

## THE SPREAD OF BACTERIAL INFECTION. THE PROBLEM OF HERD-IMMUNITY.

BY W. W. C. TOPLEY AND G. S. WILSON.

*(From the Department of Bacteriology and Preventive Medicine,  
University of Manchester.)*

*(A Report to the Medical Research Council.)*

(With 9 Charts.)

IN our previous studies on the spread of bacterial infection among mice, the resistance of the host has not been especially investigated; although the importance of this factor has been repeatedly referred to in discussing the experimental results obtained.

The recent reports on experimental epidemiology from the Rockefeller Institute contain many references to this aspect of the question, and, in particular, the careful and important investigation reported by Webster (1922) affords ample evidence of the possibility of conferring some degree of immunity on mice by the administration of dead or living suspensions of bacteria of the *B. enteritidis* group, whether by feeding or by inoculation.

The experiments recorded in the present report were undertaken in order to study any differences, which might occur in the mode of spread of enteric infection among mice, when the immunity of the population at risk was varied by including, among the normal and presumably susceptible mice, a varying proportion of artificially immunised animals.

Consideration of the results obtained during the past five years, both in experiments which have formed the subject of reports and in many others not yet recorded, has led us to believe that the question of immunity as an attribute of a herd should be studied as a separate problem, closely related to, but in many ways distinct from, the problem of the immunity of an individual host.

*Experiment 1.* For the purpose of this, and of the next experiment, a vaccine was prepared from a strain of *B. enteritidis* (Aertrycke). This was killed by heat, standardised to contain 4000 million bacteria per c.c., and preserved by the addition of 0.5 per cent. phenol.

In the present experiment 127 mice were given an intraperitoneal injection of 0.125 c.c. (500 million bacteria). Eight days later there were 119 survivors, and each of these was inoculated intraperitoneally with 0.25 c.c. of vaccine (1000 million bacteria).

The main experiment was started 21 days after the second injection. Four of the immunised mice were killed on this day, and their sera were tested

against a formalised broth culture of the strain used to prepare the vaccine. Three specimens gave agglutination to a titre of 1/10,000, the fourth to 1/80,000.

For the experiment itself, 90 immunised and 90 normal mice were employed, the latter having been carefully watched during the preceding six weeks. Six cages were put up, as follows:

Cage A	contained	30	normal	mice.
„ B	„	30	„	„
„ C	„	20	normal mice and	10 immunised mice.
„ D	„	10	„	„
„ E	„	30	immunised	mice.
„ F	„	30	„	„

On the same day, the mice in each cage were fed with bread soaked in a 24 hours' broth culture of *B. enteritidis* (Aertrycke). This feeding was repeated on the following day, and again on the fourth day of the experiment. Events were then observed for 60 days.

Charts I to VI record in sufficient detail the subsequent happenings. The block of squares to the left of each chart represents the number of mice included in each cage. An unmarked square corresponds to a normal mouse, a square with one central dot to an immunised mouse. Deaths are recorded along the base-line in the usual manner. A black square represents a death which was demonstrated to be due to enteric infection by the post-mortem findings, including the isolation of the specific organism from the tissues. An unshaded square corresponds to a death from some other cause, usually unknown, a post-mortem examination having yielded no evidence of enteric infection. A square with a diagonal cross represents a death the cause of which could not be determined, owing to the impossibility of carrying out a post-mortem examination. Where both normal and immunised mice were present in a cage the deaths of these two classes are recorded along separate base-lines. The block of squares on the right of each chart represent the mice surviving at the end of 60 days. Normal and immunised mice are indicated in the same way as before. Certain other relevant facts are recorded in individual charts.

The results may be briefly summarised. The cages containing only normal mice showed, in each case, a total mortality of 96·7 per cent., and this mortality was almost entirely specific. The cage containing normal and immunised mice in the proportion of two to one showed a total mortality of 56·7 per cent., probably all specific. The cage containing normal and immune mice in the proportion of one to two showed a total mortality of 66·7 per cent. entirely specific. The two cages containing only immunised mice, showed a total mortality of 63·3 per cent. in one case and of 50 per cent. in the other. The specific mortalities were 36·7 per cent. and 33·3 per cent. respectively.

With the exception of Cage C, therefore, in which the mortality was unexpectedly low, the results show a decreasing mortality with an increasing

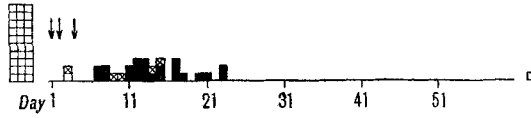


Chart I. (Exp. 1, Cage A.)

For explanation of this and other charts, see text.

In this and other charts, on dates marked ↓ ↓, mice were fed with bread soaked in a 24 hours' broth culture of *B. enteritidis* (Aertrycke).

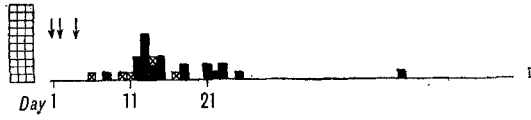


Chart II. (Exp. 1, Cage B.)

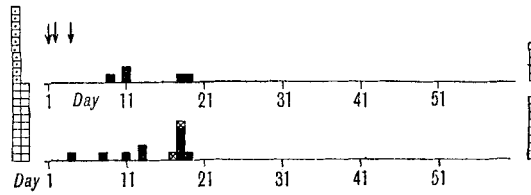


Chart III. (Exp. 1, Cage C.)

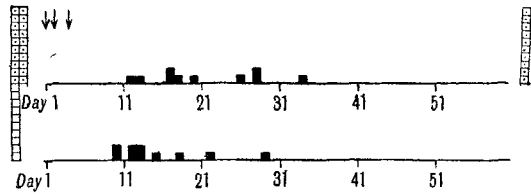


Chart IV. (Exp. 1, Cage D.)

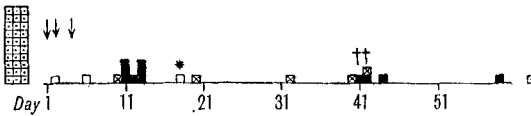


Chart V. (Exp. 1, Cage E.)

\* Mouse partially eaten. Typical lesions of enteric infection, but only lactose-fermenting bacilli isolated.

† P.-m.—typical lesions of pseudo-tuberculosis. *B.* (Aertrycke) isolated in addition to *B. pseudo-tuberculosis murium*.

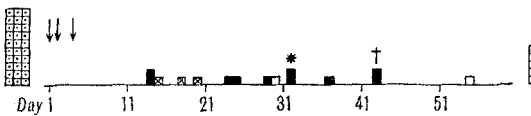


Chart VI. (Exp. 1, Cage F.)

\* One of the mice dying this day showed lesions of pseudo-tuberculosis, but *B.* (Aertrycke) isolated p.-m. in addition to *B. pseudo-tuberculosis muris*.

† One of the mice dying this day showed lesions of pseudo-tuberculosis.

proportion of immunised mice. If we consider the two cages which contained both normal and immunised mice, we find that, in Cage C, the mortality was 60 per cent. among the normal and 50 per cent. among the immunised, while in Cage D it was 100 per cent. among the normal and 50 per cent. among the immunised.

The time-relations of the deaths are of considerable interest. In Cages A and B, containing only normal mice, the deaths had practically ceased by the 24th day of the experiment. In Cage C, containing normal and immunised mice in the proportion of two to one, no deaths occurred after the 19th day. In Cage D, containing normal and immunised mice in the proportion of one to two, 25 per cent. of the total deaths occurred after the 24th day. In Cages E and F, 36.8 per cent. and 53.3 per cent. respectively of the total deaths occurred after the 24th day. There is similar evidence of delay, if we compare the deaths of the normal and immunised mice in the two cages which contained animals of both classes.

It appears, then, that immunisation by intraperitoneal inoculation of a killed vaccine confers a definite, but by no means an absolute protection against infection by feeding with massive doses of *B. enteritidis* (Aertrycke), and against the subsequent risk of infection from infected mice, since this factor will clearly operate during the later stages of such an experiment. In how far the delay in the occurrence of the deaths among the immunised mice is due to a prolongation of the infective process in mice so treated, or to the fact that they only fall victims to the disease after receiving repeated doses of the bacterial parasite from their infected companions, is a question which the evidence does not allow us to examine in any detail. The general arrangement, in time, of the deaths among the immunised mice would, however, suggest that there was delayed infection in many cases, and not merely delayed death.

*Experiment 2.* A second experiment was carried out in an attempt to determine the effect of variations in the proportion of normal to immunised mice, among aggregates submitted to the risk of infection from other individuals of their own species, the factor of massive infection by feeding being eliminated. The process of immunisation was exactly the same as in the preceding experiment, and the examination of a sample of ten immunised mice gave closely similar agglutination results, although the titres obtained were not quite so high.

Five separate aggregates of mice were arranged as follows:

Cage A	contained	30	normal	mice.
„ B	„	20	normal	mice and 10 immunised mice.
„ C	„	15	„	„ „ 15 „ „
„ D	„	10	„	„ „ 20 „ „
„ E	„	30	immunised	mice.

It is clearly not easy to devise any method of infection from mouse to

mouse which will enable us to ensure an equal risk to each of the populations under examination. The method actually adopted was as follows. Ten mice were taken, which had recently passed through a considerable epidemic of enteric infection. The faeces of these mice had been tested for the presence of *B. enteritidis* (Aertrycke) on five occasions during the preceding 14 days, and from each mouse this organism had been isolated in one or more samples. Two of these mice were added to each cage on the first day of the experiment. Next day the two infected mice in Cage A were transferred to Cage B, those in Cage B to Cage C, and so on, those in Cage E being transferred to Cage A. This process was repeated daily for the first 15 days. Each cage was thus visited three times by each pair of infected mice, the only difference being that the pair of mice which visited Cage A on the first day did not visit Cage E till the fifth day. The order of rotation was identical, but the starting-point was different. The subsequent course of events was observed over 120 days.

In Cage B, containing normal and immunised mice in the proportion of two to one, no spread of infection occurred. Several mice died during the 120 days of observation, and the deaths were proportionately more numerous among the normal than among the immunised mice, but in no instance was any evidence of enteric infection obtained post-mortem. In Cage D, containing normal and immunised mice in the proportion of one to two, there was the same failure of spread. In this case, also, many deaths occurred, and the normal mice suffered more heavily than their immunised companions. The time-relations of the deaths in this cage suggested that some infection was responsible for the mortality, since the majority of deaths occurred during a period of ten days. The post-mortem results were, however, consistently negative. Since no trustworthy information can be gained from the observations made on these two cages, they are not further considered.

The course of events in Cages A, C and E are set out in Charts VII, VIII and IX. These are constructed in the same manner as Charts I to VI and need no further explanation. Table I summarises the more important points.

Certain facts of interest may be noted. So far as was possible each cage was subjected to an equal risk of infection. In the result, a considerable epidemic was produced among the 30 normal mice leading to a mortality of 96·7 per cent. within 120 days, and a specific mortality of 70 per cent. Among the immunised mice there was no evidence of spread of specific infection. Although a total mortality of 60 per cent. occurred during 120 days, no death could be attributed to enteric infection. Among the population, half of which were normal and half immunised, an epidemic was produced of equal severity with that which occurred among the normal population. The total mortality was 100 per cent. and the specific mortality 53·3 per cent. It should, however, be noted that, while the specific mortality among the 15 normal mice was 66·7 per cent. that among the immunised was only 40 per cent.

## DISCUSSION.

These experiments are too few to allow of any but the most tentative conclusions being drawn. We believe, however, that the following inferences are allowable.

Active immunisation of mice, in the manner indicated, confers a definite degree of protection against subsequent infection by feeding. This, indeed, has already been demonstrated by Webster (1922) and by others. It also confers some protection against mouse-to-mouse infection. There is some evidence that spread of infection occurs with difficulty among a population, each individual of which has been actively immunised. There is definite

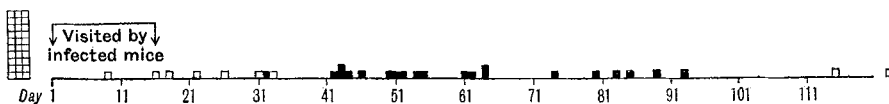


Chart VII. (Exp. 2, Cage A.)

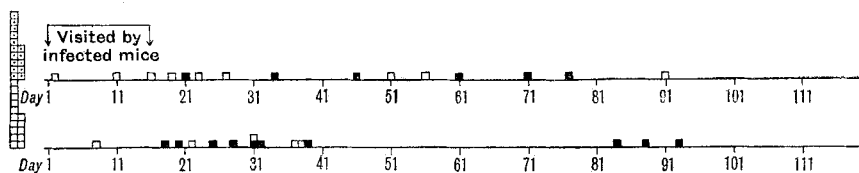


Chart VIII. (Exp. 2, Cage C.)

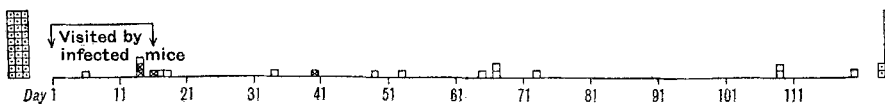


Chart IX. (Exp. 2, Cage E.)

Table I.

Cage	Mice		Mortality %	
	Normal	Immune	Total	Specific
A	30	0	96.7	70
C	15	15	100	53.3
E	0	30	60	0

evidence that, among a population containing susceptible and immunised individuals, the relative immunity of the latter does not save them from infection and death, when epidemic spread occurs. It appears that a degree of immunity, which may save individual hosts when living among equally resistant companions, is rendered of no avail when they are surrounded by highly susceptible individuals of their own species.

We have referred above to the need for a careful study of the factors determining the immunity of a herd as distinguished from the immunity of an individual. One obvious problem to be solved is the following. Assuming a given total quantity of resistance against a specific bacterial parasite to be available among a considerable population, in what way should that resistance

be distributed among the individuals at risk, so as best to ensure against the epidemic spread of the disease, of which the parasite is the causal agent?

It is, of course, impossible to suggest any measure of a "total quantity of resistance" in this sense, so far as naturally existing conditions are concerned. We might, however, attach a reasonably exact meaning to the term, if we were dealing with the production of a state of passive immunity by the inoculation of a protective serum, supposing the untreated hosts to possess no resistance of their own. When dealing with active immunisation, we may, perhaps, reasonably enquire whether it is better that some individuals shall be highly resistant, and others fully susceptible, or that all shall possess some degree of immunity, even if this be of a lower grade.

If an answer could be obtained to this question it might throw light on many problems of preventive medicine.

#### REFERENCE.

- WEBSTER, L. T. (1922). Experiments on Normal and Immune Mice with a Bacillus of Mouse Typhoid. *Journ. Exp. Med.* xxxvi. 71.