# THE RELATION BETWEEN DOSE AND MORTALITY FOR *SALMONELLA DUBLIN**

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(With 2 Figures in the Text)

## INTRODUCTION

The nature of the relationship between dose and mortality for bacterial agents appears to have received comparatively little attention. Petrie & Morgan (1931), investigating the lethal power of a strain of pneumococcus, found that for this strain at least the mortality pattern arose simply from the presence or absence of bacteria in the inocula injected; that is, the proportion dying at any given dilution was that receiving one or more bacteria. Where large numbers of organisms are required to kill, as is generally the case, this explanation will not apply, and animal variation might be assumed to determine the mortality pattern. The same problem has been discussed in some detail in connexion with the production of local lesions by viruses. A summary of the different points of view is given by Lauffer & Price (1945), and more recently by Kleczkowski (1950). The latter suggested that the sigmoidal relationship between dose and proportion of lesions is due, not to the chance distribution of virus particles, but to inherent variation in the resistance of the host.

This paper presents the results of a study of the relationship between dose and mortality for *Salmonella dublin* and mice, and the effect upon it of both active and passive protection. The data are examined under the assumption that the proportion of deaths at any dilution of a bacterial suspension is the proportion of inocula which contain one or more organisms of a type capable of growth in the host. Only a fraction, $p$, of the total number of organisms may be of this type. Assuming then a random distribution of the bacteria throughout the suspensions used, the proportion of deaths at any dilution $i$ may be written

$$P_i = 1 - e^{-pm_i},$$

where $m_i$ is the average number injected. This expression, of course, utilizes the first term of the Poisson series. $p$ might also be interpreted as the proportion of organisms which find suitable conditions for growth in the host.

While this expression might be expected to describe adequately situations where the animals are completely homogeneous, and so in effect test-tubes, any variation they exhibit would tend to modify the above pattern. Such variation would arise from fluctuation in the proportion of 'effective' organisms from one animal to another. If this varies in such a way that $\lambda = pm_i$, the average number of effective organisms received, is distributed in the form

$$\frac{c^k}{\Gamma(k)} e^{-c\lambda} \lambda^{k-1},$$

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while the actual number of organisms for mice which have the same $p$ follows a Poisson distribution, the resulting distribution of effective organisms is a negative binomial distribution with mean $\bar{p}m_i$. The mortality curve would then be of the type

$$P_i = 1 - \left(1 + \frac{\bar{p}m_i}{k}\right)^{-k},$$

where $\bar{p}$ is the average proportion capable of growth, and $k$ a measure of animal homogeneity, i.e. small values of $k$ imply large variation in $p$. The second expression is equivalent to the first when $k$ is infinitely large, and is not perceptibly different for values of $k$ greater than 10.

## METHODS

A strain of *S. dublin* was used. Cultures grown on agar slopes were made into vaccines by being suspended in saline, heated to 56° C. for 1½ hr. and preserved with 0·25% phenol; the density of organisms was adjusted to approximately $10^9$ per ml. using the McFarland standards. Six hundred mice, obtained from the colony maintained by these Laboratories, were distributed into three groups of 200 each. One group was vaccinated with 0·25 ml. of undiluted vaccine, one with 0·25 ml. of vaccine diluted $\frac{1}{10}$ and one was left unvaccinated. Four weeks after vaccination the mice were further subdivided into groups of 15–20 each, a few intercurrent deaths having occurred, and challenged intraperitoneally with a series of twofold dilutions of a suspension of *S. dublin*. The dilutions were chosen to give mortalities from 0 to 100% in each major group. Deaths occurring during the following 4 weeks were recorded, as it was found that almost all deaths from primary infection and very few from secondary spread took place within this period (MacLeod, 1954). The average number of viable organisms injected at one of the dilutions was estimated by plate counts.

Two experiments on passive protection were undertaken. In the first of these experiments, serum was obtained from mice vaccinated with five doses of vaccine. It was diluted $\frac{1}{100}$, and 0·5 ml. injected into each of 100 mice. These animals were then challenged intraperitoneally, in groups of ten, with twofold dilutions of *S. dublin* chosen to give mortalities from 0 to 100%. In the second experiment, intended to clarify the mechanism of bacterial protection, seventy mice were vaccinated with undiluted vaccine and seventy-five with vaccine diluted 1 in 10. Nineteen of the first group and twenty-two of the second were bled out for serum, giving two lots of serum, the first representative of animals which had received undiluted vaccine and the second of animals which had received diluted vaccine. From each lot of serum five dilutions, varying from $10^0$ to $10^{-4}$, were prepared and each dilution injected into groups of ten normal animals. These mice, together with the remaining vaccinated animals, were challenged with the same dose.

## RESULTS

The results of the first experiment are given in Table 1. Curves of the Poisson type were fitted to each of the series by the method of maximum likelihood (Haldane, 1939), and are presented in Fig. 1. These curves describe the observed data fairly well, though some departure seems to occur in the control group and the group

inoculated with undiluted vaccine. $\chi^2$ tests of goodness of fit are given in Table 2. As judged by these tests, this simple relationship describes the first and second set

Table 1. *Ratios of deaths (D) to number injected (I) among mice inoculated with* S. dublin

| Estimated number of organisms per dose | Control D/I | Vaccinated | |
|---|---|---|---|
| | | Vaccine diluted 1:10 D/I | Vaccine undiluted D/I |
| 1408 | — | 15/15 | 17/19 |
| 704 | — | 17/17 | 18/20 |
| 352 | — | 16/17 | 13/20 |
| 176 | — | 16/20 | 14/20 |
| 88* | 17/17 | 13/20 | 4/20 |
| 44 | 17/18 | 5/20 | 4/20 |
| 22 | 18/20 | 3/20 | 3/20 |
| 11 | 18/20 | 3/20 | 2/20 |
| 5·5 | 14/19 | 1/19 | 0/20 |
| 2·8 | 9/19 | 0/19 | 1/20 |
| 1·4 | 5/17 | — | — |
| 0·69 | 4/19 | — | — |
| 0·34 | 2/19 | — | — |
| 0·17 | 0/19 | — | — |

* Number of organisms at this dilution estimated by plate count.

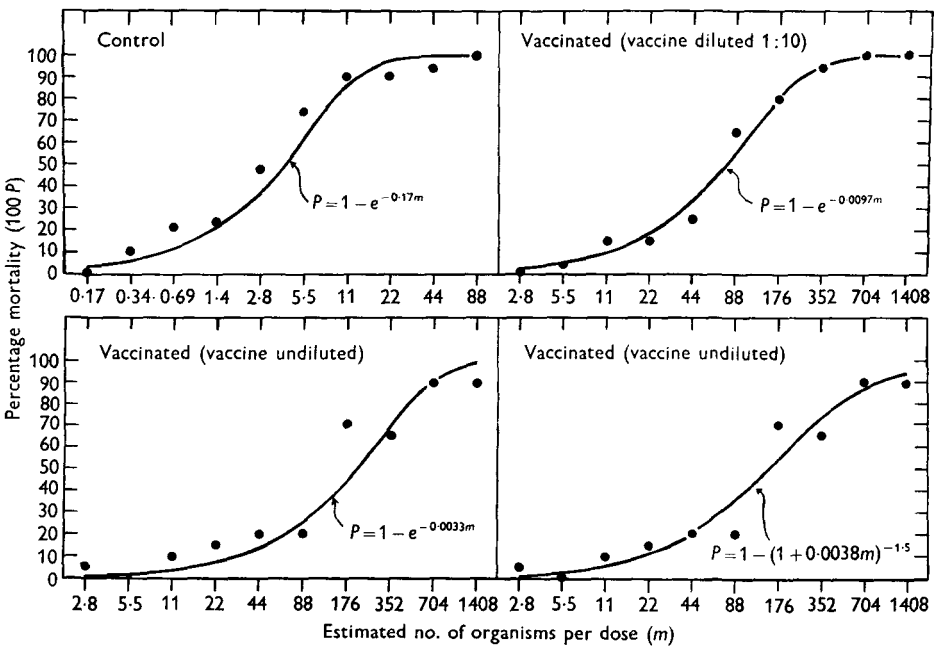


Fig. 1. Mortality patterns for control and vaccinated mice inoculated intraperitoneally with *S. dublin*.

of results satisfactorily, though not that obtained with undiluted vaccine. For this reason, a curve of the negative binomial type was fitted to this series to obtain a better description of the data. This is also given in Fig. 1.

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The constants of the various curves are given in Table 2. From these a comparison can be made of the proportions of effective organisms. On the assumption that the relationship between dose and mortality is of the Poisson type, about 1 in 6 organisms were effective in the control group; about 1 in 100 in the group inoculated with diluted vaccine; and about 1 in 300 in the group inoculated with undiluted vaccine.

Table 2. *Fit of probability curves*

| Series | Curve | Proportion of 'effective' organisms | Goodness of fit* $\chi^2$ | Degrees of freedom | $P$ |
|---|---|---|---|---|---|
| Control | Poisson | 0·17 ± 0·025 | 5·2 | 4 | 0·28 |
| Vaccinated: vaccine diluted 1:10 | Poisson | 0·0097 ± 0·0014 | 1·6 | 4 | 0·81 |
| Vaccinated: vaccine undiluted | Poisson | 0·0033 ± 0·0012 | 12·5 | 5 | 0·03 |
| | Negative binomial ($k = 1·5$) | 0·0057 | 5·3 | 5 | 0·38 |
| Serum protected | Poisson | 0·0088 ± 0·0018 | 3·0 | 3 | 0·38 |

* Frequencies grouped to give at least 2 expected deaths or survivals per cell.

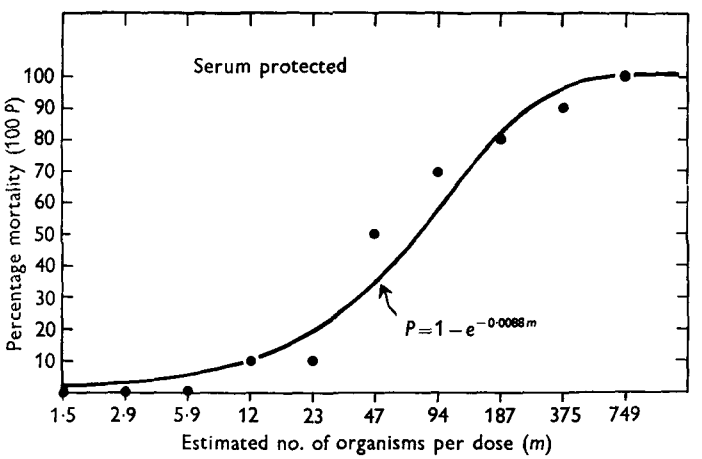


Fig. 2. Mortality pattern for serum-protected mice inoculated intraperitoneally with *S. dublin*.

The results of the first serum experiment are presented graphically in Fig. 2, together with the corresponding Poisson curve, which describes the data satisfactorily. The results of the second serum experiment are given in Table 3. Undiluted serum from vaccinated animals appears to have given almost the same degree of protection as found in the actively protected animals. As is characteristic of serum-protection tests with this organism, the concentration of serum could be varied widely with little effect on protection, tenfold changes in serum level producing little change in survival rate.

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Table 3. *Comparison of active protection and transferable serum protection*

| Type of protection | Survival rate after challenge dose of forty-nine organisms: Vaccine: undiluted | Vaccine: diluted 1/10 |
|---|---|---|
| Active: vaccinated mice | 37/51 = 73 % | 35/53 = 63 % |
| Passive: normal mice inoculated with serum from vaccinated mice | | |
| Dilution of serum: 1/1 | 9/10 | 5/10 |
| 1/10 | 5/10 | 4/10 |
| 1/100 | 7/10 | 2/10 |
| 1/1000 | 4/10 | 1/10 |
| 1/10,000 | 6/10 | 2/10 |
| Controls | 1/10 | |

## DISCUSSION

The data presented show that the mortality patterns observed in mice with this strain of *S. dublin*, injected intraperitoneally, may be described reasonably well by assuming that the receipt of one or more organisms capable of growth in the host results in death. Of the organisms injected, however, only a proportion are capable of growth; the effect of vaccination and passive protection may be chiefly, and possibly entirely, a reduction of this proportion. In general, there seemed to be no great variation among the mice used in their resistance to the organisms.

This homogeneity is surprising, particularly in the vaccinated series where animals might be expected to vary in their response to vaccination. While the fit of the simple Poisson curve is not too satisfactory for the fully vaccinated animals, even here the discrepancies are not great and are possibly due to errors of technique, which would produce departures in the direction observed. Vaccination against this organism seems to provide the host with a type of protection which might be expected with the use of serum. A possible explanation for this apparent homogeneity is suggested by the results of the second serum experiment. These indicate that the important factor in the protection of vaccinated animals against intraperitoneal challenge is probably humoral. If, then, the antibody levels of individual animals can differ greatly without materially influencing the likelihood of death, such variations would not affect the basic mortality pattern, which would be similar for normal, actively protected and passively protected animals.

Further studies are required to determine to what extent these conclusions hold for other strains of mice and organisms, and for other routes of injection.

## SUMMARY

The mortality patterns obtained with *S. dublin* injected intraperitoneally in both control and immunized mice could be described fairly well by curves of a simple Poisson type, based upon the assumption that the receipt of one or more organisms of a type capable of growing in the host resulted in death, and that both vaccination and passive protection simply reduced the proportion of this type.

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ADDENDUM

Since the authors submitted this paper, S. Peto (1953) has published an article in which a similar explanation of the relationship between dose and mortality for bacteria is given, when the probability of an organism killing the host, i.e. the proportion of 'effective' organisms, is small.

REFERENCE

PETO, S. (1953). A dose-response equation for the invasion of micro-organisms. *Biometrics*, **9**, 320.