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Comparison of the virulence for mice of *Salmonella typhimurium* given by the intraperitoneal and subcutaneous routes

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SUMMARY

Salmonella typhimurium strain 1566 administered to mice by the intraperitoneal route was considerably more lethal than the same dose administered subcutaneously. This could be measured in terms of mortality, infectivity and mean survival time of those mice that died.

INTRODUCTION

It is the general belief that localization of virulent bacteria in the tissues is beneficial to the host, whereas active dissemination and multiplication of pathogenic organisms within the body can be fatal. However, when certain pathogenic organisms are rapidly disseminated in the tissues of mice and guinea-pigs – for example, after an intravenous (IV) challenge – the mortality rate is considerably lower than when the challenge is administered by the intraperitoneal (IP) or the subcutaneous (SC) routes (Sobernheim & Murata, 1924; Lange & Gutdeutsch, 1928; Ørskov, 1940; Dutton, 1955). In the case of the organism *Salmonella typhimurium*, Dutton (1955) reported that the lethality of the organism for mice when challenged by the IV route was less than when the animals were challenged by the IP or SC routes. He found that although the mortality rates were the same after IP or SC challenge, the mean survival time of those mice that died after an IP challenge was longer than that for the group challenged by the subcutaneous route. This suggests that *S. typhimurium* given subcutaneously is more virulent for mice than when the same challenge dose is given intraperitoneally.

During the course of a study on *S. typhimurium* strain 1566 infection in mice, the observations of Dutton were not confirmed. The results are accordingly presented for consideration.

MATERIALS AND METHODS

Animals

Male Swiss white mice each weighing 19–22 g. were used.

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Challenge organism

S. typhimurium strain 1566 grown overnight in nutrient broth was washed and resuspended in sterile 0·1 M phosphate buffer at pH 8, and a dose of 20 organisms was injected into each mouse in a volume of 0·1 ml. The actual dose administered was verified by a surface viable count (Miles & Misra, 1938).

Two groups of 20 mice each were challenged by the IP route and the SC route respectively. In a third group of 20 mice the challenge inoculum was divided and one half was given IP and the other half SC to each mouse. The mice were observed daily for 28 days; deaths were recorded and the spleen and liver of each dead mouse was cultured to establish that the animal had died with an *S. typhimurium* infection. All survivors were killed on the 28th day after challenge and their spleens and livers were cultured to test for the presence of *S. typhimurium*.

Statistical analysis

The observed mortality in each group of mice was analysed by the chi-square test of probability using a formula that makes allowance for small numbers. The mean times to death of those mice that died in each group were analysed by the Students *t* test. When the values for *P* were 0·05 or less the results were considered to be statistically significant.

RESULTS

The detailed results of this experiment are shown in Table 1. With an IP challenge of 20 *S. typhimurium* the mortality was 65% and the mean time to death of those mice that died was 14 days. When the same dose was given SC the mortality was 15% and the mean survival time of those mice that died was 22 days. The difference between the results for the IP and SC experiments were statistically significant. Mice that received half the challenge dose by the IP route and the other half by the SC route showed a significantly reduced mortality of 25% when compared with the mortality of the group challenged intraperitoneally. The mean time to death was prolonged to 20 days but this result was not statistically significant. The IP challenge was more lethal than SC challenge, and when the challenge dose was divided equally and administered by both routes the animals showed a mortality rate and mean survival time that was intermediate between the corresponding values observed after IP and SC challenge. The infectivity rates also reflected this trend in that SC challenge resulted in a lower infectivity rate (70%) than the IP challenge (95%), while those mice receiving the challenge dose by both routes showed an infectivity rate (80%) that was intermediate between the other two. The reductions in infectivity were not statistically significant.

DISCUSSION

Earlier work has shown that some organisms, including *Salmonella typhimurium*, are more lethal for mice when administered by the subcutaneous route than by the intraperitoneal route (Dutton, 1955). However, the results reported above

Table 1. *Groups of* 20 *mice were challenged by different routes with* 20 Salmonella typhimurium *strain* 1566

(The experiment was terminated on the 28th day after challenge.)

Route of challenge	No. of deaths	Mortality (%)	Mean time to death (days)	No. of survivors infected	Infectivity (%)
Intraperitoneal	13/20	65	14	6/7	95
Intraperitoneal and subcutaneous	5/20	25	20	11/15	80
Subcutaneous	3/20	15	22	11/17	70

indicate quite clearly that an IP challenge with 20 *S. typhimurium* organisms of strain 1566 is more lethal for mice than a SC challenge with the same dose. It is interesting that when half the challenge inoculum was administered IP and the other half SC to the same mice, the virulence as measured in terms of mortality, infectivity and mean survival time of those mice that died was intermediate between the corresponding values for IP challenge on the one hand and SC challenge on the other.

It appears that rapid clearance and dissemination of a virulent dose of *S. typhimurium* from the intraperitoneal space augments the lethality for the mouse when compared with the outcome of a subcutaneous challenge with its slow rate of clearance. It is possible that the slower rate of release from a subcutaneous site permits the defence mechanism of the animal to deal effectively with the pathogen before the disease reaches an acute stage. Another possible factor is that the antigen is processed in different ways, depending on whether the challenge is administered by the SC or IP routes. In other words, after phagocytosis of challenge organisms injected subcutaneously, antigenic material released may be so modified as to be more efficient in the rapid stimulation of host defence mechanisms.

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