STUDIES ON AN UNCOMPLICATED CORYZA OF THE DOMESTIC FOWL

II. THE RELATION OF THE "BACILLARY" CORYZA TO THAT PRODUCED BY EXUDATE

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The isolation of a Gram-negative bacillus from the nasal exudate of fowl experimentally infected with an uncomplicated coryza is reported in the preceding paper. The introduction of this organism into the palatine cleft of normal fowl was regularly followed by an inflammation of the upper air passages with a discharge from the nares. It was of importance to determine whether any infectious agent other than the specific bacillus is etiologically associated with the disease. The observation that the coryza produced by injection of the bacillus regularly ran a shorter course than that produced by exudate had suggested that a second agent might be involved. The results of protection tests carried out to test this possibility are here reported.

EXPERIMENTAL

Seven birds that had recovered from the coryza produced by the 1931 strain of the specific bacillus were subsequently reinjected with cultures of the same strain. It may be noted that the coryza studied in 1931 (Coryza I) was characterized by a relatively short course which averaged 5 days in the case of the "bacillary" form and 11 days in that produced by exudate. The interval between the disappearance of symptoms and the second injection of the organism varied from bird to bird, with extremes of 14 and 26 days. The reinfected fowl were examined daily during a period of 2 weeks and 6 of them remained normal throughout that interval. At autopsy, there was no evidence of an inflammation of the nasal mucosa. After an incubation period of 48 hours the seventh bird developed a coryza which lasted for 7 days. In this case the interval which preceded the second injection was 14 days.

It was apparent from the above experiment that recovery from the "bacillary" coryza was attended by a considerable degree of immunity to reinfection with the bacillus. Reciprocal protection tests were then carried out to determine whether birds which had recovered from the "bacillary" disease were immune to reinfection with exudate and vice versa.

Two groups of 4 birds, confined in individual cages in separate quarantine units, were employed in this experiment. 0.5 cc. amounts of 24 hours old horse blood agar cultures of the specific bacillus were injected into the palatine cleft of the birds in one group. 0.5 cc. amounts of exudate, withdrawn from several fowl previously infected with "exudate" coryza, were similarly injected in the birds of the second group. After recovery from the initial coryza the birds of the first group were injected with exudate and those of the second with cultures of the bacillus. The interval between the disappearance of symptoms and the second injection varied from bird to bird with extremes of 7 and 20 days. Two normal birds were injected with culture and exudate, respectively, as a check on the infectivity of these agents. The reinjected birds were held under observation for 2 weeks and examined daily.

The initial injection was followed in all cases by a coryza which tended to persist for a longer time in the birds which had received exudate. Throughout the period of observation the birds which had recovered from the "bacillary" coryza showed no response to the injected exudate. In 2 cases the interval between recovery and the second injection had been only 7 days. The control bird developed a nasal discharge on the 2nd day. The fowl which had recovered from the "exudate" coryza likewise failed to respond to the second injection, in this case of the bacillus. In these birds the interval between recovery and reinjection had been 14 days or longer. The normal bird which had been injected with the bacillus showed a coryza on the 2nd day. A summary of these observations is presented in Table I.

The preceding protection tests on the first coryza (Coryza I) were carried out in the spring of 1932 and shortly after their completion the maintenance of the disease by bird to bird transfer was discontinued. In the fall attention was focused on a second coryza (Coryza II) originally obtained from a different source. Protection tests were conducted with it and also with a third coryza (Coryza III) secured from the same poultry farm as the first, but a year later.

2 The natural case of this coryza was obtained by Dr. O. Seifried and Dr. C. Cain.
Coryza II was first produced in February, 1932, by the intranasal injection of normal fowl with exudate obtained from a naturally affected bird and up to July the disease was maintained by bird to bird passage. Both Rhode Island Reds and White Leghorns were used in the experimental production of the disease. It differed from Coryza I with respect to the onset and duration of symptoms. Following the injection of exudate, symptoms were regularly delayed until the 7th to the 14th day but thereafter persisted for 2 months or longer. Exudate filtered through N and V Berkefeld candles was tested and found to be innocuous. Several unsuccessful attempts to isolate the specific bacillus by filtration were also made. The disease was maintained during the summer by contact infection and in September

<table>
<thead>
<tr>
<th>No. of bird</th>
<th>Material injected</th>
<th>Incubation period</th>
<th>Duration of symptoms</th>
<th>Period between recovery and second injection</th>
<th>Material injected</th>
<th>Result of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exudate</td>
<td>2</td>
<td>18</td>
<td>19</td>
<td>Culture</td>
<td>Normal 14 days</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>2</td>
<td>4</td>
<td>17</td>
<td>&quot;</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>2</td>
<td>17</td>
<td>14</td>
<td>&quot;</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>2</td>
<td>11</td>
<td>16</td>
<td>&quot;</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coryza 2nd day</td>
</tr>
<tr>
<td>5</td>
<td>Culture</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>Exudate</td>
<td>Normal 14 days</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>&quot;</td>
<td>14 &quot;</td>
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<tr>
<td>7</td>
<td>&quot;</td>
<td>2</td>
<td>12</td>
<td>7</td>
<td>&quot;</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>2</td>
<td>4</td>
<td>20</td>
<td>&quot;</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coryza 2nd day</td>
</tr>
</tbody>
</table>

exudate from these birds was injected intranasally in normal fowl with the finding that the incubation period of the disease had become reduced to 24 or 48 hours. The course of the coryza, however, was not altered and symptoms continued to persist for 2 months or longer. By the use of sealed plates of blood agar, the specific bacillus was readily isolated from the nasal exudate of these birds and regularly produced a coryza upon injection in normal fowl. The incubation period of the "bacillary" coryza was 24 to 48 hours, as in the case of the "exudate" coryza, but the course of the disease was much shorter, averaging 14 days in 20 cases.

It was found that birds which had recovered from the "bacillary" and "exudate" types of Coryza II were resistant to reinfection with the bacillus and with exudate, respectively. Ten birds were employed in each experiment. 2 to 4 weeks after the initial coryza, produced in
one series by injection of the bacillus and in the other by exudate, had
subsided, the birds of the first series were reinjected with the bacillus
and those of the second series with exudate. The reinjected fowl were
held under observation for a period of 30 days and during this time all
of them remained normal. Susceptible birds injected with the same
infesting agents developed a coryza on the 2nd day.

A reciprocal protection test was then carried out to determine
whether birds which had recovered from the "bacillary" coryza were
also resistant to reinfection with exudate. The outcome of this experi-
ment showed clearly that the resistance acquired as the result of in-
fec tion with the specific bacillus was at least quantitatively different
from that acquired after recovery from the "exudate" coryza.

Eleven birds were employed in this experiment. 10 to 34 days after recovery
from the initial coryza, produced by the injection of 0.5 cc. amounts of 24 hours
old horse blood agar cultures of the specific bacillus, from Coryza II, they were in-
jected with 0.5 cc. amounts of exudate, removed from the nasal passages of several
fowl affected with "exudate" coryza. Prior to injection the exudate was rubbed
up with a little bouillon in a glass tissue grinder and diluted to approximately 10
cc. with bouillon. A susceptible bird was also injected with 0.5 cc. of the same
material. The reinjected fowl were held under observation for a period of 30 days
and examined daily. Exudate was removed from each of the birds which devel-
oped a second coryza, shortly after symptoms appeared, and a 0.5 cc. portion in-
jected into a normal fowl. The birds of this series were also examined daily over a
period of 30 days.

Ten of the 11 birds which were injected with exudate, following re-
cov er y from the "bacillary" coryza, showed an inflammation of the
nasal mucosa with a discharge from the nares. In each case, how-
ever, symptoms appeared only after a long incubation period which
varied from 10 to 21 days. The eleventh bird remained normal
throughout the period of observation. The control showed symptoms
of coryza on the 2nd day. Six of the 10 birds which were injected with
exudate, removed from the nares of the reinjected fowl, developed a
coryza after an incubation period of 10 to 18 days. Four of the birds
in this series remained normal during the period of observation. A
repetition of the entire experiment with Coryza III gave essentially the
same results. Exudate from 1 recovered bird, which had developed a
second coryza following the injection of exudate, continued to produce
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a coryza of slow onset after passage through 4 susceptible birds. The experimental findings with Coryza II are presented in Table II.

Exudate from each of 10 recovered birds, which developed a coryza after the injection of exudate, was examined bacteriologically using sealed plates of horse blood agar. In all cases a culture was made shortly after the appearance of the nasal discharge and in some cases 2 or more additional cultures were made at later intervals. The specific bacillus was not isolated in a single instance from these plates.

TABLE II

Cross-Protection Tests with "Bacillary" Coryza II and Infectivity Tests with Exudate from the Reinjected Birds

<table>
<thead>
<tr>
<th>No. of bird</th>
<th>Incubation period of &quot;bacillary&quot; coryza</th>
<th>Duration of &quot;bacillary&quot; coryza</th>
<th>Interval between recovery and injection of exudate</th>
<th>Reaction of the recovered birds</th>
<th>No. of bird</th>
<th>Reaction of the susceptible birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>31</td>
<td>21</td>
<td>Coryza after 13 days</td>
<td>1-A</td>
<td>Normal 30 days</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>16</td>
<td>18</td>
<td>&quot; &quot; 21 &quot; &quot;</td>
<td>2-A</td>
<td>&quot; &quot; 30 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>21</td>
<td>22</td>
<td>&quot; &quot; 15 &quot;</td>
<td>3-A</td>
<td>&quot; &quot; 30 &quot;</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>17</td>
<td>22</td>
<td>&quot; &quot; 13 &quot;</td>
<td>4-A</td>
<td>&quot; &quot; 30 &quot;</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>18</td>
<td>34</td>
<td>&quot; &quot; 15 &quot;</td>
<td>5-A</td>
<td>Coryza after 12 days</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>10</td>
<td>22</td>
<td>&quot; &quot; 10 &quot;</td>
<td>6-A</td>
<td>&quot; &quot; 16 &quot;</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>25</td>
<td>14</td>
<td>&quot; &quot; 18 &quot;</td>
<td>7-A</td>
<td>&quot; &quot; 13 &quot;</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>21</td>
<td>10</td>
<td>&quot; &quot; 15 &quot;</td>
<td>8-A</td>
<td>&quot; &quot; 10 &quot;</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>7</td>
<td>24</td>
<td>&quot; &quot; 17 &quot;</td>
<td>9-A</td>
<td>&quot; &quot; 13 &quot;</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>13</td>
<td>14</td>
<td>&quot; &quot; 14 &quot;</td>
<td>10-A</td>
<td>&quot; &quot; 18 &quot;</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>6</td>
<td>18</td>
<td>Normal 30 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bacteria which developed on the plates were the usual organisms which inhabit the nasal mucosa of both normal and affected birds; no additional bacteria were encountered.

A bacteriological examination was also made of exudate removed on the 1st day of the coryza from Birds 5-A, 6-A, 8-A, and 9-A. Practically a pure growth of the specific bacillus was obtained on sealed plates streaked with exudate from Nos. 5-A, 8-A, and 9-A. It was not recovered, however, on the plate from No. 6-A. Exudate from each of
these 4 birds was also injected intranasally in normal fowl. The incubation period of the resulting coryza was 24 hours in each case. The specific bacillus was isolated from the fowl injected with exudate from No. 6-A. The 4 strains of the coryza organism which were isolated from the birds of this series were injected into normal fowl and in each case produced a coryza after an incubation period of 24 hours.

Protection tests were also carried out with a number of birds which had been exposed to Coryza II by direct contact. In this experiment 5 normal fowl were placed in the same pen with 5 birds affected with "bacillary" coryza and a similar number in contact with 5 affected with "exudate" coryza. In each series 4 of the exposed birds developed a coryza, those in contact with the "bacillary" cases after 6, 7, 7, and 8 days and those in contact with the "exudate" cases after 4, 5, 6, and 8 days. One bird in each series remained normal throughout the period of contact, which was continued until most of the birds had recovered. 2 weeks or more after recovery, 3 contact birds from each group were injected with the same agent to which they had been exposed. In both cases this number included 2 birds that had developed coryza as a result of exposure and the 1 unaffected bird. The injected fowl were held under observation for 30 days and during this time none of them showed symptoms of the disease.

DISCUSSION

The existence of several types of uncomplicated fowl coryza, differing from one another in the length of the incubation period and the course of the disease, is indicated by the preceding observations. Three such types were encountered; namely, Coryza I with a rapid onset and relatively short course, Coryza II with a slow onset and a prolonged course, and Coryza III with a rapid onset and a prolonged course. There is a suggestion, from the experimental study of the 3 types, that Coryza III is the basic form of the disease and that Coryzas I and II are variants which tend to revert to it with continued passage through susceptible fowl. Thus, Coryza III was obtained from naturally affected birds of the same flock from which Coryza I had been secured a year earlier. In the case of Coryza II a reduction in the originally prolonged incubation period occurred with the continued passage of exudate from bird to bird. The variant types of the
coryza may, however, retain their particular characters for a considerable period of time.

Coryzas I and III were also produced experimentally by injection of the specific bacillus isolated from the nasal exudate but in both cases the duration of the disease was much shorter than that of the coryza produced by exudate. Protection tests were carried out to determine whether this discrepancy was due to the presence of a second infectious agent, acting independently of or in conjunction with the specific bacillus, or to some other factor.

The demonstration of reciprocal protection in the case of Coryza I was directly opposed to this view but the course of events with Coryza II, after its incubation period had become reduced, and with Coryza III seemed at first to favor it. In the case of the latter coryzas most of the birds which had recovered from the “bacillary” infection showed a nasal discharge following the injection of exudate. Subsequent observations, however, suggested another explanation; namely, that the degree of immunity produced by the cultivated bacilli was not sufficiently high, in most cases, to actually destroy the bacilli present in exudate but was sufficient to retard their development. There was reason to believe, moreover, that growth of the latter bacillus in the partially immune host was attended by a temporary change in certain of its characters. Exudate from some of the reinjected fowl was infective for normal birds but with the first passage and sometimes with several additional passages it produced a coryza of slow onset. Eventually, however, the exudate returned to its normal level of infectivity and for some unknown reason the isolation of the specific bacillus was most readily accomplished immediately preceding this change.

As a working hypothesis, it is suggested that the fluctuations observed in the onset and course of the coryzas from different sources and in the “bacillary” and “exudate” forms of the disease are referable to changes induced in the specific bacillus by unfavorable environmental conditions, in the one case by growth in a partially immune host and in the other by artificial cultivation. The existence of a second infectious agent is not definitely excluded but no evidence that would support a dual etiology has been forthcoming. The non-specific effect of secondary invaders, which are numerous in the injected exu-
date, should not be overlooked in this connection. It is unlikely, however, that they play more than a minor rôle in the maintenance of the "exudate" coryza since they are also found in considerable numbers in the exudate from "bacillary" cases after the first few days.

SUMMARY

Three types of an uncomplicated fowl coryza, differing in the onset and duration of symptoms, developed after the intranasal injection into normal birds of exudate from natural cases. Protection tests were carried out with 2 of the types in an attempt to explain why the "bacillary" disease regularly ran a shorter course than the "exudate" disease. Reciprocal protection was demonstrated in one case, but in the other the birds which had recovered from the "bacillary" disease were susceptible to reinfection with exudate. There was no indication, however, that a second infectious agent was present in the exudate, and the failure to cross-immunize was ascribed, rather, to a reduction in the immunizing properties of the specific bacillus induced by artificial cultivation.

It was also noted that the coryzas produced by exudate and bacilli, respectively, could be transmitted from infected birds to normal ones by direct contact. In both cases 1 bird out of 5 failed to contract coryza on exposure. These 2 birds were later injected with the respective agents to which they had been exposed and found to be resistant.