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

VI. THE RELATIVE SUSCEPTIBILITY OF DIFFERENT STRAINS OF MICE TO PER OS INFECTION WITH THE TYPE II BACILLUS OF MOUSE TYPHOID (BACILLUS PESTIS CAVLÉ).

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During the past year, as part of the studies on experimental epidemiology being carried on at the Rockefeller Institute, routine monthly tests have been performed in this laboratory with five separate strains of mice in an effort to determine as accurately as possible their relative resistance to infection, by the gastrointestinal route, with the Type II bacillus of mouse typhoid (Bacillus pestis cavlē). The histories of these strains, so far as they are known to us, are as follows:

Bagg Mice (Albino).—About six mice were obtained from the Carnegie Experiment Station at Cold Spring Harbor in December, 1922, and were bred at the Rockefeller Institute. They were received at Cold Spring Harbor in 1922 from Dr. L. C. Strong, who had himself obtained them from Dr. H. J. Bagg in 1919. Dr. Bagg had first obtained these mice in 1913, and the strain as used by him arose from a single brother-sister mating. In our hands the strain has been strictly inbred, but not by brother-sister matings.

Hagedoorn Mice (Albino).—About ten mice were obtained from Dr. A. C. Hagedoorn of the University of California in June, 1922, and were bred at the Rockefeller Institute. Dr. Hagedoorn obtained them in 1916 from Dr. T. B. Robertson, who used them for experiments on growth. In Dr. Hagedoorn’s

hands they were inbred by brother-sister matings. In our hands, they have
been strictly inbred, but not by brother-sister matings.

_Lathrop Mice (Black, with an Occasional Brown)._—About twenty-five mice
were obtained from the Carnegie Experiment Station at Cold Spring Harbor in
December, 1922, and were also bred at the Rockefeller Institute. This stock
originally came to Cold Spring Harbor in 1920 from a breeder in Holyoke, Massa-
chusetts, successor to Miss Lathrop. It was of heterogeneous origin, having
arisen from a mixture of yellow, black, and brown mice. In our hands the strain
has been constantly inbred, though not by brother-sister mating, and as the
mice first received from Cold Spring Harbor were predominantly black, we have
eliminated as far as possible, by selective breeding, the brown individuals, though
these still arise in small numbers. Experiments on the effects of x-ray, performed
with mice of this strain, have been described by Bagg and Little.4

_Little Mice (Dilute Brown)._—About six mice were obtained from the Carnegie
Experiment Station at Cold Spring Harbor in December, 1922, and bred at the
Institute. They were brought to Cold Spring Harbor in 1919 by Dr. C. C.
Little of the University of Maine, who had inbred them since 1909 by brother-
sister mating. In our hands they have been strictly inbred but not by brother-
sister matings.

_Rockefeller Institute Mice (Albino)._—This stock originated at the Rockefeller
Institute in 1917 from a combination of two albino strains, and in 1919 two new
strains were added. Since that time our mice have been strictly inbred, without
the addition of new stock, not, however, by brother-sister mating. They are the
same mice that have been used in the work of Flexner,5 Amoss,6 and Webster,7 on
the epidemiology of mouse typhoid. Since our experience with these mice has been the most extensive, they have served as controls for the other strains
in each experiment.

The importance of using large numbers, in order to diminish error
from random sampling, has been kept constantly in mind, and whenever possible at least fifty mice of each strain were used in each of the
monthly tests, a total of 3,120 mice having been employed in the
course of the year. Intrastomachal inoculations were made according

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4 Little, C. C., and Bagg, H. J., _Am. J. Roentgenol._, 1923, x, 975; Bagg, H. J.,
and Little, C. C., _Am. J. Anat._, 1924, xxxiii, 119.

5 Flexner, S., and Amoss, H. L., _Proc. Nat. Acad. Sc._, 1921, vii, 319. Flexner,


9 Webster, L. T., _J. Exp. Med._, 1923, xxxvii, 21, 33, 231, 269, 781; xxxviii,
33, 45; 1924, xxxix, 129, 879; _Am. J. Hyg._, 1924, iv, 134.
to the technique described by Webster, each mouse receiving 0.5 cc.
of a 1:100 dilution made from an 18 hour broth culture of the mouse
typhoid bacillus, the dose received by each animal containing on an
average about 5,000,000 organisms.

EXPERIMENTAL.

The series of tests was begun in October, 1923. It was not possible
at the outset to obtain the full number desired of all the five strains of
mice, since some had bred too slowly, and in October and November
one or more strains were completely lacking. By December, however,
it was possible to obtain monthly a certain number of mice from each
strain and thereafter the full quota of fifty from each strain was used
almost every month. Occasionally this number was even exceeded.

The routine procedure in starting one of these tests was as follows:

The mice from all strains were assembled, each mouse in a separate battery
jar partly filled with shavings, on the day before inoculation. The next morn-
ing a dilution of 1:100 of an 18 hour broth culture of the mouse typhoid bacillus
was made in bouillon from which three dilution plates were poured for the pur-
pose of counting the organisms present. Each mouse then received bystomach
tube 0.5 cc. of this 1:100 dilution of the original culture, containing usually
about 5,000,000 mouse typhoid bacilli. All the animals were fed directly after
the administration of the infecting dose, with the usual daily diet of bread soaked
in pasteurized Grade B milk. At about 9 o'clock each morning the deaths for
the preceding 24 hours were recorded. For the first 7 months, autopsies were
performed on all dead mice and cultures made on green dye plates from the
spleen and feces, to determine the presence of the mouse typhoid bacillus. How-
ever, after 1,256 autopsies had been performed, of which only 17 failed to reveal
the presence of this bacillus in the spleen or feces or both, it was decided that
the labor involved in performing the autopsies was scarcely justified in view of
the small percentage of negative animals encountered, and they were conse-
quently discontinued. All experiments were allowed to run 8 weeks.

A comparison of Text-figs. 1 to 6 will give a clear indication of the
relative susceptibility of these five strains of mice to per os infection
with the bacillus of mouse typhoid Type II (Bacillus pestis cavia). Since the Rockefeller Institute mice, being the ones most intensively
studied, have served as controls for the other four strains, we shall
use them throughout as a common basis for comparison with these
strains.
Three properties may be especially mentioned in connection with the curves of the Institute mice, (a) their height, (b) their pitch, and (c) their scatter. Discussion of the first and second properties will be deferred until after the question of scatter in the curves of all strains has been considered.

Text-fig. 1 shows the twelve monthly curves for the Institute mice, together with the standard control curve previously constructed for this strain by Webster. The scatter in these curves is seen to be considerable, the highest and lowest curves being separated by a difference of 50 per cent at their termination. It must be noted, however, that this scatter, while marked, is not haphazard, the curves falling into three fairly clear-cut groups—high in the spring, low in the summer, and midway between the two extremes during the fall and winter. This phase of the work is more fully dealt with elsewhere.

Webster, p. 231.

Text-Fig. 2.
Text-Fig. 3.
Fig. 7. Five day periods.

Legend:
- Black rice
- Little rice
- Rockerbell Institute rice
Text-figs. 2 to 5 show the monthly curves for the Bagg, Hagedoorn, Lathrop, and Little mice respectively. The scatter is seen to vary considerably between the different strains and between each strain and the Institute controls. If we take as an index of the scatter in the curves for each strain the difference between the lowest and the highest mortality recorded for that strain, the following figures are obtained (Table I).

Table I brings out the differences in the constancy of response of the different strains to the inoculation. As is shown also in Text-fig. 4, the Lathrop mice were the most constant in their response throughout the year, the lowest curve recorded being separated from the highest by a margin of only 30 per cent. The Hagedoorn mice, on the other hand, showed so wide a variation (68 per cent between the lowest and the highest curves) as to render somewhat unsatisfactory any determination of the average susceptibility of this strain to infection with the bacillus of mouse typhoid. This may be clearly seen from Text-fig. 3, in which are given the curves for the Hagedoorn strain during the whole year. The Institute and Bagg mice most closely resemble each other in the matter of the scattering of their curves as well as in the tendency of their curves to fall into three fairly clear-cut groups—high in spring, low in summer, and medium in fall and winter. The Little dilute brown mice incline rather toward the Lathrops in the constancy of their response to the inoculation.

The relative height and pitch of the curves for the different strains may best be studied in Text-fig. 6, in which are shown average curves constructed for each strain from all the curves for that strain in the course of the year, together with the standard control curve previously

<table>
<thead>
<tr>
<th>Strain</th>
<th>Difference separating lowest and highest mortality rates. (per cent)</th>
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</thead>
<tbody>
<tr>
<td>Lathrop mice</td>
<td>30</td>
</tr>
<tr>
<td>Little &quot;</td>
<td>34</td>
</tr>
<tr>
<td>Institute &quot;</td>
<td>50</td>
</tr>
<tr>
<td>Bagg &quot;</td>
<td>55</td>
</tr>
<tr>
<td>Hagedoorn &quot;</td>
<td>68</td>
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constructed by Webster for the Institute mice. It will be seen from these curves that in general the pitch of the curves of the Bagg, Hagedoorn, and Institute mice is similar, while the curves of the Lathrop and Little mice are distinctly more abrupt in their rise. As to the relative susceptibility of the different strains, as shown by the final mortality figures from the average curves given in Text-fig. 6, the Littles are the least resistant, the Baggs the most resistant, while the Lathrop, Hagedoorn, and Rockefeller Institute mice fall in decreasing order of susceptibility between the two extremes.

In Text-fig. 7 are shown standard control curves constructed for each of the five strains of mice from the foregoing charts, together with Webster's standard control curve for the Institute mice. These curves have been constructed to bring out especially the variation in the pitch of the curves for the several strains, as well as the difference in the final mortality rate of all the strains. It is interesting to see how closely the curve for the Institute mice in this test approximates that previously found by Webster for this strain.

Although the differences in the final mortality figures for the various strains of mice, as shown by the average curves, given in Text-fig. 6, are not great, we feel that they are significant since practically no deaths from intercurrent disease, with a consequent natural selection of the strongest, had occurred in any of the strains before inoculation and since the same relative differences in susceptibility to mouse typhoid in general predominated in the separate monthly experiments.

DISCUSSION.

The only recent work known to us which seems to parallel our own closely is that of Wright and Lewis on tuberculosis in the guinea pig. These authors worked with several distinct families of guinea pigs inbred through many generations, and were able to distinguish clear-cut differences in the relative resistance of these families to tuberculous infection. Moreover, they showed that individuals of both sexes in the most resistant family were capable of transmitting the factor for resistance to their offspring, the progeny from such a mating prov-

ing to be superior in resistance to the more resistant parent. Variations in the susceptibility of different strains of mice to transplantable mouse tumors have been observed by many authors, most recently perhaps by Strong, who records for a great variety of strains of mice receiving transplants of a mouse sarcoma that there was practically 100 per cent susceptibility in normal individuals, except in the case of wild mice, in which there was approximately 80 per cent susceptibility. Webster has recorded dissimilarity in the susceptibility of three strains of mice to infection with the bacillus of mouse typhoid used in this laboratory. Twenty-six mice from a Pennsylvania breeder and twenty-eight mice from a New Jersey breeder, together with twenty control mice of the Rockefeller Institute strain, were inoculated per os in the usual way with a suspension of M. T. II. The resulting mortality curves showed that the New Jersey mice resembled the Institute mice in their susceptibility to the infection, while the Pennsylvania mice were far more resistant.

The interest in these observations lies not alone in the mere demonstration that variations in susceptibility to injurious agents do exist among different strains of the same species, receiving the same food and subjected to the same living conditions, but in the bearing that such an observation must have on the correlation of experimental work done in any given biological field with totally unrelated strains of the same species of animal. It emphasizes anew the importance of knowing thoroughly the normal range of variation in susceptibility for any given strain of experimental animal, before attempting to draw a sharp line between the normal and the abnormal response to an injurious agent.

SUMMARY.

Five separate strains of mice have been tested for their relative susceptibility to per os infection with the Type II bacillus of mouse typhoid (Bacillus pestis caviae), more than 500 individuals of each strain having been employed in the course of 12 months. Clear-cut differences in the susceptibility of these strains to the infection have been shown to exist.