MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

III. The Immunity of a Surviving Population.

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Topley[1] and Amoss[2] have both observed that recurrent outbreaks of mouse typhoid in an experimentally controlled community flourish more readily and are much more fatal among the fresh recruits than among the older surviving individuals left over from previous epidemics. The experiments described in this paper relate to the nature of the resistance displayed by this latter group.

The past histories of the surviving individuals of epidemics generally considered are uncertain. It is assumed that taking all the survivors together, certain of them have entirely resisted infection, certain have been infected and have recovered, others carry the disease in a more chronic form, while still others have escaped exposure entirely. In planning the present experiments, exposure to infection was insured by the administration of the cultures *per os*, and chronic forms of infection were detected by adequate bacteriological methods. In this way the number of survivors was reduced to mice which had (*a*) actually resisted or (*b*) recovered from the infection.

The purpose of the first experiment was to determine the protecting effect of different strains of paratyphoid-enteritidis bacilli against a subsequent dose of the epidemic mouse typhoid culture, M. T. II.

[1] Topley, W. W. C., *J. Hyg.*, 1921, xx, 103. Topley, W. W. C., and Wilson, G. S., *J. Hyg.*, 1923, xxi, 243.

[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.

Immunity among Survivors to Bacilli.

Experiment 1.—According to the purpose of this experiment, each of six series of mice of similar age and weight was given by stomach tube one of six paratyphoid-enteritidis strains in a known dose which varied from 3,000,000 to 6,000,000 bacteria. The technique of this procedure has been discussed, together with the characteristics of the various strains employed: Mouse Typhoid I (M. T. I), Mouse Typhoid II (M. T. II), *B. enteritidis* (Gaertner), *B. aertrycke* (mutton), *B. pestis caviæ*, and *B. paratyphosus* B.[3,4]

60 days after this injection, about 10 per cent of the *B. enteritidis* series, 20 per cent of the M. T. I series, 30 per cent of the M. T. II and of the *B. aertrycke* series, and 70 per cent of the *B. paratyphosus* B and of the *B. pestis caviæ* series were alive. These mice were temporarily considered to be survivors. Each series was placed two per jar and with twenty normal control mice were given *per os*

TABLE I.

Summary of Protocols of Surviving Mice Inoculated with Strain M. T. II.

Preliminary bacterial strain.	No. of mice.	Mice developing positive blood cultures.	Mice dead at 8 wks.	Mice surviving at 8 wks.
		per cent	*per cent*	*per cent*
No preliminary cultures (controls)	20	75	80	20
B. pestis caviæ	27	33	52	48
" *paratyphosus* B	32	31	52	48
M. T. I	15	13	33	67
" II	32	6	31	69
B. aertrycke (mutton)	22	4	27	73

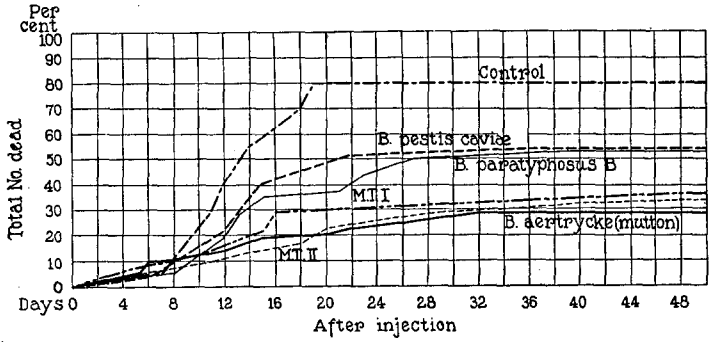
a fixed dose, 4,000,000 bacteria, of the epidemic mouse typhoid strain, M. T. II. Blood cultures were taken at frequent intervals, and all fatal cases were autopsied and cultured. Whenever the original bacillary strain administered for immunizing purposes was recovered, the mice yielding it were considered to be chronic cases and not true survivors. All such mice were excluded from the final computations.

Table I and Text-fig. 1, in which the duration of life among the survivors is compared with that of the controls, are taken from the last of three tests, all alike in respect to the treatment given, the number of mice used, and the end-results. Examination of the protocols and chart shows unmistakably that mice which have passed

[3] Webster, L. T., *J. Exp. Med.*, 1923, xxxviii, 33.
[4] Webster, L. T., *J. Exp. Med.*, 1923, xxxviii, 45.

through and survived an otherwise infecting dose of paratyphoid-enteritidis bacilli given *per os* exhibit greater resistance to a second dose of a related bacillus than do normal mice not previously exposed to infection.

In addition, a second effect is discernible; namely, that the amount of resistance displayed by each group of survivors is more directly related to the virulence of the strain of bacillus originally administered than to an antigenic similarity of this first strain to the second strain employed. Thus *Bacillus paratyphosus* B, which is of low virulence, yielded at first relatively a large group of survivors but at the end of the experiment relatively a small number of ultimate



TEXT-FIG. 1. The resistance of groups of mouse typhoid survivors to a second dose of similar organisms.

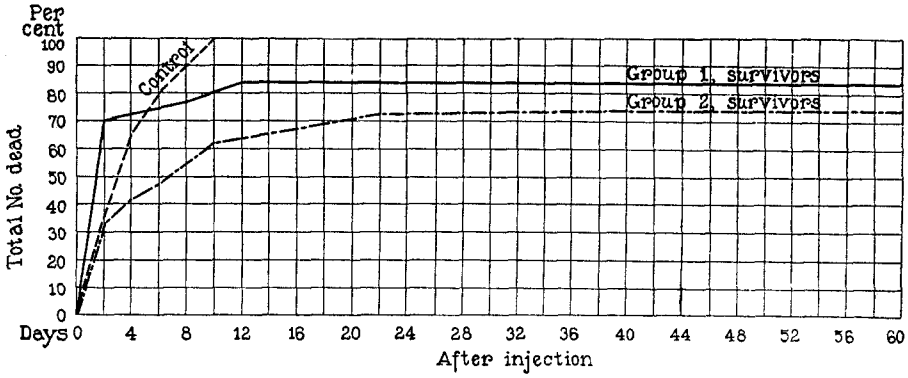
survivors to the second inoculation with Strain M. T. II; and likewise the survivors of *Bacillus pestis caviæ* which not only is also of low virulence but is antigenically identical with Strain M. T. II. On the other hand, the mice surviving the more virulent *Bacillus aertrycke* (mutton) and Strain M. T. II, itself, proved far more resistant to the second administration made with M. T. II. In a like manner, Strain M. T. I, which is highly virulent but antigenically dissimilar from Strain M. T. II, left survivors which resisted, in the way of the preceding examples, the second administration of Strain M. T. II. Hence it appears that the virulent strains of bacilli originally inoculated eliminated the susceptible individuals from the respective series of mice with the result that the survivors consisted of the naturally resistant part of the populations on which

the bacillary Strain M. T. II exerted little effect. The fact should be mentioned that too few individuals survived the original *Bacillus enteritidis* inoculation to provide survivors for the second M. T. II test.

Parallel observations by Pritchett have enabled us to eliminate the possibility of cross-protection phenomena in this experiment.[5]

Resistance of Survivors to Chemicals.

The inherent qualities on which resistance to infection is based may consist, therefore, not only of specific but also of non-specific factors. We have previously pointed out[6] that any large number of mice divides



TEXT-FIG. 2. The resistance of mouse typhoid survivors to mercury bichloride.

itself automatically into three groups according as they (*a*) become infected and die, (*b*) become infected and recover, and (*c*) escape infection altogether when given a fixed dose of mouse typhoid bacilli. A somewhat similar grouping was determined to take place when a toxic chemical such as mercury bichloride was substituted for living bacteria.[4] The question now arose as to the manner in which the surviving mice, in the sense of Experiment 1, would react to the administration of this toxic drug.

Experiment 2.—The total number of mice tested in this experiment was 81, divided as follows: 13 surviving one dose of mouse typhoid bacilli (Group 1); 48 surviving two doses (Group 2); and 20 normal or control mice of similar weight (Group 3). Each mouse received 0.0016 gm. of mercury bichloride in 0.5 cc. volume of distilled water *per os* by stomach tube.

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[5] Pritchett, I. W., *J. Exp. Med.*, 1924, xxxix (in press).

[6] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 231.

Text-fig. 2 clearly shows that the mice which survived one or more doses of mouse typhoid bacilli resist a toxic dose of mercury bichloride better than do the normal controls. This result supports and extends the results of our previous experiments with this chemical and indicates anew that in escaping or recovering from infection, certain non-specific factors operate just as they operate in preventing a fatal drug intoxication.

"Resistant" versus "Recovered" Survivors in Relation to Infection.

The data presented above emphasize the influence of a factor which for want of a better name we have called non-specific in the determination of infection or intoxication and recovery from those conditions. Still other evidence bearing on the same point will now be presented. It is to be regretted that the material available was not more voluminous, but in spite of its limited size, it has seemed to yield a significant fact.

Experiment 3.—From a series of earlier experiments, eleven mice were chosen which had survived an infecting dose of Strain M. T. II *per os* by stomach tube. Stool cultures, blood cultures, and agglutinin tests had been frequently performed. Eight of these mice had passed the bacilli with the feces for a few days but had shown no positive blood cultures (twelve tests) and no homologous agglutinins (four tests) during the 8 weeks period. Three of these mice, on the other hand, had shown symptoms of disease and had recovered. They had passed bacilli for 4 to 6 weeks; blood cultures, positive for 4 weeks, had become negative and had remained so for 4 weeks. The serum of each had agglutinated the homologous Strain M. T. II in a dilution of 1:5,000 or 1:10,000.

These eleven mice, together with eleven controls of the same weight, were injected intraperitoneally with 1 cc. of an 18 hour broth culture of Strain M. T. II diluted 1:500.

The controls were all dead on the 6th day; no deaths had occurred among either group of survivors during a 30 day period of observation.

The results of this experiment indicate that not only are the "recovered" survivors which show specific agglutinins more resistant to Strain M. T. II injected intraperitoneally than are normal control mice, but also that the "resistant" survivors which have failed to respond either with evidences of infection or the production of agglutinins are equally refractory.

Response of the Chronically Infected Mice.

In addition to the "survivors" among the inoculated mice, which term as used in this paper applies to those which resisted as well as those which recovered from infection, there is a third group of mice which we designate as "chronic cases." These animals, few in number, which apparently had recovered, were reinoculated *per os* 8 weeks after the original inoculation. They succumbed not only to the second but to the first inoculation or to the combined action of the two infections. The determination in each instance depended upon the cultures recovered at autopsy. Apparently these animals

TABLE II.

Chronic Cases among Several Survivor Series.

Series No.	Survivor group.	Total No. of survivors.	No. of chronic cases.
1	M. T. I	20	3
2	" I	20	1
2	Enteritidis.	8	2
3	M. T. I	15	1

were able to cope with one infectious process but not with two. The original infection was indeed not mastered in part without damage, because the organs (liver and spleen) showed chronic lesions; hence they readily succumbed to a second inoculation which either revived the original infection or started a second equally fatal one (Table II).

CONCLUSIONS.

Mice which survive a preliminary dose *per os* of paratyphoid-enteritidis bacilli are more resistant to a second dose *per os* of a similar epidemic mouse typhoid strain than are mice which have received no preliminary culture.

The amount of this resistance is related to the pathogenicity of the preliminary strain more than to its antigenic similarity to the second strain.

Mice which survive a preliminary dose *per os* of paratyphoid-enteritidis bacilli are more resistant to a lethal dose of mercury bichloride *per os* than are mice which have not received the preliminary bacterial culture.

Mice which have resisted and mice which have recovered from a preliminary dose *per os* of paratyphoid-enteritidis bacilli are more resistant to a lethal intraperitoneal dose of an epidemic mouse typhoid strain than are mice which have not received the preliminary culture.

Mice in which the preliminary dose of paratyphoid-enteritidis bacilli has induced a chronic infection readily succumb to a second dose of such bacilli.

These findings indicate that the resistance mechanism of the host contains important non-specific factors which vary in degree with the individual mice.