BACTERIOPHAGE ISOLATED FROM THE COMMON HOUSE FLY (MUSCA DOMESTICA).

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INTRODUCTION.

It is known, through the work of others, that the house fly, when fed cultures of various pathogenic bacteria, harbors them for a longer or shorter time but that they finally disappear entirely. Various explanations of this elimination have been offered or are suggested by the facts presented. Wollman (1) regarded the process as entirely mechanical. In the case presented by Jones and Little (2) an actual rapid sterilization was observed in which more subtle factors are obviously concerned.

Glaser and Sanderson, in unpublished observations from this laboratory, found in house flies bacteriophage active against *Staphylococcus muscae*, a microorganism which gives rise to a definite disease of this insect (Glaser (3)). This observation offered the suggestion that bacteriophage might be found more widely active and might be concerned in the natural process of removal of foreign bacteria when these are fed to the fly. Duncan (4) has recently studied the same problem and discovered a bactericidal principle active against many species of bacteria in the gastrointestinal tracts of various insects, the fly among them. The references above generally commented on are more specifically as follows.

Graham-Smith (5) found that, with flies fed on cultures, *B. prodigiosus* (page 96) could survive in the crop only about 17 days. *B. enteritidis* Gärtnner (page 146) could be recovered from the gut contents up to the 7th day. *B. typhosus* (page 130) up to the 6th day, and *V. cholerae* (page 173) for 2 days after ingestion by the insect. Manson-Bahr (6) found that *B. dysenteriae* Shiga, from a culture, survived in the gut contents of flies 4 days. Naturally infected flies, he thought, carried the organ-
isms for a much longer time. Wollman (1) observed that flies fed on cultures of
*B. typhosus* and *B. dysenteriae* and then transferred daily to aseptic surroundings
became free of the specific bacteria in from 8 to 10 days. Jones and Little (2),
in conducting an investigation on infectious ophthalmia of cattle, made the ob-
servation that the causative diplobacillus was not capable of surviving as long as
5 minutes in the gastrointestinal tract of the house fly. Duncan (4) isolated a
bactericidal principle from the alimentary tracts of a number of insects and arach-
nids. He included *Musca domestica* in the series of insects found by him to con-
tain this bactericidal principle in their gastrointestinal contents. He considered
that this principle might be a bacteriophage but was forced to discard the possi-
bility as the substance he described exhibited none of the essential properties of
this principle.

The experimental work recorded in the following pages was con-
ducted with the purpose of determining any bactericidal action which
might pertain to the house fly, and its result has been to identify
bacteriophage very active against a number of bacteria, as well as an
inhibitory substance, not identified with bacteriophage, and active still
more widely.

**EXPERIMENTAL.**

1000 house flies were obtained from the vicinity of a hog lot, etherized and
ground in a mortar with physiological salt solution in the proportion of 10 flies
per cc. This mixture was filtered first through paper, then through a Berkefeld N
filter. A clear, dark, straw-colored fluid was obtained which gave no bacterial
growth when incubated on the ordinary culture media. This fluid was kept at
refrigerator temperature and was used as the starting point for most of the ex-
perimental work here reported.

Two organisms were used at the outset to determine whether or not the fly
filtrate just described contained substances which were either bactericidal or cap-
able of inhibiting bacterial growth. One of these was a non-mucoid strain of
*B. coli* 223 of calf origin (7), and the other *B. paratyphi Type I* of guinea pig origin
(8).

Plain bouillon (pH 7.5), 5 cc. per tube, was used as the culture medium and the
fly filtrate was added to the bouillon before inoculation. 1, 0.5, 0.25, and 0.1 cc.
amounts of fly filtrate were added to four separate tubes in each series and one
tube in each was kept as a control. One loopful of a 24 hour bouillon culture was
used in making each inoculation. The tubes were read at 6 and 24 hours with the
results given in Table I.

This experiment made it evident that the fly filtrate contained some
substance which was inhibitory or bactericidal for *B. coli* (calf) but
which exerted no such action upon \textit{B. paratyphi} Type I (guinea pig). This apparent specificity suggested the possibility that the factor might be bacteriophage. Each tube of the two sets of cultures was therefore passed through a Berkefeld N filter and the experiments were repeated using the bouillon filtrate. The dilutions were made this time from tube to tube increasing by successive powers of ten. A single pipette was used for each series. The results are given in Table II. After 48 hours incubation, each tube of these two sets of cultures was passed through a Berkefeld N filter and the experiment repeated with results similar to the above.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Dilution} & \textbf{B. paratyphi Type I (guinea pig)} & \textbf{B. coli (calf)} \\
\hline
\hline
$10^{-1}$ & C.* & M.T. & M.T. & C. & V.S.T. & S.T.; agglutinated \\
$10^{-2}$ & " & " & " & " & " & " \\
$10^{-3}$ & " & " & " & " & " & " \\
$10^{-5}$ & " & " & " & " & " & " \\
$10^{-6}$ & M.T. & " & " & " & " & " \\
$10^{-7}$ & " & " & S.T. & " & " & " \\
$10^{-8}$ & " & " & " & " & " & " \\
$10^{-9}$ & " & " & " & " & " & " \\
\hline
\textbf{Control} & T. & T. & M.T. & T. & T. & \\
\hline
\end{tabular}
\caption{TABLE II.}
\end{table}

* C. = clear; V.S.T., very slightly turbid; S.T., slightly turbid; M.T., moderately turbid; T., turbid.
<table>
<thead>
<tr>
<th>Dilution</th>
<th>B. typhosus (Rawlings)</th>
<th>B. paratyphi Type I (guinea pig)</th>
<th>B. coli (calf)</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 hrs.</td>
<td>24 hrs.</td>
<td>48 hrs.</td>
<td>6 hrs.</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>C.*</td>
<td>C.; Sed.</td>
<td>C.; Sed.</td>
<td>C.</td>
</tr>
</tbody>
</table>

* C. = clear; S.T., slightly turbid; M.T., moderately turbid; T., turbid; Sed., sedimentary growth.
Plates were next made and these showed typical plaque formation in both series. Individual colonies on solid media showed notched and stellate forms. The inhibition of growth in series by dilutions of the filtrates and the formation of plaques definitely characterizes the action as that of bacteriophage.

The activity of the original fly filtrate against a wider range of bacteria was then tested. Preliminary determinations were carried out using a single pipette for each set of dilutions, while the experiments to determine the definite end-points of activity, those given in Tables III and IV, were done using a fresh pipette for each dilution.

Table IV.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Highest dilution showing inhibition of growth at 6 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>1:1000 (complete)</td>
</tr>
<tr>
<td>Streptococcus C 54</td>
<td>1:100 &quot;</td>
</tr>
<tr>
<td>&quot; 744</td>
<td>1:10 (complete), 1:10,000 (partial)</td>
</tr>
<tr>
<td>&quot; C 55</td>
<td>1:10,000 (partial)</td>
</tr>
<tr>
<td>Pneumococcus I</td>
<td>1:10 (complete), 1:10,000 (partial)</td>
</tr>
<tr>
<td>&quot; II</td>
<td>1:10,000 (complete)</td>
</tr>
<tr>
<td>Bacillus of swine plague</td>
<td>1:1000 (partial)</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>No inhibition of growth</td>
</tr>
<tr>
<td>Friedländer's bacillus</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>Bacillus proteus</td>
<td>&quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

Whenever evidence of inhibition of growth was obtained filtrates were made and tested in order to develop any potential transmission in series. Inhibition, displayed in successive filtrates, was thus uncovered in the case of B. typhosus (Rawlings) and Staphylococcus muscae in addition to the two species previously considered. The complete record, as finally determined for the four species, is shown in Table III.

The original filtrate inhibited the growth of certain other species in various dilutions but filtrates in these cases had no inhibitory action. The end-point of activity for these bacteria and the species for which no inhibitory action could be demonstrated are contained in Table IV.

The bacteriophage isolated was very active against Staphylococcus muscae, giving complete inhibition of growth to a dilution of $10^{-9}$. The lysis of this organism was complete and permanent and no secondary growth ever occurred in the tubes that were completely lysed at 48
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hours. It was fairly active against *B. typhosus* (Rawlings), giving lysis to a dilution of $10^{-8}$, but in the case of this organism all lysed tubes showed some secondary growth in the form of a sediment. Against *B. coli* (calf) it was also fairly active, giving evidence of lysis in a dilution of $10^{-9}$. With this organism a secondary growth occurred in 24 hours also. The lytic principle active against *B. paratyphi* Type I (guinea pig) was a relatively weak one and a secondary growth always occurred. Lysis in this case seemed to progress for at least 48 hours but complete clearing of the cultures never resulted.

The nature of the inhibitory action which differs so definitely from bacteriophage is of considerable interest. One possibility seemed to be that it might be related as a precursor or "building stone" for the bacteriophage. To test this, one of the species susceptible to inhibition, but for which bacteriophage was not developed, was fed to flies for a period. These flies were extracted as before and the filtered extract was tested for the characteristic transmissible lysis for the bacterium fed. The result was negative. The detailed experiment follows.

Streptococcus C 55, a non-hemolytic strain originating in bovine mastitis (9), which was inhibited but not susceptible to lysis by the bacteriophage in the original fly filtrate, was used. About 200 flies were placed in a large glass jar and fed bouillon cultures of Streptococcus C 55 daily for 8 days. At the end of this time the flies were etherized and ground in a mortar with physiological salt solution using 5 flies per cc. This material was passed through a Berkefeld N filter for sterilization and, from the filtrate, attempts were made to obtain bacteriophage capable of lysing cultures of Streptococcus C 55. These attempts were all unsuccessful and no bacteriophage active against this organism could be obtained.

DISCUSSION.

Physiological salt solution extracts of the house fly present bacteriolytic or inhibitory phenomena of two types which may have a bearing on the inability of certain pathogenic microorganisms to exist for more than a short period of time in the gastrointestinal tract of the insect. Bacteriophage active against at least four species of bacteria was found in a salt solution extract of flies; and another substance, growth-inhibiting, but not showing the essential characteristics of bacteriophage, was also present. This was active against four additional species.
In so far as relates to the bacteriophage, it is very likely that the fly filtrate contains a mixture of lytic principles, rather than a single bacteriophage capable of causing lysis of the four species. Thus neither the broth filtrate active against *B. coli* (calf) nor the one active against *B. paratyphi* Type I (guinea pig) had any lytic action on *Staphylococcus muscae*. The filtrate active against *B. paratyphi* Type I (guinea pig) was also strongly lytic for *B. typhosus* (Rawlings). The filtrate active against *B. coli* (calf) was somewhat active for *B. typhosus* (Rawlings), giving lysis to a dilution of $10^{-4}$. The filtrate active against *Staphylococcus muscae* failed completely to cause lysis of *B. typhosus* (Rawlings). The filtrate active against *B. coli* (calf) caused no lysis of *B. paratyphi* Type I (guinea pig). No effort was made to adapt a lytic principle, active against one organism, to another of the group.

Because of the possibility that the non-bacteriophagic growth-inhibiting substance might be a precursor to true bacteriophage, the feeding experiment described was planned. No bacteriophage against the streptococcus fed could be obtained in this way. It is very likely that this non-bacteriophagic growth-inhibiting factor is the same as that observed by Duncan (4).

Within the range of the experimental observations, the lytic principle present in the fly filtrate may have been obtained either from the external parts of the fly or from its digestive tract. A much more elaborate technical procedure would be required to make this discrimination.

**SUMMARY AND CONCLUSIONS.**

1. Bacteriophage active against four species of bacteria was found in a salt solution extract of house flies.

2. A growth-inhibiting principle, not bacteriophage, active against four other species of bacteria was found to be present in the same extract.

3. An attempt to secure streptococcus bacteriophage by feeding to flies a streptococcus susceptible to the inhibitor but not to the bacteriophage of this filtrate was unsuccessful, indicating that the two activities are quite unrelated.