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Evidence for a two-stage model of microbial infection

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Any model for microbial infection must account for the heterogeneity in response that invariably characterizes infections of all kinds. Some hosts die, and at different times, while others survive: and at every stage of infection the number of viable organisms differs from individual to individual, even under experimental conditions where dosage and factors like the age and weight of the hosts are kept constant. This heterogeneity can be considered as arising in two ways: either it could be fully established almost immediately after inoculation (e.g. if the inoculated organisms were randomly distributed amongst susceptible and resistant sites in the host: Meynell & Stocker, 1957; Meynell, 1957), or it could increase progressively as time passed (e.g. if infection followed a birth–death process: Williams, 1965 *a*,*b*; Armitage, Meynell & Williams, 1965; Williams & Meynell, 1967; Shortley & Wilkins, 1965). The first possibility was suggested by viable counts on mice infected with *Salmonella typhimurium* which showed that the counts made only 1·5 hr. after inoculation already differed considerably and that the degree of heterogeneity did not appear to increase during the following 10–14 days. A large number of other colony counts was then examined, with similar results. Thus, it appears that salmonella infections comprise two stages: (1) an initial stage lasting no more than a few hours in which heterogeneity is established; and (2) the subsequent stage in which the degree of heterogeneity often remains unchanged so that the infection may be progressing in much the same way in all the hosts. It will be seen that this interpretation corresponds to the course of events suggested by Miles (1963) on quite different grounds: namely, that there is an early 'decisive period' in which primary lodgement of part of the inoculum occurs, followed by multiplication of the surviving organisms.

Figure 1 shows the two extreme cases to be discussed: in Fig. 1*a*, the colony counts (n) differ more and more as time passes, whereas in the two-stage model of Fig. 1*b*, the scatter is constant once the initial period is past. In each case, it is assumed that log n follows a parabola; that an individual falls ill or dies if n reaches the morbidity or mortality threshold respectively (Williams & Meynell, 1967); and that the mean rate of increase in log n at a given time is the same for all doses (Williams & Meynell, 1967; Mackaness, 1962, fig. 2). In fatal infections, n will appear to increase exponentially, as suggested by the linear relation generally observed between log dose and mean response time (see Meynell & Meynell, 1958; Shortley & Wilkins, 1965).

The experiments from which the colony counts were derived are shown in

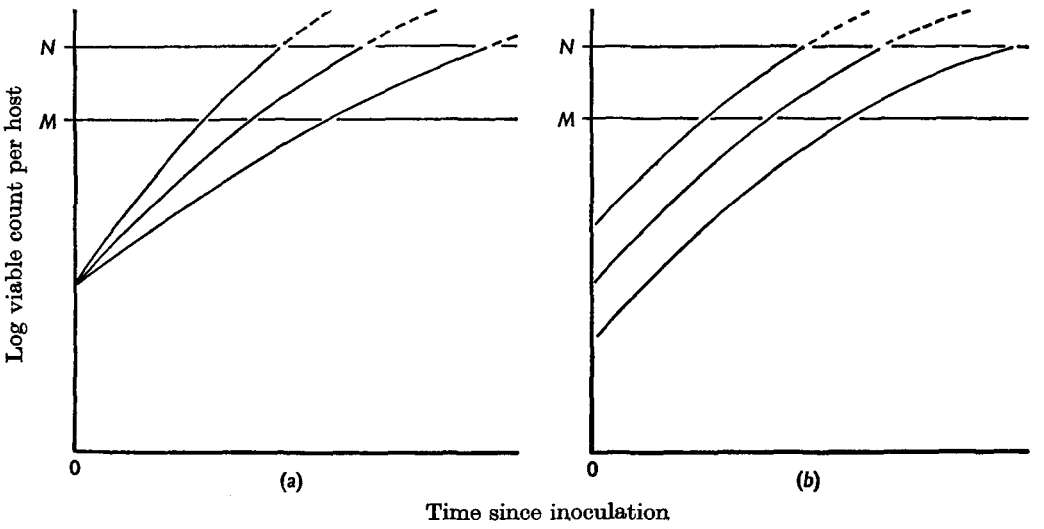

Fig. 1. Two models for the origin of heterogeneity in microbial infection. Each assumes that individual viable counts follow a parabolic course and that the host falls ill or dies if the count reaches the morbidity threshold (M) or the mortality threshold (N). In (a) individual counts differ more and more as time passes; whereas, in (b), these differences are fully established within a short time of inoculation.

Table 1. *Details of the experimental infections*

Organisms	Dose in number of: Organisms	Dose in number of: LD 50	No. of counts based on x colonies: 1–10	11–20	21–30	31–40	41–50	51–100	101–300	> 300	Reference
S. typhi	10^7	10^{-1}	—	—	1	4	5	10	12	12	Meynell (unpubl.)
S. typhimurium	2×10^6	$6{\cdot}3\times10^3$	—	—	—	—	—	—	2	10	Meynell & Meynell (1958), Fig. 10*a*
	8×10^4	$1{\cdot}3\times10^2$	—	—	1	—	—	1	3	13	Meynell & Meynell (1958), Fig. 10*b*
	3×10^3	$2{\cdot}6\times10^{-1}$	—	1	2	3	—	3	15	15	Meynell & Meynell (1958), Fig. 10*c*
S. paratyphi B	5×10^5	5×10^{-2}	3	—	—	6	3	17	17	6	Meynell & Meynell (1958), Fig. 11
	3×10^5	$1{\cdot}2\times10^{-4}$	—	2	1	3	1	17	23	12	Williams & Meynell (1967)
	3×10^5	$1{\cdot}2\times10^{-4}$	1	—	—	1	2	22	26	7	
	7×10^2	$4{\cdot}8\times10^{-2}$	5	3	3	1	3	8	19	17	
	9×10^2	$4{\cdot}8\times10^{-2}$	6	6	4	1	5	10	12	12	
S. typhimurium	10^7	5×10^{-1}	—	—	—	—	—	2	6	6	Maw & Meynell (1968), Exp. 1
	10^7	5×10^{-1}	1	—	—	1	—	6	17	6	Maw & Meynell (1968), Exp. 2
	10^7	5×10^{-1}	—	—	4	1	—	12	30	24	Maw & Meynell (1968), Exp. 3
	10^7	5×10^{-1}	—	—	—	—	1	8	17	12	Maw & Meynell (1968), Exp. 4

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Table 1. The counts were analysed by calculating $\sigma_{\log n}$, the standard deviation of log n, for each time after inoculation. Values of log n rather than n were used because plots of the individual counts show that log n is clearly more symmetrically distributed than n itself (e.g. Hobson, 1957; Meynell & Meynell, 1958; Williams & Meynell, 1967). The values of $\sigma_{\log n}$ were then plotted against time since inoculation (Fig. 2) and showed that their values fluctuated around a mean of 0·8–1·0

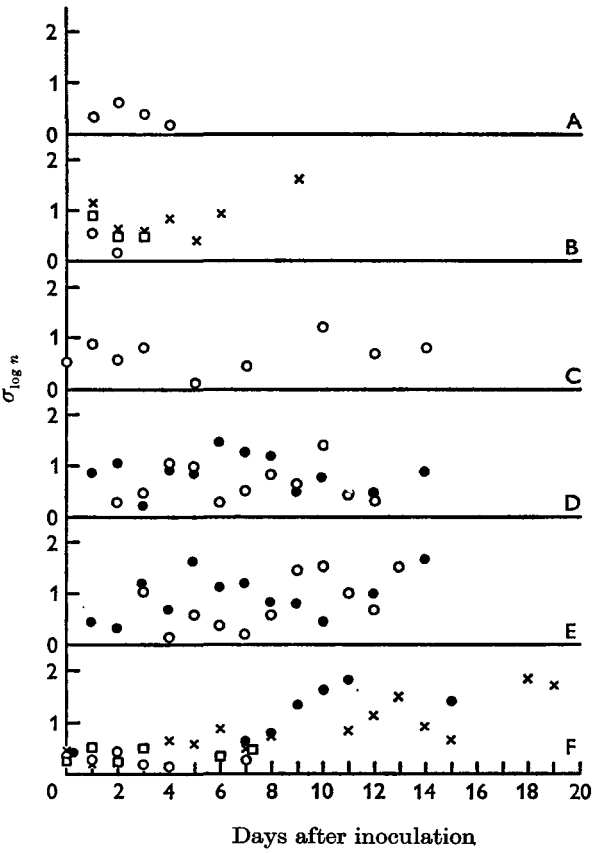

Fig. 2. Values of $\sigma_{\log n}$ observed in the experiments of Table 1. A: *Salmonella typhi* (Meynell, unpublished). B. *Salm. typhimurium* (Meynell & Meynell, 1958. ○, Fig. 10*a*; □, Fig. 10*b*; ×, Fig. 10*c*). C: *Salm. paratyphi* B (Meynell & Meynell, 1958, Fig. 11). D,E: *Salm. paratyphi* B (Williams & Meynell, 1967; Figs. 2*a*,*b*, respectively. The points come from duplicate experiments, indicated by ○, ●). F: *Salm. typhimurium* (Maw & Meynell, 1968: ○, Exp. 1; □, Exp. 2; ×, Exp. 3; ●, Exp. 4.).

during the first 10 days, although they might increase subsequently. The observed scatter in n arose almost entirely from the nature of the infection and not from trivial causes like the sampling error of the doses or colony counts, since the numbers of organisms per dose and the numbers of colonies counted were so large that sampling error was negligible (Table 1). For example, a typical value of $\sigma_{\log n}$ was derived from counts on five mice. Supposing each count to be based on x colonies, then $\sigma_{\log n} \simeq 0{\cdot}4343/\sqrt{5x}$. Hence, the values of $\sigma_{\log n}$ for groups of five

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counts, each based on 10, 20, 40 or 100 colonies, are 0·061, 0·043, 0·031 and 0·019 respectively, which are considerably less than the observed values of approx. 0·8 shown in Fig. 2. The constancy of $\sigma_{\log n}$ for the first 10 days therefore suggests that during this period at least, these salmonella infections are better described by the two-stage model of Fig. 1*b*, in which $\sigma_{\log n}$ is constant, than by the alternative model of Fig. 1*a*, in which $\sigma_{\log n}$ increases progressively from zero. After 10 days, $\sigma_{\log n}$ sometimes increased, at about the time the viable counts began to decline, possibly because new host processes then appeared which increased the heterogeneity in count by having a different intensity or time of onset in different individuals.

The validity of the two-stage model can, in principle, be tested further, by determining how the percentage of responses varies with size of dose and by examining the times at which individual hosts respond to inoculation of a given dose.

The dose-response curve

In general, the larger the number of organisms inoculated, the larger the proportion of hosts that subsequently respond. Both models imply that this occurs because, as the dose is increased, a growing proportion of counts reach the morbidity or mortality thresholds. On the two-stage model, the distribution of n from the end of the decisive period determines the distribution of response on dose. In practice, log n appears to be symmetrically distributed, so that response will be symmetrically distributed on log dose, as is observed (see Meynell, 1957). If log n differs considerably from host to host, a given increment in dose will produce a smaller increase in the proportion of hosts responding than would be the case if log n differed less. The standard deviation of log n (namely, $\sigma_{\log n}$) therefore determines the slope of the log dose-response curve which in turn determines the values of $\sigma_{\log \mathrm{ED}50}$, the standard deviation of the log dose causing 50 % of hosts to respond. In fact, since the response thresholds are constant and the viable counts appear to increase exponentially in fatal infections, $\sigma_{\log n} = \sigma_{\log \mathrm{ED}50}$. Values of $\sigma_{\log \mathrm{ED}50}$ have been determined for many infections and are always 0·5 or greater (Meynell, 1957) which is consistent with the values of $\sigma_{\log n}$ shown in Fig. 2. In other words, frequency of response increases with increase in dose to about the extent predicted from the scatter in counts.

Distributions of individual response time (t)

It follows from Fig. 1*b* that a symmetrical distribution of log n leads to a symmetrical distribution of t. At first sight, this is inconsistent with the distributions found in practice, many of which can be reasonably well depicted as normal distributions of log t (see Meynell, 1963; Sartwell, 1950, 1966). The explanation comes from Sartwell's investigations of the distributions observed in naturally occurring outbreaks, which showed that although t differed, it did so to only a limited extent. Sartwell assumed a normal distribution of log t, and could therefore express its spread by Δ, the 'dispersion factor':

$$\Delta = t_{50}/t_{16} = t_{84}/t_{50},$$

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Table 2. *Values of Δ observed in experimental infections*

Pathogen	Host	Δ	Author
Mouse encephelomyelitis virus	Mice	1·3	Gard (1940)
Poliomyelitis virus	Mice	1·5	Gard (1943)
Streptococcus pneumoniae	Mice	1·28	Cavalli & Magni (1943)
Mycobacterium tuberculosis	Mice	1·1	Martin (1946)
M. tuberculosis	Mice	1·2	Litchfield (1949)
Leukosis virus	Chickens	1·42	Eckert, Beard & Beard (1951)
Avian erythromyeloblastic leukosis virus	Chickens	1·11	Eckert, Beard & Beard (1954)
Salmonella dublin	Mice	1·53	Reid & Macleod (1954)
Avian erythroblastosis virus	Chickens	1·15	Eckert, Beard & Beard (1956)
Salm. gallinarum	Chickens		
	(Barred Rock)	1·4	Williams Smith (1956)
	(Light Sussex 3)	1·33	Williams Smith (1956)
Pasteurella pestis	Guinea-pig	1·37	Druett *et al.* (1956)
Bacillus anthracis	Mice	1·2	Roth, DeArmon & Lively (1956)
Salm. typhimurium	Mice	1·38	Meynell & Meynell (1958)
Salm. typhimurium	Mice	2·00	Meynell & Meynell (1958)
Salm. typhimurium	Mice	1·33	Meynell & Meynell (1958)

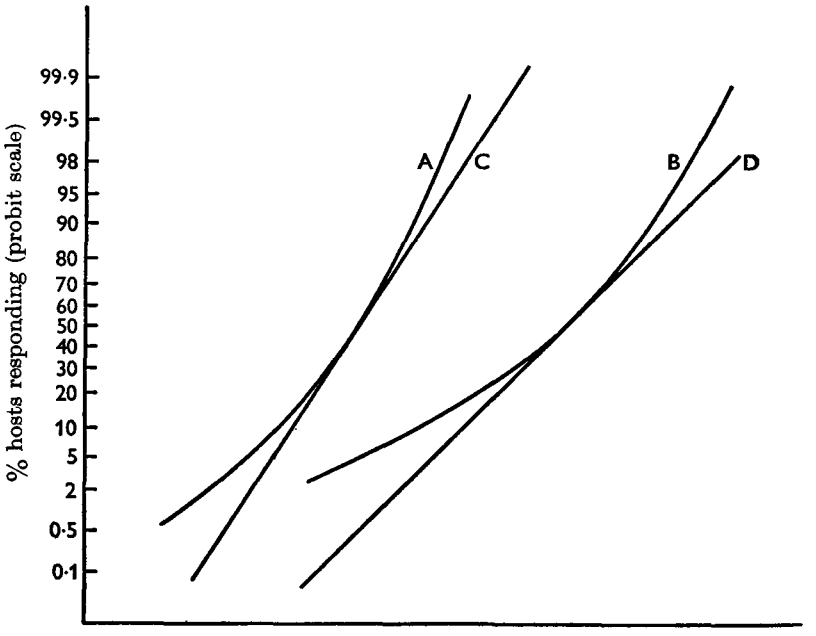

Fig. 3. A comparison of normal distributions of t and of log t. Curves A, B: normal distributions of t with $\sigma_t = 1{\cdot}0$ and $1{\cdot}7$, respectively. Curves C, D: normal distributions of log t with $\Delta = 1{\cdot}3$ and $1{\cdot}5$, respectively. Curves A and C (or B and D) would be indistinguishable in practice unless an exceptionally large number of hosts was used.

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where t_{16}, t_{50}, and t_{84} are the times corresponding to 16, 50 and 84% responses. Thus, $\Delta = 1$ if all the responses occur simultaneously and its value rarely exceeds 1·5 in outbreaks. The scatter of t is also limited in experimental infections (Table 2). Because of this, the corresponding normal distributions of t, log t and $1/t$ will be extremely hard to distinguish in practice because their differences are most marked at the tails of the distributions which can rarely be determined precisely because of the large number of hosts required. Thus, normal distributions of log t with $\Delta = 1{\cdot}1$ and 1·5 give virtually linear curves when $1/t$ is plotted (Meynell & Williams, 1967) and, as Fig. 3 shows, analogous normal distributions of t appear almost linear when plotted on a logarithmic time scale. The empirical use of log-normal distributions of t is therefore unlikely to be inconsistent with t being symmetrically distributed, as suggested by Fig. 1*b*. Thus, the dose-response curves and distributions of individual response times generally observed in bacterial infections are not inconsistent with the distributions of viable counts found in these experiments.

A two-stage process therefore provides as good a description of salmonella infections as any previous model. It could, of course, be tested far more rigorously by obtaining many more values of n and t and by determining the dose-response curve in the same experiment, although, to be useful, this might have to be done on a very large scale. Considered purely as a model, a two-stage process has the disadvantage of a large number of parameters. In addition to the morbidity and mortality thresholds, there are $\sigma_{\log n}$ and the mean proportion of the dose that survives the decisive period, for the first stage, and, for the second stage, the true division and death-rates of the organisms *in vivo* and the rates at which these change with the passage of time.

The existence of two stages is not implausible. The inocula were given by intraperitoneal or intravenous injection, and the bacteria were therefore initially in the peritoneal cavity or the blood, while subsequently they were confined to the organs. The presence of two stages is also indicated by measurements of the true rate of division of *Salmonella typhimurium* in the mouse spleen, where the true mean generation time is 90 min. during the 1·5 hr. after inoculation but thereafter increases to 5 hr. or more (Maw & Meynell, 1968). Whatever the antimicrobial mechanisms of the host may be, the first stage appears 'decisive' in the sense suggested by Miles (1963). During this phase, a varying proportion of the inoculum is killed within a short time, leaving a fraction of survivors whose numbers in part determine the mortality. The fact that two unrelated investigations, one concerned with organisms injected intravenously or intraperitonally and the other with infections of the skin (see Miles, 1956, 1963), have each led to a two-stage model is a strong indication that analogous processes may well come to be found of importance in determining the outcome of other types of infection.

SUMMARY

Colony counts on mice given the same number of *Salmonella* always differ considerably. However, the standard error of the mean log count does not increase

after the first 1·5 hr. of infection until the 8th or 10th day. These infections therefore appear to pass through an initial stage lasting a few hours, in which a varying proportion of the inoculum is killed, followed by a prolonged second stage in which the scatter in individual colony counts remains constant.

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