STUDIES ON A PARATYPHOID INFECTION IN
GUINEA PIGS.

IV. THE COURSE OF A SECOND TYPE OF SALMONELLA INFECTION
NATURALLY APPEARING IN THE ENDEMIC STAGE.

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The appearance of a second type of Salmonella in a guinea pig popula-
tion during the endemic stage of a spontaneous outbreak of para-
typhoid disease was reported in an earlier paper (1). The earliest cases
of the first infection occurred in the summer of 1924. The associated
organism became rapidly disseminated throughout the population;
particularly through the breeding stock, and for a period of 8 weeks a
moderately high specific death rate ensued. The epidemic subse-
quently declined to an endemic level which has persisted up to the
present. The course of the infection was followed by a bacteriological
examination of all animals that died in the population under observa-
tion.

During July and August, 1926, several strains of a second type of
Salmonella were isolated from fatal cases. Subsequently there was a
slow but general spread of the second organism. These natural
occurrences, among the guinea pigs, afforded an opportunity for study-
ing the course of a second infection in an animal population which had
been exposed for a considerable period of time to a related but sero-
logically distinct type of organism.

A somewhat similar condition in a mouse population was described by Lynch
(2). A spontaneous outbreak of paratyphoid infection, of 2 years duration, was
followed by a second outbreak caused by a serologically different type of Sal-
monella. The two types of organism were designated Mouse Typhoid I and
II. Some 18 months prior to the second outbreak half of the population was
vaccinated with a strain of Mouse Typhoid I. The specific death rate for the
ensuing 7 months was low. There followed a period of fluctuation, with the
dearth rate in general on a higher level, which terminated in a sharp rise. At
time this the second type of organism was isolated.

Later, Pritchett (3) encountered a reverse replacement during the course of an
artificially induced mouse epidemic. This was started by placing normal individ-
uals in contact with mice which had received per os injection of Mouse Typhoid
II. After an interval of several months the original Mouse Typhoid II strain
was almost entirely replaced by Mouse Typhoid I. The latter type was known
to be mildly endemic in the breeding stock and was thought to have been intro-
duced into the particular population by the chance addition of fecal carriers.

The recovery of a second type of Salmonella from mice under similar circum-
stances was reported by Topley (4). An epidemic was started by feeding broth
cultures of Bacillus Gaerlner. Some of the mice which died during the early
stage of the epidemic yielded pure cultures of a different Salmonella type. The
organism showed a close serological relationship to Bacillus suipedefter (mutton).
In a later stage of the epidemic both types were frequently isolated from the
same animal. It should be added that the earlier type corresponded with Mouse
and Guinea Pig Type I, and the second organism with Type II.

It may be pertinent to refer, in passing, to the relationship of the mouse and
guinea pig types of organism and to their position within the Salmonella group.
It was previously shown that Mouse Typhoid I and Guinea Pig Paratyphoid I
of our epidemic were intimately related if not identical strains of the same Salmo-
nella type (5). A similar relationship was established for Mouse Typhoid II
and Guinea Pig Paratyphoid II. That the latter are an aerlrycke form of Bacillus
paratyphi likewise appears established (1). The relationship of the former, the
initial strain, to a distinct Salmonella type is less definitely indicated. Amoss
and Haselbauer (6) have related Mouse Typhoid I to Bacillus enteridis. For,
of three Bacillus enteridis strains, the first was agglutinated to the titer limit
by a Mouse Typhoid I antiserum, the second to less than 50 per cent and the
third not at all. Upon absorption the first strain reduced the agglutinin content
50 per cent, the second 60 per cent and the third anomalously 50 per cent. A
similar relationship, on the basis of direct and reciprocal absorption tests with
specific agglutinating serums, was indicated by Sakai (7). The absorption
tests, however, revealed a considerable variation in the absorptive capacity of
the strains employed. Nelson and Smith (5) reported only a remote relation-
ship between the two rodent strains and two strains of Bacillus enteritis. A
specific antisera against which agglutinated the former in a dilution of 1:51,200 agglu-
tinated the latter in a dilution of 1:800 and 1:400 respectively. Absorption tests
were not run. There appears to be nearly as wide a variation between different
strains of Bacillus enteritis and between the same and the Type I rodent strains
as there is between the latter and the Type II rodent strains.

A general discussion of the rodent forms of the paratyphoid-enteritis group
is given by Jordan (8). He divided them into two main types, one related to
Bacillus enteritis, the other to an aerlrycke form of human Bacillus paratyphosus.
In the several papers of the present series the two organisms under discussion have been consistently designated as *Bacillus paratyphi* of a given type, I or II. This broad designation has been adopted as a matter of convenience pending more satisfactory evidence for the species relationship of the initial form.

The course of the Type II infection is considered first with reference to its percentage mortality within the population at large and secondly with reference to its cage to cage spread among the breeding stock. The former is computed from the specific deaths occurring within the total estimated population by months. The figures are only approximations inasmuch as the general population is not stable because of the removal of individuals for experimental purposes. Moreover, it is difficult to classify specific deaths as active cases or carriers which died from some other cause. This applies particularly to the unweaned guinea pigs among which gross lesions may not be apparent. Hence, the specific deaths undoubtedly include a few cases which should be classed as carriers. It is believed, however, that the percentage mortality indicates roughly the progress of the infection. The population by months, the total deaths, the specific deaths and the per-

### TABLE I.

Population, Total Deaths, Deaths from Paratyphoid and Percentage Mortality from Paratyphoid, July, 1925, through June, 1926.

<table>
<thead>
<tr>
<th>Month</th>
<th>Population</th>
<th>Total deaths</th>
<th>Deaths from paratyphoid</th>
<th>Mortality from paratyphoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>401</td>
<td>51</td>
<td>10</td>
<td>2.49</td>
</tr>
<tr>
<td>August</td>
<td>460</td>
<td>8</td>
<td>5</td>
<td>1.08</td>
</tr>
<tr>
<td>September</td>
<td>438</td>
<td>27</td>
<td>16</td>
<td>3.65</td>
</tr>
<tr>
<td>October</td>
<td>500</td>
<td>7</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>November</td>
<td>540</td>
<td>9</td>
<td>3</td>
<td>0.55</td>
</tr>
<tr>
<td>December</td>
<td>581</td>
<td>15</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>January</td>
<td>470</td>
<td>22</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>February</td>
<td>455</td>
<td>31</td>
<td>11</td>
<td>2.41</td>
</tr>
<tr>
<td>March</td>
<td>493</td>
<td>24</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>April</td>
<td>491</td>
<td>17</td>
<td>2</td>
<td>0.40</td>
</tr>
<tr>
<td>May</td>
<td>502</td>
<td>18</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>June</td>
<td>478</td>
<td>33</td>
<td>7</td>
<td>1.46</td>
</tr>
</tbody>
</table>
Percentage mortality for the period between July, 1925, and July, 1926, are given in Table I.

The data pertaining to the combined infections for the year beginning with July, 1926, when the second type first appeared, are given in Table II.

The percentage mortality for the two periods is presented in graphic form in Fig. 1.

The percentage mortality for the Type I infection displayed a considerable monthly fluctuation during the 2 endemic years beginning

### Table II.

<table>
<thead>
<tr>
<th>Month</th>
<th>Population</th>
<th>Total deaths</th>
<th>Deaths from Paratyphoid Type I</th>
<th>Mortality Type I</th>
<th>Deaths from Paratyphoid Type II</th>
<th>Mortality Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>571</td>
<td>14</td>
<td>7</td>
<td>1.22</td>
<td>1</td>
<td>0.17</td>
</tr>
<tr>
<td>August</td>
<td>468</td>
<td>48</td>
<td>30</td>
<td>6.41</td>
<td>3</td>
<td>0.64</td>
</tr>
<tr>
<td>September</td>
<td>382</td>
<td>32</td>
<td>17</td>
<td>4.45</td>
<td>3</td>
<td>0.78</td>
</tr>
<tr>
<td>October</td>
<td>463</td>
<td>11</td>
<td>0</td>
<td>0.00</td>
<td>5</td>
<td>1.07</td>
</tr>
<tr>
<td>November</td>
<td>432</td>
<td>13</td>
<td>2</td>
<td>0.46</td>
<td>5</td>
<td>1.15</td>
</tr>
<tr>
<td>December</td>
<td>417</td>
<td>64</td>
<td>4</td>
<td>0.95</td>
<td>22</td>
<td>5.27</td>
</tr>
<tr>
<td>January</td>
<td>436</td>
<td>40</td>
<td>3</td>
<td>0.68</td>
<td>13</td>
<td>2.98</td>
</tr>
<tr>
<td>February</td>
<td>424</td>
<td>35</td>
<td>1</td>
<td>0.23</td>
<td>11</td>
<td>2.59</td>
</tr>
<tr>
<td>March</td>
<td>406</td>
<td>49</td>
<td>1</td>
<td>0.24</td>
<td>24</td>
<td>5.91</td>
</tr>
<tr>
<td>April</td>
<td>454</td>
<td>57</td>
<td>5</td>
<td>1.10</td>
<td>18</td>
<td>3.96</td>
</tr>
<tr>
<td>May</td>
<td>407</td>
<td>42</td>
<td>1</td>
<td>0.24</td>
<td>11</td>
<td>2.70</td>
</tr>
<tr>
<td>June</td>
<td>449</td>
<td>35</td>
<td>0</td>
<td>0.00</td>
<td>5</td>
<td>1.11</td>
</tr>
</tbody>
</table>

July, 1925, and July, 1926, respectively. From the curve it may be seen that the highest rates, with a single exception, occurred during warm weather, namely: in July and September of 1925 and in February, June and Augusts of 1926. In general the infection showed a wave-like progress throughout the entire endemic stage. After the high rate of August, 1926, the percentage mortality declined and remained on a low level thereafter. The Type II infection appeared just prior to this peak rate of the initial type, in July, 1926. A relatively quiescent period of 5 months duration followed. During this time there was a slight but steady increase in the percentage mortality. The
period terminated in a sharp rise during December, 1926. There was a decline the 2 succeeding months with a second rise to a slightly higher level in March, 1927. Both of the peaks, it will be noticed, occurred during cold weather. The percentage mortality again declined and continued to fall through June at which time accurate observations were discontinued.

The breeding stock was the only group within the population which was sufficiently stable for a study of the cage spread of the Type II infection. The breeders are segregated in cages containing either four or five sows and one boar. Since the appearance of the second form of paratyphoid the sows have been removed to individual isolation units shortly before parturition. The young are born here and are left with their dams for a period of about 2 weeks when they are weaned. At this time the young are transferred to the stock, for general laboratory use, and the sows are returned to their original breeding cages. Until the suckling period is over the majority of young guinea pigs are subjected to direct contact only with their dams or within the litter. Occasionally a premature birth occurring in the

Fig. 1. Percentage mortality from Type I infection July, 1925, to June, 1927, and from Type II infection July, 1926, to June, 1927.
### TABLE III.

*Spread of Type II Infection in the Breeding Cages July, 1926, to June, 1927.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>43.</td>
<td>46.</td>
<td>52.</td>
<td>45. 50. 53.</td>
<td>47. May 27 D.</td>
<td></td>
</tr>
</tbody>
</table>

C., carrier; D., fatal case.
breeding cage brings them in contact with adult animals other than their dam.

Fatal cases of paratyphoid among the unweaned guinea pigs may be attributed in most cases to transfer of the organism from the dam. The transfer may be direct during intra-uterine life or indirect through the ingestion of contaminated feces or milk. Frequently the sow presents a normal appearance. These cases have been common with both types of infection. Formerly the sows were killed and autopsied. Throughout the second infection, however, most of them have been returned to the breeding cages and kept under observation. All of them were designated carriers. In addition, fatal cases have occurred from time to time among the sows, either in the breeding cages or in the isolation units. A cage record of the so called carriers and fatal cases has been kept since the onset of the second infection. A presentation of the data is given in Table III. Each square represents a cage in the order of its arrangement in the breeding room. The notations refer to individual sows. There are three sections of cages, as indicated.

Within a year after the first death from the second infection either fatal cases or carriers were detected in 32 out of 48 cages. The inmates of the remaining cages may have escaped infection entirely. It is more probable, however, that the repeated fecal examination of individuals would have revealed a small proportion of carriers. It is unlikely that there was any carriage of the infection from cage to cage except through the agency of the handlers.

The cages are of metal with solid tops and bottoms and are placed in rows on iron pipe racks. The rows are separated from each other by a wide air space. The cage arrangement precludes any possibility of direct leakage. In some instances infection may have been introduced through the addition of young sows to replace older ones removed by death or because of old age. These sows are drawn from the general stock which always contains a small number of carriers. With the exception of Cage 9, however, replacements have not been added to those cages with a high incidence of infection. It may be said that the breeding stock is cared for by two handlers. The feeding and cleaning in cages from 1 through 47 is always done by the same helper. Similarly, Cages 48 through 54 and in addition the isolation units are attended to by one man.

The spread of infection from cage to cage and within individual cages was gradual. The irregularity which is apparent with the cage
spread may be associated with the nature of the particular group. The breeding stock is composed largely of sows which normally breed and bear young four times a year. During pregnancy it is to be expected that the sow becomes more susceptible to bacterial invasion than during the postparturient periods. The ingestion of a normally tolerated dose of culture during the pregnant state might result in active disease. Or, in the absence of actual disease the lodgment of organisms in the uterus might result in a carrier state with subsequent infection of the young. Again, the ingestion of a sublethal dose of culture during a resting period with localization of the organism in the spleen, liver or intestinal tract might be followed by active disease during a subsequent pregnancy. It is suggested that the continual shifting of the sows together with periodic changes in the susceptibility of individuals were important factors in determining the cage spread of the organism.

In this connection it may be noted that no boars succumbed throughout the course of the second infection. It is to be expected that the number of fatal cases would be less than among the sows since the latter outnumber them five to one. The chances of the boars acquiring infection in the breeding cages are, however, somewhat greater since they remain there continuously except for a short interval during the summer. The sows, on the other hand, are periodically removed to isolation units while the young are suckling. It may be that the more vigorous nature of the boar is accompanied by a more active native resistance. It is believed, rather, that the weakening effect of successive pregnancies on the sow is accountable for the seeming difference in susceptibility. Among younger animals which have not been bred the incidence of fatal cases has shown no consistent difference.

DISCUSSION.

Two years after the initial outbreak of paratyphoid the second infection appeared in the population. Following a quiescent period of 5 months the mortality from the latter type increased abruptly, declined slightly and again increased with a second decline. Both of these waves occurred during cold weather, the first in December, the second in March. Throughout this period the mortality resulting from the initial type was consistently low. The course of the second
infection was somewhat different from that of the initial one. The onset of the latter was abrupt, while that of the former was gradual. The mortality was greater with the initial disease and there was a corresponding increase in the number of fatal cases among adult animals. Apparently the population after long continued exposure to the first infection was better able to resist the second.

It seems clear, however, that the factors instrumental in holding the Type I infection to a low level were not equally active in combating the second type. A number of possible factors were discussed in a preceding paper (9). These will be reconsidered from the standpoint of their bearing on the spread of the second infection. It may be said that prior to the initial outbreak the guinea pig population had been free from specific disease, of epidemic proportions, for a period of 6 years. It was suggested that during this period there was an accumulation of individuals of low resistance. The natural removal of these animals during the early part of the epidemic left a stock better able to resist invasion and among which the infection ultimately declined to a low level. It may be supposed that natural selection continued to operate throughout the endemic stage tending to maintain a stock of normal resistance. It might be argued, however, that the less severe environmental conditions imposed upon the population during this long period would favor the reappearance of animals of low resistance. The unequal course of the two infections is, in part, opposed to such a view. Moreover, the reappearance of a weak strain of guinea pig should tend to increase the number of Type I cases. Actually these declined. It is suggested that the difference in the onset of the two infections may be attributed to the more uniform nature of the population, as regards natural resistance, which obtained at the appearance of the second type. The latter enjoyed a long free period which was not observed with the former.

With due regard to the significance of natural resistance and selection in checking the initial infection and delaying the second it is apparent that some additional factor or factors must have been operative. Fluctuation in the killing power and the invasiveness of the parasite are naturally suggested. As previously noted the question of a possible alteration in these characters with the Type I organism was not satisfactorily answered from experimental evidence (9). It was
tentatively suggested, however, that the organism was gradually adjusted to the population on a lowered level of virulence. Likewise, the possibility of similar differences between the second type and the initial one after its long sojourn in the guinea pig population has not been settled. Intraperitoneal injection of graded amounts of the two types into guinea pigs and mice failed to demonstrate any consistent variation. The inaccuracies inherent in the method employed are regarded as too great to warrant any final statement concerning the comparative invasive power of the two types.

Specific immunity was earlier considered as a factor in the decline of the initial infection. Its importance in the population at large was minimized but it was not denied that the resistance of certain groups might have been raised. It is certain that conditions were favorable for immunization within the breeding group during the endemic stage of the earlier infection. The Type I organism was widely disseminated throughout the cages of the unit and the ingestion of small doses of culture by individual sows must have commonly occurred. The postmortem examination of so-called carriers, sows whose litters had shown one or more cases of paratyphoid, gave indirect evidence of an acquired immunity. In some instances healed or inactive lesions were found in the abdominal organs. The blood serum frequently agglutinated the homologous organism. It may be said, too, that a small number of sows examined shortly after the appearance of the second infection showed a much higher agglutinin titer against the initial organism than against the second type. There is a suggestion that natural immunization with the earlier form afforded a protection which was largely type-specific and accountable in part for the subsequent dominance of the second infection.

**SUMMARY.**

The course is considered of a second type of Salmonella infection naturally appearing in a guinea pig population during the endemic stage of an earlier outbreak. After a quiescent period of 5 months the percentage mortality increased abruptly; fluctuated, with a second rise during the 9th month; and then declined. With the exception of a high rate during the 2nd month the percentage mortality from the initial infection tended to remain on a low level.
The spread of infection in the cages of the breeding stock is recorded from the time of the first fatal case. There was a slow but general dissemination of the second organism through the group. Fatal cases were confined solely to the sows. It is suggested that a lowered individual resistance occurring during pregnancy might be associated with the irregular cage spread and with the apparent difference in susceptibility of the sexes.

Natural host resistance, virulence of the organism and acquired host resistance are discussed from the standpoint of their bearing on the unequal distribution of deaths from the two infections.

BIBLIOGRAPHY.