STUDIES ON AN UNCOMPLICATED CORYZA OF THE DOMESTIC FOWL

I. The Isolation of a Bacillus Which Produces a Nasal Discharge

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Under natural conditions the domestic fowl is subject to a number of infections which affect the nasal passages and result in a discharge from the nares. Laryngotracheitis, fowl pox, and fowl cholera may be accompanied by an inflammation of this character but in these cases the coryza is generally a transient manifestation which precedes the appearance of lesions elsewhere. The fowl is also susceptible to an uncomplicated coryza, not unlike the common cold of man, in which the inflammatory reaction is limited to the mucosa of the nasal passage and the communicating portions of the orbital tract. This form of coryza, which is here considered, is probably identical with the condition known as catarrhal roup in the older literature and later designated contagious catarrh.

De Blieck[1] recently studied a fowl coryza of this type in Holland and isolated a hemophilic bacillus from a blood agar plate streaked with nasal exudate from a naturally affected bird. He stated that the organism resembled the human influenza bacillus and named it *B. haemoglobinophilus coryza gallinarum*. Normal fowl injected intranasally with pure cultures of the bacillus regularly developed a coryza. McGaughey[2] in England reported the isolation of a para-influenza bacillus, dependent only on V factor for growth, from the upper respiratory tract of fowl affected with coryza and also from normal birds. The bacillus was not pathogenic for fowl upon subcutaneous or intravenous injection. Intranasal injection was evidently not carried out. The significance of the organism as the cause of the coryza was considered to be uncertain.

[1] De Blieck, L., *Vet. J.*, 1932, **88**, 9.

[2] McGaughey, C. A., *J. Comp. Path. and Therap.*, 1932, **45**, 58.

Isolation and Cultivation

The present work on fowl coryza was begun in the fall of 1931.[3] The disease was established in a group of normal birds by the introduction of nasal exudate from naturally affected fowl obtained on a nearby poultry farm and was subsequently maintained by transferring exudate to additional birds from time to time.[4] The material was injected into the palatine cleft and its introduction was followed after a short incubation period by an inflammation of the nasal passages and a discharge from the nares.

Most of the experimental fowl were drawn from a flock of Rhode Island Reds maintained for many generations at The Rockefeller Institute and known to be free from respiratory disease. As the work advanced it became necessary to use Rhode Island Reds from another flock and also White Leghorns but in both cases the birds were from disease-free stocks. The infected birds were kept indoors in cages or enclosed runways which were maintained under a strict quarantine.

Exudate from the experimentally affected fowl was first studied by ordinary bacteriological methods. A 1.5 mm. loop of the material removed from either the nares or the nasal canals was streaked over the surface of an open plate of 10 per cent horse or chicken blood agar. This examination served only to catalogue the bacterial flora of the affected air passages. Exudate withdrawn after the 2nd day of the coryza generally contained large numbers of bacteria, a single loopful often giving a nearly confluent growth of small colonies. Gram-positive cocci, diphtheroids, and Gram-negative bacilli predominated but rarely with any approach towards the pure growth of a particular organism. The former group included staphylococci, a greenish haloed streptococcus, a tetrad, and unidentified micrococci. The latter group embraced some 10 unidentified species and in addition several strains of a para-influenza bacillus. Bacilli of the Pasteurella group were not encountered. Representative cultures of the Gram-negative bacilli were injected into the palatine cleft of normal fowl and failed in all cases to produce a coryza. Most of these bacteria

[3] A preliminary report of the early work was presented in *Proc. Soc. Exp. Biol. and Med.*, 1932, **30**, 306.

[4] The original coryza cases were obtained by Dr. O. Seifried and Dr. C. Cain.

may be isolated from the nasal mucosa of normal birds but are present in much smaller numbers.

Exudate diluted with bouillon and filtered through short Berkefeld V candles was likewise innocuous.

As a control on the sterility of the filtrates a 0.5 cc. portion was added to fluid horse blood at the base of slanted nutrient agar. There was no macroscopic evidence of growth in these tubes. Fluid removed from the base of such a tube, after it had been incubated at 37°C. for 4 days, was injected into the palatine cleft of a normal fowl. 2 days later the injection was followed by a typical discharge from the nares. This was the first instance in which a nasal discharge had been produced in the absence of exudate. The uncultured filtrate in this case was non-infective, as usual. Exudate was removed from the nasal passages of the affected bird, filtered through the same candle after sterilization, and portions before and after cultivation injected in normal birds. Identical results were obtained with this series and again with a third in which exudate from the second bird was utilized. A repetition of the entire experiment modified only by the use of fluid chicken blood in the culture medium yielded similar findings throughout.

In all cases the birds which had received filtered exudate before cultivation remained normal, whereas those which had received the filtrate after cultivation, in the presence of fluid blood, developed a coryza.

In the experiments described above exudate was removed from the nasal passages, at autopsy, and rubbed up with a little bouillon in a glass tissue grinder. The suspension was diluted to a volume of approximately 5 cc. with bouillon and rapidly filtered through a 3.5 cm. Berkefeld V candle. 0.5 cc. portions of the filtrates were used in the inoculation of culture tubes, which were generally incubated at 37°C. for 24 to 48 hours, and in the injection of fowl. Blood agar plates were streaked with exudate prior to filtration and in all cases showed many colonies of miscellaneous bacteria.

As noted above, there was no macroscopic evidence of bacterial growth in the incubated tubes of filtered exudate, the fluid from which was infective. At first, some difficulty was experienced in detecting bacteria microscopically. It was shortly found, however, that films made from the fluid portion of the medium, not later than the 2nd day and after the tube had been shaken to disperse the sedimented red blood cells, regularly showed small Gram-negative bacilli. The supernatant portion of the fluid blood remained clear for several days and prior to shaking contained only an occasional bacillus. Upon

prolonged incubation the fluid becomes turbid due to the precipitation of serum constituents. After the 2nd day of incubation the bacterial cells tended to fragment and lose their staining properties. In hanging drop preparations made from 24 hour old cultures the bacilli showed no motility. On open blood agar plates streaked with fluid from both young and old cultures and examined daily under low magnification there was no evidence of colonization.

Cultivation of the specific bacillus was continued by serial transfer to horse blood agar slants at weekly intervals. Normal birds were injected with fluid from such cultures from time to time and in every case through the 25th subculture the injection was followed by a coryza. A total of 35 birds varying from 5 weeks to 2 years in age but mostly between 8 and 12 weeks of age, were employed in these experiments which were carried out between January and July.

No differences were noted in the inflammatory changes produced by exudate and by cultures of the specific bacillus. There was a difference, however, in the course of the 2 coryzas, the nasal discharge continuing for a longer time in birds injected with exudate. The average duration of symptoms was approximately 11 days in 20 cases of the "exudate" coryza and 5 days in 20 cases of the "bacillary" disease.

Weekly transfers of the organism were made during the summer of 1931 but the maintenance of the coryza in fowl was discontinued. In September the infectivity of the 35th subculture was tested by intranasal injection, with the finding that the organism had become avirulent.

Before setting out to recover a new strain of the bacillus, an attempt was made to perfect the method of isolation. Differential filtration, while a useful procedure, is not suitable for routine examinations. Only certain candles function in that capacity and an adequate supply cannot be kept on hand.

It was found that the avirulent organism would colonize on the surface of blood agar plates that were tightly sealed. For this purpose a closely fitting cover of modeling clay was employed instead of the usual glass lid. Small, slightly rounded, clear colonies were produced on plates sealed in this way. With the aid of a dissecting microscope, transfers could be readily made from individual colonies to blood agar slants.

The covers used in sealing the plates were made from one of the commercial modeling clays. A ball of clay was pressed into a circular disk about 5 mm. thick and 1 cm. larger than the bottom half of a Petri plate. The projecting portion was moulded to form a raised rim or flange. A sterile paper disk was used to cover the inner portion of the clay cover, the inoculated dish inverted over it, and the flange firmly pressed to the side of the dish. The medium is thus in contact only with sterile surfaces and accidental contamination rarely occurs. The cover is readily removed intact by running a spatula inside the flange and may be used over and over again.

This method of isolation was employed with birds which had been injected with exudate from naturally infected fowl. The latter were obtained in October, 1932, on the same poultry farm from which the original cases had been secured during the preceding fall. Exudate was removed from the nasal canals of the experimentally infected fowl on the 1st or 2nd day of the coryza and streaked on plates, from which the specific bacillus was readily isolated. A coryza was regularly produced in normal birds by the intranasal injection of the organism in pure culture. The bacilli were again obtained on sealed plates prepared from the exudate of these fowl.

The incubation period of both the "exudate" and "bacillary" coryzas was 24 to 48 hours as in the case of those of the preceding year. The duration of the second "exudate" coryza was much longer however, than that of the earlier corresponding coryza. Symptoms regularly continued for 2 months or longer. The difference between the "exudate" and "bacillary" coryzas with respect to the duration of symptoms was more marked than in the case of the respective types of the preceding year. The average period in 15 cases of the second "bacillary" coryza was 11 days. It may be noted that White Leghorn fowl were used in addition to Rhode Island Reds in the above experiments. No difference was observed in the susceptibility of the 2 varieties.

Aside from variations in the duration of the disease, no differences were noted in the symptoms or the pathology of either the "exudate" or the "bacillary" forms of the coryzas studied in 1931 and 1932, respectively.

The experimentally infected birds showed a unilateral or a bilateral nasal discharge after a short incubation period. An initial unilateral discharge generally became bilateral as the coryza advanced. Early in the disease the exudate was

largely a thin mucus which was relatively poor in cells. Later the mucus became thick and tenacious and mixed with large numbers of leucocytes and epithelial cells. Small cheesy granules made up largely of bacteria were also present in some of the advanced cases. The exudate was rarely limpid enough actually to drop from the nares but tended, rather, to collect there forming a plug which partially occluded the lumen. After the first few days the exudate was generally covered by a crust of dust and grain particles. At autopsy, a large volume of exudate was commonly found in the nasal passages and not infrequently in the orbital sinuses. Aside from the increased secretion of mucus the nasal mucosa showed little or no gross evidence of injury.

There were no other consistent manifestations or symptoms in the infected fowl. Occasionally the inflammation extended to the external periorbital tissues, with a transient watery discharge. At autopsy, a few birds showed a catarrhal inflammation of the trachea. There was no specific mortality and in most cases the infected birds did not appear ill. The majority of the birds showed no respiratory abnormalities; a few birds with a heavy bilateral discharge breathed through the mouth with the beak open and in those with an involvement of the trachea respiration was accompanied by a distinct gurgling sound. In the case of the persistent "exudate" coryza, growth and egg laying were noticeably retarded.

The appearance of naturally affected fowl was often quite different. Some of the birds received from the poultry farm where the coryza was endemic showed emaciation, diarrhea, a labored audible respiration, and edema or inflammation of the periorbital tissues. The latter was characterized by either a copious purulent discharge or a massive accumulation of exudate in the orbital cavity. In our experience the transference of nasal exudate from such birds to normal fowl maintained under controlled conditions was followed only by the development of a coryza.

DISCUSSION

The passage of the specific organism through certain coarse Berkefeld candles, which held back other bacteria, was a particularly fortunate circumstance in view of its inability to colonize on open blood agar plates. This peculiarity in growth obviously precluded plating, under ordinary conditions, as a method of separating the bacillus from the miscellaneous bacteria present in the nasal exudate of fowl affected with coryza.

The filtered exudate after cultivation in a suitable fluid medium showed intact bacterial cells in an uncontaminated condition. The precursors of these cells, present in the filtrate before cultivation, were incapable of producing a coryza in normal fowl, either by reason of insufficient numbers or of an altered biological state. After cultivation the filtered exudate was regularly infective.

Earlier attempts to induce colonization of the bacillus on solid media had been unsuccessful and the evidence that only one organism was present in filtrates from different birds was somewhat circumstantial. The subsequent discovery that colonization could be initiated by sealing the plates used for cultivation furnished a useful method of isolation and by the uniformity of the colonies supplied additional evidence that the same organism was present throughout.

The question which arises concerning the relationship of this bacillus to the one isolated by de Blieck[1] cannot be answered at present. The latter organism was described as resembling *B. influenzae* of man but a detailed statement of its growth requirements was not made. It apparently colonized on open blood agar plates. In the morphology of its cells and colonies the present bacterium bears a resemblance to the influenza bacillus and like it is soluble in bile. Unlike the latter, however, it fails to grow in bouillon containing X and V factors of plant origin, suggesting that its growth requirements may not include these accessory factors; and it fails to colonize on open blood agar plates. The present organism is readily differentiated from the para-influenza bacillus which was occasionally isolated from the nasal exudate of fowl affected with coryza. The latter bacillus required V factor only in the medium of growth, as did the strains isolated by McGaughey,[2] and it grew well in bouillon enriched with a plant extract instead of blood. There appears to be no good reason for regarding the present bacterium as an influenza bacillus, although it probably belongs to the group of hemophilic bacteria which includes that bacillus. Its final classification as well as a detailed description of its characters is, however, held in reserve.

SUMMARY

By a method combining filtration and cultivation an unidentified Gram-negative bacillus was isolated from the nasal exudate of fowl experimentally infected with an uncomplicated coryza. Isolation was accomplished by cultivation on sealed blood agar plates after unsuccessful attempts to produce colonies on open plates. Injection of the organism into the palatine cleft of normal birds was regularly followed by an inflammation of the nasal mucosa and a discharge from the nares. A para-influenza bacillus which was also recovered from the nasal tract of affected fowl was innocuous. Certain cultural characters of the bacillus, bearing on its classification, are discussed.