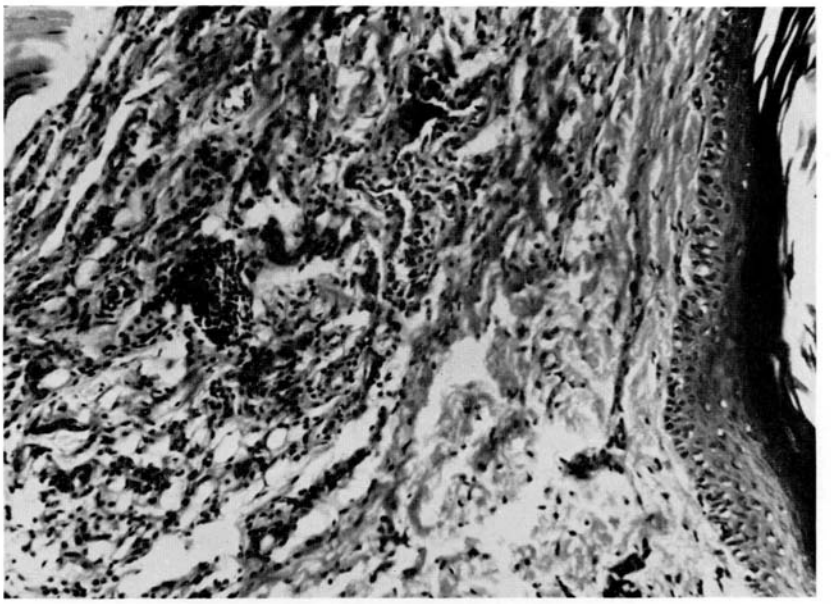

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