THE BEHAVIOUR OF A MUTANT STRAIN OF *SALMONELLA TYPHIMURIUM* IN EXPERIMENTAL MOUSE TYPHOID

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(With 3 Figures in the Text)

Laboratory investigation into the pathogenesis and control of typhoid fever is hampered by the fact that *Salmonella typhi*, and the paratyphoid bacilli are not natural patogens for any of the common laboratory animals, and experimental infections with these organisms bear little relation to the natural disease in man. The problems that arise in the control of typhoid fever are concerned with a particular host-parasite relationship, and the aim of laboratory methods should be to simulate this relationship as closely as possible in small animals. The pioneer work of Ørskov and his colleagues (Ørskov, Jensen & Kobayashi, 1928) has shown that mouse typhoid caused by *Salm. typhimurium* runs a very similar course to typhoid fever. *Salm. typhimurium* is a natural pathogen of the mouse, and has a somatic antigenic constitution identical with that of *Salm. paratyphi* B (Felix, 1952). Thus, experimental mouse typhoid may be accepted as a satisfactory model of a natural systemic Salmonella disease.

Previous investigations on the course of experimental mouse typhoid have shown that bacterial multiplication occurs in the liver and spleen, and that infection is widely disseminated at the time of death (Ørskov, Jensen & Kobayashi, 1928). Quantitative studies with one virulent strain (Hobson, 1956, 1957*a*) showed that the intraperitoneal injection of 5000 organisms, which was fatal to 96% of mice, resulted in progressive bacterial proliferation in the first few days. The peak of mortality was at the seventh day of infection, and bacterial populations of the order of ten million organisms were found in most mice killed at this time.

Reduction of the infective dose to 10 organisms appeared to affect the speed and probability of attaining a critical final bacterial population. Only 50% of mice died, but many of the survivors continued to carry potentially virulent bacteria. As with the larger dose, moribund mice always contained 10^8–10^9 viable organisms. Quantitative experiments showed that progressive bacterial multiplication occurred in all mice in the first 7–14 days after infection with 10 organisms. Thereafter, in mice which were destined to survive, overt bacterial multiplication gradually ceased, and bacterial counts of the viscera fell slowly to the twenty-eighth day.

The course of events in mice infected with less virulent strains of *Salm. typhimurium* has received less attention. The failure to kill mice could theoretically be explained in several different ways. Either the avirulent strain fails to establish

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infection except in those mice which subsequently die, or infection may be established but not prove lethal. A non-lethal result of infection may be because a given population of avirulent organisms is less harmful than equivalent numbers of virulent bacteria, or because the avirulent strain is incapable of attaining the critical final bacterial population characteristic of mice dying of infection with a virulent strain.

The present experiments were undertaken to investigate these aspects, using as a strain of *Salm. typhimurium* of reduced virulence a streptomycin-resistant mutant derived by a single step from a virulent strain S 2/446 previously studied.

MATERIALS AND METHODS

A pure strain of white mice was used, at weights of 22–25 g. All mice were segregated into individual glass jars immediately after inoculation, to obviate cross-infection. The mice were shown to be free from natural salmonellosis throughout the course of the experiments, and were maintained on a salmonella-free pellet diet.

All bacterial inoculations were of logarithmic phase organisms derived after one subculture from freeze-dried ampoules. The method of subculture and standardization of bacterial suspensions for intraperitoneal injections has been previously described (Hobson, 1956).

The course of infection was followed by killing mice chosen at random at various intervals after inoculation. Quantitative bacterial counts were made on the blood and on each organ by a standard procedure (Hobson, 1956). All mice which died spontaneously were examined post-mortem, and the identity of the strain of *Salm. typhimurium* recovered was confirmed by inoculation on to nutrient agar plates containing streptomycin (200 μg/ml.) or as described in the text. The day to death of each mouse was recorded.

EXPERIMENTAL RESULTS

The derivation of the bacterial strain

In four experiments, approximately 10^{11} organisms of the virulent strain of *Salm. typhimurium* S 2/446, derived after one subculture from freeze-dried ampoules, were planted on to nutrient agar plates containing 5000 μg/ml. of streptomycin. After overnight incubation there were five to thirty colonies on each plate; thirty-two colonies were picked off on to Dorset-egg slopes. In preliminary animal tests, 500 bacteria of each of the thirty-two strains were injected intraperitoneally into groups of ten mice. All were significantly less virulent than the parent strain S 2/446. From one of these single-step mutant strains designated S 2/R, a large stock of freeze-dried ampoules was prepared. This strain has been employed throughout the present experiments.

The behaviour of Salm. typhimurium S 2/R in vitro

On nutrient agar plates incubated overnight, the colonies of the strain S 2/R were smaller (1–1·5 mm. diameter) than those of the parent strains S 2/446 (2–3 mm.). Cultures of S 2/R organisms were examined by the methods of Wilson

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(1930); the colony-structure appeared smooth by direct or transmitted light, and there was no spontaneous agglutination of bacterial suspensions in saline. The growth rate of S 2/R was compared with that of S 2/446, using initial inocula of 300–400 organisms per ml. in continuously agitated tubes of PLYP broth (Challice & Gorrill, 1954). The mean generation time calculated by the method of Wilson & Miles (1955) was 22·3 min. for the parent strain S 2/446, and 27·1 min. for the mutant S 2/R; six of the other streptomycin-resistant strains were similarly tested, and were found to have mean generation times 3–7 min. longer than that of the parent strain S 2/446. Despite this difference in growth rate, there was no evidence that the mutant S 2/R had acquired any new metabolic requirements, nor was it streptomycin-dependent. From washed bacterial suspensions of the strains S 2/446 and S 2/R saline dilutions containing 10^7–10^2 organisms per ml. were prepared and inoculated on to nutrient agar, and on to plates of a defined minimal medium (Davis & Mingioli, 1950). There were no significant differences between the two strains on either medium, other than the previously observed difference in colony size.

It was shown by Smith, Oginsky & Umbreit (1949) that there was a marked difference in final growth between streptomycin-resistant mutants of *B. coli* and the sensitive parent strains. This difference was tenfold greater in aerated than in stationary broth culture, and was associated with a failure of the mutants to complete the oxidation of oxalo-acetate and pyruvate. When *Salm. typhimurium* S 2/446 was compared similarly with S 2/R the difference in final growth was slight, and was not accentuated by aeration of the cultures.

There was no detectable difference in the behaviour of the two strains S 2/446 and S 2/R in agglutination tests (method of Felix & Bensted, 1954) with standard Salmonella antisera, or with sera from mice recovering from infection with either strain. In cross-absorption experiments of the type described by Felix & Pitt (1951), live or killed suspensions of each strain were equally capable of removing O agglutinins from standard sera or from the pooled serum of mice vaccinated with either strain, or surviving actual infection.

The possibility that the relative avirulence of S 2/R might be due to an increased sensitivity to normal bactericidal mechanisms was considered. From logarithmic phase cultures of S 24/46 and S 2/R saline dilutions containing 10,000 organisms per 0·1 ml. were prepared; 0·2 ml. volumes were added to 1·8 ml. volumes of normal mouse serum, and the mixture was incubated at 37° with constant rotation. At intervals, 0·1 ml. aliquots were removed and plated out for subsequent bacterial counts. The behaviour of the two strains was similar; there was no significant fall in the bacterial count, and after a period of bacteriostasis varying from ½–1 hr., the counts began to rise, attaining levels 300–400 % greater than the original count by the fourth hour. It was suggested by Schütze, Gorer & Findlayson (1936) that the failure to demonstrate bactericidal activity in normal mouse serum may be due to its lack of haemolytic complement, and they devised a test in which *Salm. typhimurium* was incubated in a mixture of mouse serum and fresh guinea-pig serum. The same technique employed with the strains S 2/446 and S 2/R failed to demonstrate any differential enhancement of bactericidal

activity, though in each case the period of apparent bacteriostasis was increased to 1½–2 hr.

The susceptibility of each strain to phagocytosis was determined by inoculating samples of normal whole mouse blood containing 10 μ/ml. heparin with organisms from logarithmic-phase cultures; the final concentrations of bacteria were varied from 10^6 to 10^3 per ml. The mixtures were rotated constantly at 37° and samples were taken at intervals; blood smears were made and suitable dilutions of each sample were plated out for bacterial counts. Phagocytosis occurred equally rapidly with both strains, but there was little evidence of intracellular digestion in either case, and after 2 hr. many polymorphs were packed with bacteria, and were beginning to disintegrate. Bacterial counts remained relatively constant over the first ½–1 hr., and then progressive multiplication occurred. The behaviour of the two strains did not differ significantly.

The common origin of the virulent streptomycin-sensitive strain and the less virulent streptomycin-resistant strain was confirmed by demonstrating that the strain S 2/R yielded the same three types of temperate bacteriophage as the virulent S 2/446, and that both strains were equally readily induced to undergo mass lysis by ultra-violet irradiation (Hobson, 1957*a*).

The behaviour of Salm. typhimurium *S 2/R* in vivo

The comparative virulence of the parent and mutant strains. Groups of twenty mice each were inoculated intraperitoneally with 10^7, 10^5, 5×10^3 or 10 organisms of the strain S 2/R. Similar groups of mice were inoculated with the same doses of S 2/446. All mice were placed in separate cages immediately after inoculation. Mice which survived for 28 days after infection were killed and examined bacteriologically. The results (Table 1) showed that an inoculum of 10^7 bacteria of either strain caused a rapid and uniformly fatal disease. With decreasing dosage, however, the total mortality fell more abruptly in the S 2/R-infected groups than in mice inoculated with the strain S 2/446, and the mean time to death was increased. Despite the reduced lethality of the strain S 2/R even small inocula could cause persistent infection.

Table 1. *The mortality following varying infective doses of* Salm. typhimurium *S 2/446 and S 2/R injected intraperitoneally*

	S 2/446 dose					S 2/R dose				
	10^7	10^5	5×10^3	10^3	10	10^7	10^5	5×10^3	10^3	10
Deaths in 28 days (out of 20)	20	20	20	18	11	19	13	9	4	0
No. of survivors with persistent infection	—	—	—	2/2	8/9	1/1	7/7	11/11	15/16	16/20
Mean time to death (days)	2·0	5·2	7·3	8·5	13·6	3·2	10·0	17·1	12·2	—

To obtain a more accurate picture of the pattern of mortality and of the incidence of bacterial carriage, the result of infection with 5000 or 1000 organisms of the strain S 2/R was examined in a series of similar experiments.

A total of eighty mice were inoculated intraperitoneally with 5000 organisms; twenty-nine mice (36 %) died within 28 days of infection. The deaths began on the seventh day, and continued sporadically throughout the succeeding 21 days. The mean time to death was 16·1 days after infection; forty-eight (96 %) of the fifty-one survivors showed persistent infection of the liver and spleen, although all appeared healthy when killed.

After infection with 1000 organisms only twenty-seven (13·5 %) of 205 mice died. Most of the survivors killed on the twenty-eighth day of the disease appeared healthy, but 166 (93 %) of the 183 animals were carriers of the streptomycin-resistant *Salm. typhimurium*. The mean time to death, 11·2 days, was shorter than with the larger dose; 78 % of the deaths occurred in the first 14 days, and only 3·5 % of the mice alive at this time died in the next 2 weeks. Thus, although infection was established in almost the entire mouse population the probability of the infection attaining a lethal level was significantly reduced in mice which had survived the first 14 days of the disease (χ^2, with Yates' correction = 6·3. $n = 1$. $P < 0{\cdot}01$).

The chronic carrier state

In the survivors of the foregoing experiments, the distribution and numbers of bacteria were similar to those previously recorded (Hobson, 1956, 1957*a*) in survivors of infection with *Salm. typhimurium* S 2/446. Blood cultures were consistently negative, and only 5 % of mice yielded positive lung cultures. Liver and spleen were both infected in most animals, and bacterial counts ranged from 1000 to less than 30 organisms per organ. The greatest fluctuations occurred in the kidneys; positive cultures were obtained from eighty mice, but in most of these cases less than thirty bacteria per organ were present. In a few cases, however, there were obvious renal abscesses, and in four mice, all apparently healthy when sacrificed, one kidney was converted into a pyonephrosis. From the kidneys with visible lesions bacterial counts ranging from 10^5 to 10^8 organisms were obtained. It is interesting that liver and spleen bacterial counts were no higher in these animals than in survivors with normal kidneys. This may be analogous to the type of renal localization noted with other Gram-negative bacteria (Gorrill, 1952).

The duration of carriage of *Salm. typhimurium* S 2/R was shown by keeping twenty mice under observation in single cages for 56 days after inoculation with 1000 organisms. Four mice died in the first month, and none in the second; thirteen (81 %) of the survivors killed on the fifty-sixth day were still infected. The bacterial counts in liver and spleen were similar to those at 28 days.

The course of infection with Salm. typhimurium *S 2/R*

Fifty mice were injected intraperitoneally with 1000 organisms; two mice were killed and examined each day except Sunday until the tenth day after infection. Twenty mice of the same age and weight were similarly inoculated with 1000 organisms of the virulent strain S 2/446. In the latter group of mice bacterial counts rose rapidly and progressively, and eight animals died spontaneously before the tenth day. The sequence of events in the spleens is shown in Fig. 1. In the S 2/R-

infected mice, the distribution and total number of bacteria 24 hr. after infection were similar to those in mice infected with the virulent strain S 2/446. The bacterial counts increased less rapidly than in the S 2/446-infected animals during the next 3 days of the infection, and thereafter, at a time when bacterial counts of S 2/446 organisms were rising rapidly, there was little further rise in the population of S 2/R organisms.

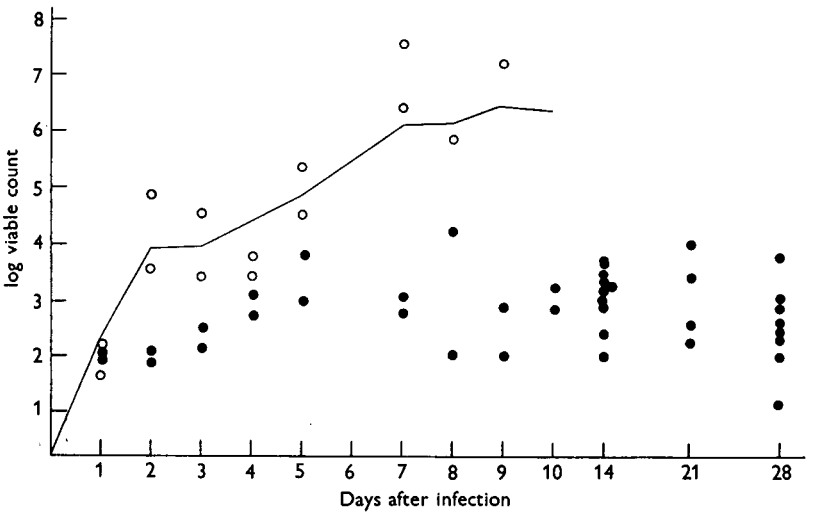

Fig. 1. The distribution of spleen bacterial counts after infection with *Salm. typhimurium* S 2/R (1000 bact. i.p.). ●, infection with S 2/R; ○, infection with virulent strain S 2/446; —, average trend of bacterial counts in S 2/446 infection *c.* 5000 bact.

Eight mice died spontaneously between the sixth and twentieth day of the disease. Ten mice were killed on the fourteenth day of the infection, four on the twenty-first day and eight on the twenty-eighth day. All these mice were still infected; the spleen bacterial counts are included in Fig. 1. The liver yielded positive cultures in all cases, but cultures of blood and lungs were all sterile. Kidney bacterial counts fell gradually from a mean count of 500 organisms at 14 days to less than 30 organisms at 28 days.

The bacterial population of moribund mice

After inoculation with 10^3 or 10^7 organisms of the strain S 2/R eleven mice which were seen to be moribund and unlikely to survive for a further 12 hr. were killed, and counted as deaths for that day. From the sum of the bacterial counts of blood, liver, spleen and kidneys an estimate was made of the total bacterial population. The distribution and numbers of bacteria (Table 2) were not significantly different from those found in mice dying after infection with the virulent strain S 2/446 (Hobson, 1956*b*).

The nature of the bacteria recovered from mice post-mortem

Since most mice infected with 1000 S 2/R organisms showed no great increase in bacterial counts, the small number of deaths might represent a selective growth in

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those mice of back-mutant organisms of greater virulence than the average members of the strain S 2/R.

Table 2. *The distribution of bacteria in moribund mice infected intraperitoneally with* Salm. typhimurium *S 2/R*

Infecting dose	Blood (bact./ml.)	Spleen (bact./organ)	Liver	Kidney	Total bacterial count
10^2	$1{\cdot}84 \times 10^4$	$4{\cdot}1 \times 10^6$	$3{\cdot}98 \times 10^9$	$3{\cdot}28 \times 10^7$	$4{\cdot}02 \times 10^9$
	$1{\cdot}0 \times 10^5$	$9{\cdot}0 \times 10^7$	$1{\cdot}2 \times 10^8$	$1{\cdot}0 \times 10^8$	$3{\cdot}1 \times 10^8$
	$6{\cdot}7 \times 10^6$	$2{\cdot}4 \times 10^8$	$7{\cdot}6 \times 10^8$	$3{\cdot}78 \times 10^7$	$1{\cdot}03 \times 10^9$
	$6{\cdot}0 \times 10^5$	$4{\cdot}7 \times 10^8$	$6{\cdot}0 \times 10^8$	$4{\cdot}4 \times 10^7$	$1{\cdot}11 \times 10^9$
	$1{\cdot}13 \times 10^7$	$2{\cdot}6 \times 10^8$	$3{\cdot}0 \times 10^8$	$2{\cdot}9 \times 10^7$	$6{\cdot}0 \times 10^8$
	$2{\cdot}0 \times 10^5$	$1{\cdot}6 \times 10^8$	$2{\cdot}6 \times 10^8$	$1{\cdot}56 \times 10^8$	$5{\cdot}76 \times 10^8$
	$1{\cdot}1 \times 10^5$	$1{\cdot}9 \times 10^9$	$1{\cdot}24 \times 10^8$	$4{\cdot}38 \times 10^8$	$3{\cdot}54 \times 10^9$
	$5{\cdot}0 \times 10^2$	$2{\cdot}5 \times 10^7$	$2{\cdot}8 \times 10^7$	$3{\cdot}0 \times 10^6$	$6{\cdot}0 \times 10^7$
10^7	$4{\cdot}4 \times 10^5$	$2{\cdot}0 \times 10^9$	$1{\cdot}1 \times 10^8$	$1{\cdot}3 \times 10^9$	$3{\cdot}4 \times 10^9$
	$1{\cdot}4 \times 10^6$	$4{\cdot}9 \times 10^7$	$9{\cdot}9 \times 10^7$	$1{\cdot}4 \times 10^8$	$2{\cdot}88 \times 10^9$
	$3{\cdot}8 \times 10^5$	$3{\cdot}8 \times 10^7$	$1{\cdot}4 \times 10^8$	$1{\cdot}36 \times 10^8$	$3{\cdot}14 \times 10^8$

The blood and spleen homogenates of mice dying early or late in the course of S 2/R infection, and spleen homogenates from chronic carriers were plated in duplicate on nutrient agar and on to agar containing 200 μg/ml. streptomycin. There was no evidence of any reversion to streptomycin sensitivity. The growth rate of a strain isolated from a mouse dying on the sixth day of the disease was compared with that of the laboratory stock culture of *Salm. typhimurium* S 2/R. There was no evidence that a faster-growing variant organism had been selected *in vivo*.

The same mouse-passaged strain was reinjected into twenty normal mice. The culture used was the primary growth obtained from the homogenate of spleen; an estimated inoculum of 5000 organisms was injected intraperitoneally. Twenty similar mice were injected with the same dose of the laboratory strain S 2/R; 28 days later there were eight and seven deaths, respectively, in the two groups. There was no significant difference in the extent of bacterial carriage in the survivors.

A comparison of the toxicity of killed suspensions of Salm. typhimurium *S 2/446 and mutant S 2/R*

Nutrient agar slopes inoculated with the strain S 2/R or the virulent strain S 2/446 were incubated for 18 hr., and the growth was washed off in normal saline. The bacterial suspensions were adjusted turbidimetrically to an estimated concentration of 10×10^{10} organisms per ml.

The bacterial suspensions were killed by heating in a water-bath at 56° for 1 hr.; sterility was confirmed by incubating 1 ml. aliquots for 4 days in meat broth. Groups of mice were injected intraperitoneally with 0·5 ml. of the neat suspensions, or with 0·5 ml. of a 1 in 10, 1 in 100 or 1 in 1000 dilution in saline. Ten mice were used for each dilution of each strain. There was no significant difference in the

mortality caused by the two strains. The LD_{50} calculated by the method of Reed & Muench (1938) was equivalent to $9{\cdot}1 \times 10^9$ organisms for the parent strain S2/446 and $8{\cdot}5 \times 10^9$ for the strain S2/R.

The fate of Salm. typhimurium *S2/446 and S2/R after intravenous injection*

To determine whether the two bacterial strains differed in their initial resistance to blood-clearance mechanisms after their first introduction into the body, the following experiments were performed. For the purposes of comparison with subsequent groups the animals were selected at 20 g. and kept for 28 days before injection.

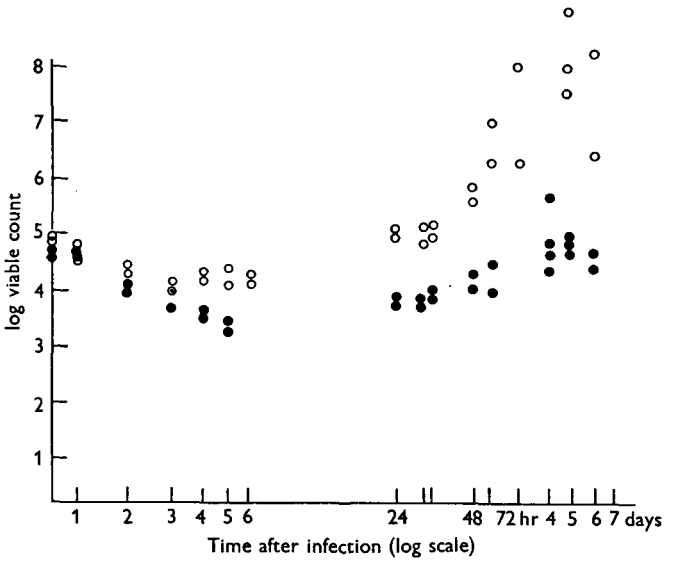

Fig. 2. The trend of bacterial multiplication *in vivo* after intravenous injection of 2×10^5 *Salm. typhimurium*. Total bacterial population: ○, strain S2/446; ●, strain S2/R.

In each experiment thirty-two mice were inoculated intravenously with 0·2 ml. of a saline suspension containing 2×10^5 organisms derived from cultures of *Salm. typhimurium* S2/446 or S2/R in the logarithmic phase of growth. Mice were sacrificed at intervals after infection and bacterial counts made on the blood and each organ. The first two mice (0 hr.) were killed immediately after completion of the intravenous injection.

The 'total' bacterial population at each interval was estimated as the sum of the counts of liver, spleen, kidney and blood; at each interval the lungs contained less than 100 organisms. These total counts are shown in Fig. 2, and blood-clearance data are shown separately in Fig. 3.

The rate of blood-clearance was similar for both bacterial strains over the first 5 hr. after infection. The removal of S2/R organisms from the circulation continued, and blood cultures were sterile in all but one of the mice examined 24 hr. or more after infection; after the fifth hour there was no further reduction in the number of circulating S2/446 organisms, and blood bacterial counts began to rise after 24 hr.

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During the first 5 hr. of the infection the total bacterial population fell progressively in both groups of mice, and there was little difference in the rate of bacterial destruction. Between 5 and 24 hr. after infection, bacterial counts increased in both groups of mice, but to a greater extent in S 2/446-infected animals; by 24 hr. the numbers of S 2/446 organisms were ten times greater than the numbers of S 2/R. Between 24 hr. and 5 days after infection, there was rapid bacterial multiplication in the S 2/446-infected animals to levels 160 times greater than the original inoculum; in the same period the population of S 2/R organisms increased only 18-fold.

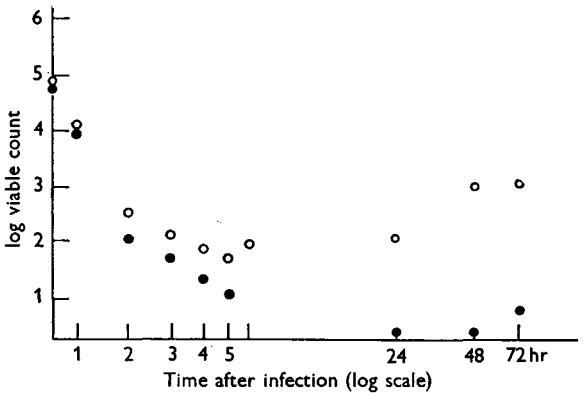

Fig. 3. The rate of blood clearance of *Salm. typhimurium* after intravenous injection of 2×10^5 bact. ○, strain S 2/446; ●, strain S 2/R. Each point in the first 5 hr. is the mean of two mice. Each point thereafter is the mean count of all mice killed during that day (see Fig. 2).

DISCUSSION

A mutant of a virulent strain of *Salm. typhimurium* has been shown to differ from the parent strain in its behaviour *in vivo*, but the difference was only marked when the bacterial inoculum which initiated infection was small.

There was no apparent difference in antigenic constitution of the two strains. The possibility of small quantitative differences, or of the possession of minor antigens, will require further investigation. However, killed suspensions were as toxic as those of the virulent strain S 2/446, and experiments to be reported later (Hobson, 1957*b*) show that infection with the strain S 2/R induces marked resistence to subsequent infection with the virulent strain. Boivin (1939) showed that chemical fractionation of virulent and avirulent strains of *Salm. typhimurium* yielded the same amount of polysaccharide of equal toxity and immunizing potency. Mackenzie, Pike & Swinney (1940) failed to find any differences in antigenic structure of virulent and avirulent strains.

The demonstration of equal degrees of toxicity in organisms of different virulence could indicate that infections with *Salm. typhimurium* are fatal for some reason other than release of endotoxin. This is a possible explanation for the finding of Hill, Hatswell & Topley (1940) that mice selectively bred for resistance to endotoxin retained full susceptibility to infection with live organisms. However, the present experiments suggested that, whatever the mode of action, the two

bacterial strains caused death by the same mechanism. When the two strains were injected in an inoculum of 10^7 organisms there was an equally rapid high mortality in both cases, although the original inoculum was not of toxic size. The final bacterial population of moribund mice appeared to be relatively constant whatever the size of the original inoculum, or whichever strain had been injected. It is interesting to note that the final bacterial population of moribund mice was less than the LD_{50} of killed bacteria.

A reduction in the inoculum of virulent organisms, or reduction of the virulence of the inoculated strain, appeared to have comparatively little effect on the frequency of established infections, but did affect the speed and the probability of the bacterial population attaining a critical final population and causing death of the animal.

The difference in virulence of the two strains was not associated with any difference in sensitivity to normal bactericidal mechanisms *in vitro*, nor was the rate of primary blood-clearance *in vivo* significantly greater for the mutant than for the virulent parent strain. Similarly, Wright (1927) in studies of experimental pneumococcal infection of rabbits, found it impossible to predict the future behaviour of an infection from the events during the first 5 hr., even with bacterial strains of wide differences in virulence.

The important difference between the two strains of *Salm. typhimurium* was their fate in the tissues after clearance from the blood-stream. During the first few hours after infection, bacteria of both strains appeared to die off at approximately the same rate. Thereafter, bacterial multiplication occurred in both cases, beginning earlier with the virulent strain, but at 24 hr. after infection there was only a tenfold difference between the two populations. From that time, the difference in *in vivo* growth rate of the two strains increased progressively. The majority of S2/R-infected animals yielded relatively constant low bacterial counts throughout the rest of the period of observation, while S2/446 organisms multiplied rapidly.

Since the two bacterial strains were of similar antigenic constitution, it is a reasonable assumption that the injection of 1000 organisms of either strain should cause the same initial stimulus to the mobilization of host immunity, and that the initial response to this stimulus should be manifest after the same lag period, and be of the same degree in each case. The difference in outcome of infections with the two strains could thus be attributed to differences in their behaviour during the period before the first immune-reactions develop. In the case of S2/446-infected animals, the total bacterial population increased rapidly, and may be assumed to have exceeded a critical level capable of control by the developing host defences. In S2/R-infected animals, bacterial multiplication was slow, and the total population at the time when host defences were mobilized was presumably small enough to be controlled and gradually suppressed.

The outcome of mouse typhoid infection could thus be considered to depend on an equilibrium between the rate of bacterial growth and the rate of development of host resistance. The balance could be tipped in favour of the organism by increasing the size of the inoculum, so that a large bacterial population was achieved

early before host defence mechanisms could be mobilized. Previous experiments (Hobson, 1956) have shown that host survival from S2/446 infection could be increased significantly by maintaining bacteriostasis with furazolidone throughout the first few days of infection; although all animals remained carriers at the end of treatment few relapsed, and the subsequent course was closely similar to that seen in the present experiments with the strain S2/R.

The reason for the failure of *Salm. typhimurium* S2/R to grow rapidly *in vivo* has not been determined, but does not appear to depend on added nutritional requirements, as was the case with avirulent mutants of *Salm. typhimurium* described by Bacon, Burrows & Yates (1950). The slow growth of the mutant S2/R was equally manifest *in vitro*.

The ability of the mutant strain to grow to final populations of 10^8–10^9 organisms in some mice, and to cause a certain number of deaths was probably an indication of differing susceptibility in different members of the mouse population, since no evidence of reverse mutation could be discovered in organisms recovered from these animals.

SUMMARY

A streptomycin-resistant mutant of a virulent strain of *Salm. typhimurium* was less virulent to mice than the parent strain. The difference in virulence was only observed when mice were infected with small numbers of organisms. Although the mutant strain caused fewer deaths than the parent strain it remained capable of establishing persistent infection in the majority of animals.

The significant difference in the behaviour of the two strains *in vivo* was that after clearance from the blood multiplication in the tissues began more rapidly with the virulent strain and was progressive, whereas multiplication of the mutant was delayed, was less in degree and was apparently suppressed within a few days of infection.

The mutant strain was apparently identical antigenically with the parent strain, and was equally toxic. The mice dying of infection with this strain did so when the bacterial population had reached a level similar to that in the terminal stage of infection with the virulent strain.

The essential difference between the two strains was the speed and probability of attaining a critical final population. The mutant strain of *Salm. typhimurium*, both *in vitro* and *in vivo*, had a slower growth rate than the parent strain. A possible hypothesis to explain the importance of this finding in relation to the outcome of infection has been discussed.

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