Infection of mice by the respiratory route with *Salmonella typhimurium*

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INTRODUCTION

There is an increasing volume of circumstantial evidence suggesting that salmonellosis may sometimes be transmitted in man by the agency of air-borne particles. Netter (1950), Varela & Olarte (1942), Varela & Ochoa (1953) and Datta & Pridie (1960) have isolated salmonellae and other faecal organisms from the nose, pharynx and sputum. This raises the question of the role of the respiratory tract as a possible portal of entry. Trillat & Kaneko (1921), succeeded in infecting mice with aerosols of *Salmonella typhimurium* (Danysz strain), and showed that the dose required to initiate infection was very much smaller by the respiratory route than by percutaneous, conjunctival and oral routes. Further Clemmer, Hickey, Bridges, Schliessmann & Shaffer (1960) have shown that chicks can be infected with salmonellae by the respiratory route; they used ten different strains, and showed that the organisms proliferate in the lungs.

Darlow & Bale (1959) showed that the flushing of a lavatory pan could produce an aerosol of particles sufficiently small to be inhaled into the pulmonary alveoli, and they suggested that some faecal organisms might infect in this way. The investigations described below were primarily undertaken as a sequel to this, and secondarily to repeat the experiments of Trillat & Kaneko using modern techniques.

MATERIALS AND METHODS

The infective agent

Salmonella typhimurium was chosen for a variety of reasons. First, it is the commonest cause of salmonellosis in man in the British Isles, and it is also pathogenic for laboratory animals. Secondly, it is commonly regarded as being transmitted by the ingestion of contaminated substances, as opposed to the inhalation of infected dusts. In addition to this, preliminary tests showed that it would withstand the traumata involved in aerosol production and subsequent sampling, and that it could be mono-dispersed; it also survived in air for long enough to ensure the inhalation of an infective dose.

The choice of strain of organism was initially determined by intraperitoneal and oral inoculation of several strains of *Salm. typhimurium* into *Porton* mice. All but one were found to be relatively avirulent for this mouse colony, even after passage. The exception was 'strain VI' (Phage-type 12) provided by our colleague Dr J. S. Paterson, who isolated it from a calf, and it was used throughout the work.

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The organism was grown on tryptic meat agar at 37° C. for 24 hr., washed off with phosphate buffer and counted by a modified Miles & Misra technique (Miles & Misra, 1938). Counts were also made in the same manner of inocula, spray suspensions and cloud samples.

Experimental animals

A number of mouse colonies was screened with a view to the choice of suitably susceptible strains; those used were: (i) the *Porton* strain; (ii) a strain known as *Strong A*. The former showed a marked resistance to infection with *Salm. typhimurium* (strain VI). In the absence of adjuvants the I.P. LD_{50} dose lay between 10^4 and 10^5 organisms and the oral LD_{50} was greater than 10^8 organisms. The *Strong A* mouse strain, on the other hand, was highly susceptible; the oral LD_{50} was about 100 organisms and the I.P. LD_{50} dose was less than 10 organisms.

Experimental technique

Three experiments were made. In the first there were two lots each of 90 Porton mice weighing 20–22 g. Each lot was divided into three groups. In group I each mouse was inoculated intra-peritoneally with 5×10^4 organisms in 0·1 ml. of phosphate buffer. Group II received the same dose in one drop of buffer placed on the tongue. Swallowing appeared to be normal, and there was no spluttering. Group III was exposed to a mono-dispersed aerosol in a modified Henderson apparatus (Henderson, 1952). The spray and sampling fluids were phosphate buffer (pH 7·6). The spray concentration was adjusted to give a lung retention dose as close as possible to the dose given by the other two routes, the calculations being based on the work of our colleague G. J. Harper (unpublished), who has shown that the number of mono-dispersed organisms (labelled with ^{32}P) retained by similar mice in 1 min. approximates to the viable cell content of 7·5 ml. of aerosol.

After inoculation each mouse was placed in a separate compartment to reduce cross-infection to a minimum. In the first lot post-mortem examination was carried out on all dead mice in the three groups, and the survivors were sacrificed after 4 weeks and similarly examined. In the second lot mice were sacrificed at intervals for the study of pathogenesis. The lungs, spleen, liver and gut were removed for histological examination, and fixed in formol saline or formol corrosive (Lendrum, 1941). In addition, heart blood and spleen were streaked on tryptic meat agar and MacConkey agar plates, and culture identification was confirmed by slide agglutination.

The second experiment was a repetition of the first, except that only one lot of 90 mice was used, and none were sacrificed until the 28th day. In the third experiment *Strong A* mice were used in place of Porton mice, and the dose was reduced to 100 organisms by each of the three exposure routes. In all other respects the technique of the first experiment was repeated.

RESULTS

The mortality rates and bacteriological findings of the three experiments are summarized in Table 1. In the third experiment seven mice that died showed rapid development of putrefaction and *Salm. typhimurium* could not be isolated from the cultures; the plates were overgrown with other organisms, notably *Proteus* spp. It is considered, however, that death was, in fact, due to *Salm. typhimurium* infection, since half (i.e. 15) of the lot sacrificed for histological purposes showed changes consistent with infection, and half of both lots remained unaffected.

Table 1. *Death-rates and bacteriological findings*

	Dose			No. of mice dying in 4 weeks		Survivors sacrificed at 4 weeks	
	I.P. (group I)	Oral (group II)	INHAL (group III)	Positive culture	Nega-tive culture	Positive culture	Nega-tive culture
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Expt. 1. (Porton mice)	5×10^4	—	—	20	—	9	1
	—	5×10^4	—	—	—	6	24
	—	—	$4 \cdot 56 \times 10^4$	17	—	12	1
Expt. 2. (Porton mice)	5×10^4	—	—	22	—	8	—
	—	5×10^4	—	—	1	9	20
	—	—	$6 \cdot 63 \times 10^4$	17	—	13	—
Expt. 3. ('Strong A' mice)	100	—	—	30	—	—	—
	—	100	—	7	7	1	15
	—	—	86	26	—	4	—

Table 2. *Distribution of deaths in time*

Expt.	Dose	Deaths occurring in 4 weeks			
		1	2	3	4
1	I.P.	4	11	4	1
	Oral	—	—	—	—
	INHAL	—	6	9	2
2	I.P.	3	15	3	1
	Oral	1	—	—	—
	INHAL	—	4	11	2
3	I.P.	11	19	—	—
	Oral	3	10	—	1
	INHAL	2	13	7	4

The results of the first and second experiments are in close agreement, and those of the third experiment follow a similar pattern, modified only by the low resistance of the *Strong A* strain. The figures in columns 5 and 7 show that the intra-peritoneal and inhalation doses were equally infective, though in the latter case the course of the disease was more prolonged, as can be seen more clearly in Table 2.

The *Porton* mice, given an inhalation dose, developed numerous well defined foci of pneumonitis within 24 hr., which enlarged centrifugally and extended along

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the adventitia of the blood vessels and bronchi, involving considerable areas of lung tissue. If the animal survived long enough, massive involvement of the lung with suppuration and mediastinitis ensued.

In the *Strong A* mice the initial foci were relatively few in number in keeping with the lower dose, and were difficult to find until after the fourth day. The foci enlarged and developed in the same way as those in the *Porton* mice, and the terminal lung lesions in those mice which survived long enough were as severe. Abscess formation was a common feature. A few of the *Strong A* mice, however, died at a rather earlier histological stage with less extensive lesions, possibly resulting from a greater susceptibility to toxin.

In the *Porton* and *Strong A* mice infected by the intra-peritoneal route no lung lesions apart from hyperaemia, patches of pleuritis and associated pleural effusion were present until the third or fourth day, when small secondary lesions began to appear beneath the patches of pleuritis. Later, minor inflammatory changes developed in the lung parenchyma adjacent to the mediastinum after the onset of mediastinitis and arteritis. All these secondary lesions were minimal and quite different from those found in the inhalation groups.

No significant pulmonary pathology was found in any of the orally inoculated mice, though in some of those that actually succumbed to infection post-mortem changes were too rapid and extensive to permit proper examination.

It would appear, therefore, that in both strains of mice inhalation produced a specific, primary, pulmonary disease quite distinct from that initiated by the other two modes of inoculation, but there was also a significant difference in the histological picture produced in the intestine. In both strains of mice intestinal lesions were absent in the inhalation groups. Intraperitoneal inoculation produced the classical picture of peritonitis, though there was a strain-wise difference in that massive thrombosis of the mesenteric vessels was a constant feature in the *Porton* mice, but was very rare in the *Strong A* mice. In the orally inoculated groups no evidence of intestinal pathology was found in the *Porton* mice, which is not surprising in view of the high resistance to infection. Lesions were scanty in the *Strong A* mice, and consisted of slight sub-mucous congestion and leucocytic infiltration of the small intestine, caecum and colon, accompanied by occasional inflammatory changes in the mesenteric lymph nodes.

The pathological changes found in other organs such as liver spleen and kidney broadly followed classical description of the disease in mice.

DISCUSSION

The results show that two strains of white mice can be infected with *Salm. typhimurium* by the inhalation of a mono-dispersed aerosol; that the disease so produced is histo-pathologically different from that resulting from intra-peritoneal and oral inoculation; and that the organism is as infective and as lethal when inhaled, as when the dose is administered by the intra-peritoneal route.

Infection by the oral route was much more difficult to establish than by the other two routes. This fact, combined with the lung changes and negative intestinal

findings in the inhalation groups, shows that swallowing of part of an inhaled dose by the mice under the selected experimental conditions played no significant part in infection.

No precautions were taken to protect the conjunctivae during exposure to the aerosol. Trillat & Kaneko (1921) concluded from their mouse experiments that the infective dose by conjunctival inoculation was 200 times greater than the inhalation dose. It seems unlikely, therefore, that the conjunctivae played any part in infection in this instance. Certainly no signs of conjunctival involvement were observed. Moore (1957), however, has reported natural and experimental infection of guinea-pigs via the conjunctivae, but the disease so produced bore no resemblance to that described above.

The pathological condition produced in the respiratory tract of mice does not appear to have been paralleled in the human, though secondary lung involvement in typhoid and para-typhoid fever is by no means rare. However, Christian (1947), describing typhoid fever, states, 'Lobar pneumonia develops rarely at onset. After an indisposition of a day or so the patient is seized with a chill, has a high fever and pain in the side with signs of consolidation and the evidences of an ordinary lobar pneumonia. The intestinal symptoms may not occur until the end of the first week or later; the pulmonary symptoms persist; crisis does not occur; the aspect of the patient changes, and by the end of the second week the picture is that of typhoid fever.... This condition depends on the early localization of the typhoid bacillus in the lung.' This syndrome is in sharp contrast to the more usual picture of pulmonary complications, and may well represent a true primary inhalation pneumonia.

The majority of organisms classified as *Salmonella* are pathogenic for man, and the number of strains hitherto considered specific for other animals is rapidly becoming smaller (Dubos, 1958). The commonest clinical manifestation in man is enteritis, though many other organs can be involved as a result of systemic or lymphatic spread, and some strains habitually produce the syndrome of enteric fever. The gut, however, appears to be the principal focus of infection, and as ingestion of contaminated materials can often be shown to have occurred, it is reasonable to regard the intestinal mucosa in man as the most susceptible tissue. This does not exclude the respiratory tract as an effective portal of entry to the host. Here multiplication may occur without undue constitutional disturbance. If the respiratory tract can become so infected, then one method of extension to the gut could occur simply by the agency of swallowing sputum. There is no convincing reason to dismiss this possibility solely on the ground that gut lesions in mice did not appear. The disease in this species clearly takes a more fulminating course than salmonellosis in man, and tissue susceptibility in the two species may vary widely.

SUMMARY

Experiments have been described in which mice were infected by the inhalation of a mono-dispersed aerosol of *Salm. typhimurium*. The disease so produced was characterized by a specific primary pneumonia. The lethal dose was very much

smaller than that required by ingestion, and was approximately equal to the intra-peritoneal lethal dose. Possible implications have been discussed.

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