Study of Coryza of the Domestic Fowl with special reference to its Bacteriology.

by

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C O N T E N T S.

C O N T E N T S (contd.)

PREFACE

It was the desire of the author of this piece of work, since he qualifed in Veterinary Science, 1933, to study thoroughly the nature of the various existing animal diseases rampant in his country (Iraq) and taking a heavy toll of life of animals and birds.

With its two main sources of fertility (the rivers Euphrates and Tigris), Iraq was destined to be one of the foremost agricultural countries in the Near East. Consequently, animals and animal industry play an important role in its economic existence. The fowl is not a new bird to Iraq, where it existed as long ago as in other parts of the world i.e. ever since the domestication of the bamboo-jungle fowl of India or Java.

It is therefore but natural for the author to take a serious interest in the animal welfare and investigate the numerous obscure animal diseases prevailing in those parts. The writer has been fortunate enough to have the opportunity of gaining a first-hand information on the practical aspect of the investigation of veterinary problems in general, by working as a post-graduate scholar at the Imperial Veterinary Research Institute, Muktaser, India, for a period of one year.

This period of practical research-training has been amplified by his two years domicile in Great Britain, during which time he has carried out investigations on Infectious Coryza of the fowl.

The aetiology of several animal diseases is still obscure, one of which is Infectious Coryza of the fowl. At the present time, with improved facilities for diagnosis and investigation, the veterinarian still finds many problems awaiting solution. Infectious Coryza in the fowl is one of the immediate problems awaiting satisfactory elucidation. At present, there exists considerable lack of agreement as to the cause of this condition, and in the course of his investigations the author has made a careful examination of the various agents which have been incriminated as the causal factor of the disease, and he does not desire to add to the already existing confusion with regard to the cause of the condition. The writer felt that should it be possible to control the disease as a result of the determination of the true causal agent, his investigation into the subject would have considerably aided the maintenance of a healthy poultry world with resulting benefits to the profession and poultry industry alike. Under modern conditions of domestication and particularly in the intensive and semi-intensive methods of poultry-keeping, the natural resistance of

the fowl to certain infectious diseases appears to be lowered, and the birds fall victims to maladies which they could resist while in free, natural life. The most outstanding of these ailments are those of the respiratory passage, and their aetiology is still a matter of controversy. Of these, Infectious Coryza occupies the foremost place in the investigations of the poultry-pathologist of to-day, both in the old and in the new worlds.

DEFINITION AND NOMENCLATURE.

Coryza Infectiosa Gallinarum, Contagious Nasal Catarrh, Cold in the Head, and Roup are terms applied to describe an infectious disease of the upper respiratory tract of the fowl, characterised by a series of inflammatory changes in the mucous membrane of the nose, larynx and conjunctiva together with the infra-orbital sinuses, and manifested by discharge from the nostrils, sneezing, conjunctivitis, sinusitis, respiratory distress and general depression on the part of the bird. The disease is often fatal.

A review of the literature shows confusion to exist with regard to the nomenclature of this disease. Each of the symptoms and lesions seems to have been used as a

basis for specific name for the disease without recognition of the fact that the disease occurs as an independent pathological condition, involving the upper respiratory tract, conjunctiva and facial sinuses; clinically similar lesions occur as secondary manifestations of other specific diseases such as Fowl Cholera, Fowl Pox and Vitamin A deficiency.

While the term "Roup" has been generally accepted to indicate Infectious Coryza or Contagious Catarrh (Barger and Card, 1938 '3', Hutyra, Marek and Mannin--ger, 1938 '46', De Blieck, 1935 '21', The Advisory Committee on Poultry Diseases of the N.V.M.A., 1937 '1' and others), there are other authorities who believe that Roup (Schleimhauterkrankungen) and Fowl Pox (Geflugelpocken) are two different manifestations of the same disease (Reischauer, 1906, '76'). Jowett (1909 '50'), on the other hand, was convinced that Avian Diphtheria (commonly termed diphtheritic roup) is separate from Fowl Pox, and Booth (1911 '10') was of the opinion that "Roup" and "Avian Diphtheria" are one and the same condition.

Other workers, such as Harrison and Streit (1903 '43'), Streit (1904 '87'), Booth '10', Jackley (1918 '48'), Crofton (1924 '16'), and Edwin and Johnson (1927 '26') seemed to have used the expressions "Roup" and

"Avian Diphtheria" as synonymous terms describing one and the same disease. There are still others, among whom are Hadley and Beach (1913 '41'), Verge (1926 '94'), Popov (1928 '72'), Doyle (1929 '23') and Reis (1930 '74') who express the view that "Roup", "Avian Diphtheria", "Coryza", "Oculo-nasal Roup", and often "Contagious Epithelioma" are all different manifestations of the same condition.

Some workers, however, are of the opinion that "Roup" is only a complicated form of Coryza (Bushnell and Hinshaw, 1924 '14', Bushnell and Brandly, 1929 '13'). Sawyer and Worley (1927 '78'), Beach and Freeborn (1930 '5') and Stafseth (1931 '85') express the same view in as much as they believe that "Roup" is really Cold or Coryza which has been neglected. Blount, '11', on the other hand, claims that Contagious Catarrh is wrongly termed "Roup".

Foley (1926 '30') describes Roup as "it covers a multitude of sins", while Reis (1926 '75') is inclined to believe that "Roup" is a term under which various diseases are grouped. He was supported by Dalling (1934 '17'), who suggested that "Roup" is a common name for different conditions which could be classified as

(a) Pox
(b) Infectious Catarrh

(c) Nutritional Roup
(d) Roups due to other causes.

The situation, as it has been summarised above, clearly shows that poultry investigators have been confused as a result of the numerous names applied to diseases in the upper respiratory tract of the fowl. This state of affairs gives support to the view held by the author that this multiplicity of names makes it all the more difficult for the student and the practitioner to recognise which disease it is that an author may be describing, with a resulting check to the development of the science of poultry pathology. The writer suggests that, following the modern tendency to simplify nomenclature in order to facilitate the recording of diseases, the use of the terms "Roup" and "Avian Diphtheria" should be abandoned.

INCIDENCE.

Hutyra, Marek and Manninger '46' state that Infectious Coryza may affect birds of all species, but that it occurs principally in fowls, especially in the young ones. Workers on this subject ('1', '73', '63', '79' etc.) are fully agreed that the condition mainly affects the young stock. It is the author's experience that young chickens are more susceptible to the disease under

investigation than adult birds. The greater resistance of the adults was demonstrated by means of experimental inoculations. Such experiments demonstrated that the incubation period in the inoculated adults was much longer than it was in the case of the young. On the other hand, under conditions of natural infection, adult stock seems to be at times equally susceptible as the young. Personal observations on several outbreaks of the disease have convinced the writer of this point of view. In a letter to the author, a poultry farmer says "the disease usually appears in birds of about ten weeks. I had cases where considerably young stock, say six weeks, has been infected but have always noticed that older birds start the trouble".

The disease, as described in text-books and by various investigators, makes its appearance in cold, damp months - mostly in the spring and autumn. Outbreaks, however, have been brought to the writer's notice which occurred in early summer months and which persisted till late in the autumn.

Infectious Coryza of the fowl seems to have a world-wide distribution. It was as far back as the year 1898 that it was reported by Gratia and Lienaux '37' to have occurred in France. On this occasion, these workers referred to "Coryza Contagieux ou Morve des Poules" as

a separate entity from "La diphterie Aviaire". Later the disease was reported by several investigators, of whom Nelson '62' has come to the conclusion that three types of the disease exist naturally:

Type I. Coryza of rapid onset and short duration
Type II. Coryza of slow onset and prolonged duration
Type III. Coryza of rapid onset and prolonged duration.

Among other reports from Europe on the condition are those made by Van Dorsen '92' and Kessen '53' in which each describes two types of the disease; the first being Coryza with rapid onset and the second Coryza with a slow onset.

In Africa the condition has been reported from Eritrea by Marcato '59' and from Kenya by Purchase '73'.

According to Hutyra, Marek and Manninger '46', the malady may affect all species of birds. Eber (1934 '25') also states in his book that the domestic fowl is the usual victim whereas water-fowl and pigeons rarely suffer from this condition. Bushnell and Brandly (1929 '13') are of the opinion that although the chicken is the most susceptible to Coryza, other types of poultry may also be infected.

Nelson '64', Beach and Schalm '79' and others failed to transmit the disease to ducks or pigeons. De Blieck '19' states that Coryza is an infectious disease which

can be transmitted to chickens and turkeys only.

During the course of his investigations, the author was unable to reproduce the disease either in ducks or in pigeons. Six ducks and twelve pigeons were inoculated intra-nasally with virulent nasal exudate, the virulence of which was controlled by inoculation into healthy chicks; such chicks reacted as usual to infection. The ducks and pigeons did not exhibit any symptoms of the disease over a period of eight weeks in the case of pigeons, and ten weeks in the case of ducks, at the end of which time they were destroyed and post-mortem examination failed to reveal any changes in the whole of the respiratory system and the infra-orbital sinuses.

Three turkey poults were inoculated with virulent exudate and these developed lesions of the disease similar to those found in the chickens.

The writer is in complete agreement with Nelson '64', Schalm and Beach '79' and De Blieck '19' in stating that Infectious Coryza is a disease of fowls and turkeys, and is not communicable to other domesticated avian species.

The following Table summarises the writer's experiments in connection with this part of the subject.

TABLE I

Subjects		Route	Reaction.
Species of birds tested	Control chickens	and material	
12 pigeons	3047 3051 3176 3223 3254	Nasal exudate intra-nasally	Pigeons were normal for a period of 8 weeks whereas 2 of the controls exhibited symptoms of the disease 24 hours after inoculation and the other 3 after two days.
6 ducks	3165 3205	Ditto	Ducks remained normal for a period of observation of 10 weeks whereas the controls showed symptoms of the disease 24 hours after inoculation
3 turkey poults		Ditto	2 turkeys reacted after 24 hours and the third after 48 hours.

SYMPTOMATOLOGY

Symptoms of the disease as it occurs naturally are elaborately discussed by Hutyra, Marek and Manninger '46'; these are in accordance with the picture of the disease as found by the author in the course of his investigations into the malady, occurring both under natural conditions and subsequent to experimental infection.

When crude nasal exudate is inoculated into experimental subjects, the symptoms usually take from twelve to forty-eight hours to develop in the case of

chickens between the ages of six and ten weeks, while in adult subjects the incubation period may be as long as from eighteen to forty days.

In all subjects, the disease commences with a general dullness; the bird sits with ruffled feathers and drooping wings, in a corner of the cage (Fig.I). Soon after, the appetite begins to diminish and the subject exhibits signs of uneasiness manifested by frequent shaking of the head and sneezing, which is accompanied in most cases by lachrymation and nasal discharge either unilateral or bilateral.

Lachrymation, when present, is manifested at first by the appearance of a frothy, clear, thin fluid which gradually becomes turbid and sticky; later it dries at the corners of the affected eyes and on the edges of the eyelids, which become ultimately stuck together by thin yellowish crusts (Fig.2). At this stage conjunctivitis is conspicuous and when the eye is opened, the cornea appears to have lost its healthy lustre. In more severe cases, the glued eyelids of the affected eye are bulged out, under the influence of the thick, yellowish mucopurulent material which occupies the whole of the conjunctival sac and exerts considerable pressure on the cornea, but the latter is rarely found ulcerated, although cloudiness of the cornea is frequently the result

of the presence of this substance. In this connection it might be well to note that Hardtwigk (1936 '42') has recorded that purulent panophthalmia may complicate Infectious Coryza in the fowl. This condition, he states, may often be met with as a complication of Fowl Pox, Vitamin A deficiency and Tubercular conjunctivitis in the fowl. Only one case has been found by the writer to be complicated with panophthalmia. Microscopical examination of the yellowish substance present in the conjunctival sac showed the presence of pus cells and desquamated epithelium. Examination of smears prepared from the same material, and stained by Gram's method, revealed the presence of occasional Gram positive cocci and diphtheroid bacteria.

With regard to the nasal discharge, this starts as a thin, colourless fluid changing gradually to a tenacious, yellow mucus emitting an offensive odour. The mucoid discharge dries in the nostrils, forming mucous plugs which, by interfering with respiration, give rise to rattling and snuffling breathing through the open beak (Fig.3). Tracheal rales may often be heard. In chronic cases, when the base of the nose is gently pressed, beads of yellow purulent material similar to that found in the conjunctival sac are expressed.

The tissues over the infra-orbital sinuses of the affected side appear to be reddened, hot and swollen. In the early stages this swelling is found to be soft but gradually hardens and enlarges, giving rise to what is known as "swell-head" in the fowl. In some cases the swelling may even cause sufficient pressure on the palatine bone of the affected side to cause it to bulge, thus forming a convexity in the buccal cavity.

The palatine cleft at this stage is packed with the same purulent material mentioned earlier.

Occasionally, a spot or two of yellowish deposit is found in the buccal cavity. Thin, dry, yellowish brown pseudo-membranes have also been found in a few cases.

Oedematous swellings are often met with in the submaxillary region and other parts of the head (Fig.4). In the case of cockerels, the wattles may also be affected, and in the early stages they are soft to the touch, becoming gradually hard at various places, giving the wattles a shrivelled appearance (Fig.5). The feathers on the back become matted together by mucus. this being due to constant rubbing of the face along this part.

In addition to the local lesions, general symptoms are also found e.g. the comb is generally pale and

shrivelled, but deeply coloured at the rim. The bird becomes rapidly emaciated, this fact being accounted for by the inability of the bird to take its food as a result of (1) temporary blindness and (2) difficulty in deglutition. In the later stages of the disease, some of the subjects become unconscious - especially in acute cases.

In accordance with the findings of other workers, (Nelson '63', Purchase '73', McGaughey '97' and others) the writer was unable to demonstrate any significant thermal reaction in the affected birds, beyond the occasional subnormal temperature noted in a few acute cases just before death.

On the other hand, the infection may be so mild as to last only for a short period, after which the birds begin to recover and the disease continues to run a mild course manifested only by the presence of nasal discharge. This condition is mainly met with in adult birds.

The course of the disease varies in duration, and in acute infections the disease runs a comparatively short course i.e. a few weeks, and such cases usually terminate fatally. In other cases, the course of the disease may persist for as long as eight months: the average

duration is, however, between four and five months. Mortality is rather high, reaching to about ninety per cent in young experimental subjects while in older fowls the death rate is much lower.

POST-MORTEM FINDINGS

As Eber '25', Barger and Card '3', and others stated that post-mortem findings alone would not be of much help in giving a diagnosis specific of the disease, due to the fact that many variable conditions of the head region of the fowl show at some stage similar lesions to those found in this disease, the writer proposes to discuss separately the external and internal lesions met with in carcases of subjects which had died from Infectious Coryza.

External Lesions:

Extreme emaciation of the carcase; pale and shrivelled comb and wattles; matting together by mucus of the feathers on the back, and soiling of the cloacal region (suggestive of diarrhoea) were sometimes observed.

Depending on the stage of the disease, the nasal chambers were found to be packed with gelatinous mucus, or completely inspissated masses. Smears prepared from the gelatinous nasal contents and stained by the Romanowsky

stains showed the presence of lymphocytes and a few varieties of bacteria. As the condition advances, monocytes and polymorphonuclear leucocytes and occasional eosinophiles along with cast-off epithelial cells made their appearance, and the nasal bacterial flora is seen to include a greater variety of types of bacteria. Sections prepared from the inspissated masses showed on microscopical examination the presence of pus cells, indicating the purulent nature of the material examined. On staining by Gram's method, these sections when examined showed no evidence of bacteria.

The nasal mucosa appeared reddened, and microscopical examination of a cross-section of the face showed catarrhal rhinitis, manifested by the presence of inflammatory exudate, capillary engorgement and round-cell infiltration which was restricted to the sub-mucosa (Figs 6 and 7).

As already stated, the infra-orbital sinuses were found to be packed with hard, yellowish purulent material (Fig.8). In the buccal cavity, pseudo-membranes and yellow deposits have also been met with. The oedematous submaxillary region often showed, on incision, the presence of a hard, yellowish inspissated mass which seemed, on microscopical examination, to consist of sero-fibrinous exudate with a large number of round cells distributed

throughout. No bacteria were observed in sections stained by Gram's method, and cultural examinations proved negative.

Internal Lesions:

Of the internal organs involved by the disease, the larynx was found to show petechia in addition to congestion of the mucosa, this being true of the majority of cases. Microscopical examination revealed the presence of round-cell infiltration, together with capillary engorgement and the presence of petechial spots which were restricted to the lymphoid cells of this structure (Fig.9).

The mucous lining of the trachea was usually found to be normal, only on rare occasions slight congestion of the upper part of the syrinx being noticed. Catarrhal inflammation of the mucous lining of the trachea was rarely seen. Pulmonary complications were not found to be a usual feature of the disease; pneumonia having been rarely observed. The various abdominal air-sacs were sometimes found to be packed with large, yellow inspissated masses, structurally similar to those described in the submaxillary region.

In the alimentary tract the oesophagus, crop, proventriculus and gizzard were usually normal. The crop, however, was frequently observed to be empty. The small

intestines were inflamed in some cases, and microscopical examination showed desquamation of the epithelial lining and oedema of both the sub-mucosa and the muscular layers.

The heart was usually normal, but occasionally haemorrhagic spots were observed on the external wall of the myocardium. This was often accompanied by the presence of a clear straw-coloured fluid in the pericardial sac. This fluid was bacteriologically sterile, and on microscopical examination it showed the presence of occasional endothelial cells and eosinophiles. Further, no coagulation of this fluid took place when glacial-acetic acid was added to it, this suggesting that the effusion was more of the nature of a transudate than of an exudate.

The ovaries and testes were under-developed as compared to those of normal birds of the same age. Other internal organs, on the other hand, were normal. The presence or absence of one of the above-described lesions depends mostly upon the severity and duration of the disease with which they are reciprocally proportionate.

DIFFERENTIAL DIAGNOSIS

As previously mentioned, diseases such as Fowl Pox, Laryngo-tracheitis, Parasitic tracheitis ("Gapes"), and Avitaminosis A produce in the head and respiratory tract lesions that are liable to be confused with those of Infectious Coryza.

1. Fowl Pox:

The cutaneous form of fowl-pox (contagious epithelioma) is unmistakable and should be easily recognised, as the growths which form the main feature of the condition may attack any part of the skin in the head region. The ophthalmic and buccal forms of fowl pox differ from Infectious Coryza inasmuch as in the former disease both eyes are sometimes simultaneously affected, in association with other pathognomic lesions of the disease. The croupous pseudo-membranes are firmly adherent to the buccal mucosa, with a tendency to increase in size and when these are removed, the underlying mucous membrane appears to be inflamed and raw. After the removal of a false membrane, another quickly replaces it. There is also a tendency for these false membranes to appear afresh in the unaffected mucosa of the mouth, pharynx and larynx. The membranes are often accompanied by wounds. In the case of Infectious Coryza, such false membranes are of a very rare occurrence and when they do

occur, they are found to be loosely adherent to the buccal mucosa. When these are removed, the underlying mucous membrane is found to be undamaged and there is no tendency for these membranes to recur.

Thermal reaction is another constant feature of fowl pox, whereas in Infectious Coryza no temperature reaction was recorded. Animal inoculation tests also serve to differentiate between the two conditions. Emulsion of the suspected material is applied either to the scarified comb and buccal cavity of susceptible subjects, or on to the plucked feather follicles of the legs of the fowl and the pectoral region in the pigeon. In the case of fowl pox, typical lesions of this disease are seen after a period of incubation of three to fifteen days on the inoculated areas of the experimental birds, whereas in the case of Coryza such results are not encountered. In addition, pigeons are resistant to Infectious Coryza and at the same time are susceptible to fowl pox.

Nasal and conjunctival discharge, collected from subjects artificially infected with the disease under investigation, was applied to the scarified combs of two healthy cockerels. In another experiment, the discharge was applied to the scarified buccal mucosa at the base of the tongues of two chickens. For a further test, two healthy

pullets were used after having the feathers plucked off their legs. The discharge was then rubbed on to the bare part of one leg of each of these birds, while the other leg was left as a control. Over a period of observation extending to one month no symptoms of fowl pox have been exhibited by any of these subjects.

Later, these birds were inoculated with pox virus at the same sites as mentioned above. The absence of immunity against pox in these birds (manifested by typical lesions of fowl pox) is sufficient to distinguish between fowl-pox and the disease under investigation.

2. Infectious Laryngotracheitis:

Although in some cases of infectious laryngotracheitis, the conjunctival sac and the nasal cavity along with the accessory facial sinuses may exhibit lesions similar to those found in Infectious Coryza, the lesions in the former condition are confined, as a rule, to the trachea, bronchi and to a lesser extent to the lungs. Further important points with regard to differential diagnosis of infectious laryngotracheitis and Infectious Coryza are:

a. Infectious laryngotracheitis is a febrile condition, whereas fever was never encountered in any of the cases of Infectious Coryza studied by the writer, whether naturally attacked or artificially infected.

b. In cases of infectious laryngotracheitis as described by Hutyra, Marek and Manninger '46', Eber '25', and other authorities, there is a spasmodic coughing accompanied by expectoration of blood-stained mucus or blood-clots, and frequent shaking of the head in an attempt to throw out something abnormal present in the air passages which in such cases are found to be occluded with cheesy or fibrinous deposits. The comb is cyanosed and later dyspnoea supervenes. On post-mortem examination, the trachea and bronchi in cases of laryngotracheitis are often found to be partially or completely filled with blood-clots, and blood-stained mucus or cheesy deposits which are easily removed. The tracheal mucosa is haemorrhagic and inflammed. This inflammatory process is sometimes found to extend to the bronchi and lungs giving rise to broncho-pneumonia which is frequently accompanied by small haemorrhages in the lung substance. In the author's experience, the trachea is seldom involved in cases of Infectious Coryza. Nevertheless, the lining of the upper part of this organ sometimes seemed to show evidence of a slight congestion and often catarrhal inflammation.

Histologically the lesions in infectious laryngotracheitis show evidence of degeneration and desquamation of the superficial layers of the epithelial

lining of the trachea along with oedema and small-cell infiltration of the sub-mucosa, whereas microscopical changes in lesions of Coryza are restricted to those of ordinary inflammatory alterations and round-cell infiltration in the sub-mucosa. Furthermore, injection of the exudate into the trachea of a healthy fowl from a case of laryngotracheitis will produce the disease with its typical symptoms after a period of incubation of from two to twenty-one days. On the other hand, in the case of Infectious Coryza, nasal and conjunctival exudates inoculated intratracheally failed to produce symptoms of laryngotracheitis even after a period of observation of one month. On post-mortem examination, no lesions suggestive of this disease could be found in these subjects.

3. Parasitic Tracheitis "Gapes":

This specific condition is caused by a nematode; S. trachea (Montague 1811 '98'). The presence of eggs of the causal parasites in the faeces of the affected bird (Mönnig 1934 '99') and the finding at autopsy of a good number of these red worms that are attached to the mucosa of the posterior part of the trachea when they are found embedded in blood-stained mucus (Hutyra, Marek and Manninger '46'), is sufficient proof to exclude

"gapes" from being mistaken for the disease under investigation, in which case examination of the faeces from infected cases was negative for the presence of eggs; the trachea also being free from worms.

In the case of suspected gapes, an oiled feather or a twisted horse hair may be passed down the trachea and twisted round: on withdrawal, gape-worms if present in the trachea may be found entangled in the feather or horse hair.

4. Avitaminosis A:

Haring, Jaffa and Beach (1920 '6') in the U.S.A. were the first to describe a condition in poultry produced by dietetic errors. These workers, supported later by others in Germany (Seifrid and Schaff 1928 '100'), who described the condition as giving rise to symptoms clinically similar to those observed in cases of "roup" in the fowl, defined "nutritional roup" to be a condition regularly manifested by the presence of whitish deposit in the nasal cavity, conjunctival sac and in the mouth particularly at the base of the tongue. When histologically examined, the lesions show evidence of keratomalacia with desquamation of the superficial keratinised layers. This description is dissimilar to that observed in the disease investigated by the writer; the striking histological feature of which was the presence of a round-cell infiltration and the absence of

keratomalacia from the tissue involved. Pseudo-membranes are sometimes found in the eye of the affected bird, causing the eyelids to glue together. When found in the mouth, these false membranes are seen to have no tendency to extend as in the case of the corresponding membranes found in fowl-pox. At necropsy, pustular eruptions the size of a pin-head are observed on the base of the tongue, the hard palate, larynx and oesophagus. These pustules show the same microscopical changes described above. Urates are also found deposited on the serous surface of the kidneys, liver, pericardium and often the epicardium (Eber '25'). In the advanced stages of Vitamin A deficiency, nervous symptoms and inco-ordination of the muscles, especially those of the legs, appear. The absence of these symptoms from cases infected with the disease under investigation strongly suggest that Vitamin A deficiency is a separate entity.

THEORIES OF AETIOLOGY.

Various theories regarding the aetiology of Infectious Coryza have been presented by workers on the subject, each of whom supports his views by experimental data and personal observations. Therefore it was thought that it would not be inappropriate to briefly summarise

the literature available on the subject for scrutiny and consideration.

While investigating an outbreak of avian diphtheria, Loeffler (1884 '56') was able to isolate from the false membranes in the mouth a bi-polar staining organism which caused extensive necrotic lesions on inoculation into healthy pigeons and rabbits, while inoculation of white mice led to a fatal termination, with the production of numerous necrotic areas in the liver.

In their studies of avian diphtheria in Tunis, Loir and Ducloux (1894 '57') isolated a cocco-bacillus, the description of which was incomplete. At the same time, these authors were positive that the organism was in no way similar to the Klebs-Loeffler's bacillus of the human diphtheria. Fatal results were encountered on intravenous inoculation of this bacillus into pigeons, rabbits and fowls. Guinea-pigs and cattle were found to be resistant to the organism. The workers claimed that a single attack of the disease rendered the bird immune against another attack, and advised the administration of a vaccine prepared from this organism for prophylactic purposes.

Dealing with avian diphtheria, Moore (1895 '101') recovered a Gram-negative organism closely similar to that of fowl cholera, with which he failed to reproduce the disease.

Gallez (1896 '32') was reported by Gratia and Lienaux '37' to have isolated from the nasal exudate of a fowl affected with Infectious Coryza, an organism morphologically similar to the Klebs-Loeffler's bacillus. These workers seemed also to believe that Infectious Coryza, avian diphtheria and contagious epithelioma are separate entities. They also encountered from the false membranes found in avian diphtheria a bacterium similar to that reported by Gallez.

Guerin (1901 '38'), in his investigations on avian diphtheria, reported a cocco-bacillus which he called the "bacillus of avian diphtheria" and expressed the opinion that this organism was neither a pasteurella nor a salmonella, but was similar to that reported by Loir and Ducloux '37'. Although highly pathogenic to laboratory animals, this pathogenicity of the organism was lost after passage through rabbits. To render birds immune against avian diphtheria, the intraperitoneal inoculation of two doses of a vaccine prepared from this organism was recommended by this investigator.

Harrison and Streit (1903 '43') in Canada, and later Streit (1904 '78') claimed to have isolated from outbreaks of roup (avian diphtheria), two types of bacteria with each of which they were able to reproduce the disease. They gave the name B. cacosmus (ill-smelling)

to one type which afterwards they referred to as the "roup bacillus". The virulence of the roup bacillus, it was stated, was enhanced by passage through pigeons. The second type of organism incriminated by these authors as a cause of roup in the fowl was B. pyocyaneous. They also demonstrated that diphtheria anti-toxin has no protective value against the fowl disease which was in no way transmittable to man. These findings are in complete agreement with earlier observations recorded by Gratia and Lienaux '37'. Further, these findings disagreed with those of Colin '102', who claimed that he was able to reproduce diphtheria in the fowl by inoculating them with the Klebs-Loeffler's bacillus. They also described as "wrong and untenable" the theory of Stevenson '85', who believed that this bacillus was the cause of roup in the fowl.

Studying the relationship between avian diphtheria (roup) and contagious epithelioma, Falby (1908 '28') gave an account of a series of experiments which led him to believe that both of these conditions are aetiologically different. He agreed with Bordet '103', who in turn thought that a cocco-bacillus was aetiologically responsible for diphtheria in poultry, which birds were found to remain susceptible to contagious epithelioma after surviving an attack of diphtheria. Furthermore,

Falby disagreed with Carnwath '104' who considered that both contagious epithelioma and avian diphtheria are aetiologically identical.

Hadley (1909 '40') presented details of examinations made on six fowls that died with symptoms of "roup". The reports claim that a coccidium was the causal agent of these, and probably all,other cases of roup. This is shown by the following statement which was quoted from the original report. "In the cases reported, no bacteriological examinations were made. It was apparent, however, that the factor of coccidiosis of the mucous membranes was, in all cases examined, sufficient to produce, without the assistance of bacteria, nearly all the pathological conditions observed. Just as blackhead so-called, is a coccidiosis of the caeca and liver of turkeys, and as white diarrhoea is a coccidiosis of the caeca, small intestines and duodenum of young chicks, so the writer believes that many, and perhaps all cases of the disease popularly called 'roup' are instances of coccidiosis of the mucous membranes of the head region with or without intestinal complications".

Hauser (1909 '44') suggested a colon-like organism as a cause of diphtheria in the fowl. Boggero (1911 '9') isolated a bi-polar staining cocco-bacillus from the blood of a fowl which died from avian diphtheria.

This organism was reported to be pathogenic to all laboratory animals except the guinea-pig.

In a paper on "roup", Booth (1911 '10') has given a full description of the disease, referring to its infectious nature, and he hinted that outbreaks are commonly associated with colds and after the exhibition of birds at "shows", but he did not define its aetiological factor. Salmon (1913 '77') has elaborately discussed "roup or contagious catarrh", and noted the contagious nature of the disease which he thought to be disseminated by means of wild birds, or pigeons flying from one poultry yard to another. He also remarked that the nature of the causal agent is not known. Rice (1926 '75') expressed the same views regarding the aetiology of the disease.

Describing an oedematous condition of the wattles of fowls in Australia, Seddon (1914 '83') recovered from the fluid inside the wattles a pasteurella organism apparently identical with that causing fowl cholera. Reporting on a similar condition, with which conjunctivitis and sinusitis were described as additional symptoms, Thomas (1927 '89') reported an avirulent strain of P. avicida which he found to be similar to that recorded by Seddon.

While investigating "roup", Jackley '47' and '48' concluded that the condition is caused by a pasteurella

organism which was culturally and biochemically similar to P. avisepticus, and by means of which he was able to reproduce the disease at will. Furthermore, he pointed out that the organism may be present on the mucosa of apperently normal fowls, and irritation alone permitting access to the tissues, thus setting up the disease. On the other hand, inoculation of filtrates was stated to have been inocuous. Weaver and Mitchell (1924 '96') and Weaver (1927 '95') reported similar results by demonstrating a bi-polar staining organism in lesions of "roup". This organism reproduced the disease when inoculated into healthy fowls. In a comparative study on P. avisepticus isolated from cases of roup and fowl cholera, Bushnell (1924 '12') was unable eith no notice any difference in the general characters of both strains or to explain what influences the lowering of the invasive power of the organism or to cause it to localise itself to the head region. In 1929 Bushnell in collaboration with Patton '15' noted that vaccines prepared from this organism reduced the death rate in the vaccinated poultry yards to one-fifth of that in the unvaccinated flocks.

Nakamura (1925 '61') has encountered a bi-polar staining organism which he was convinced was the cause of diphtheria in the fowl. It was easily isolated from

the local lesions in the early stages of the disease. On the other hand, Edwin and Johnston (1927 '26') recorded an avirulent form of P. avicida from cases of "avian diphtheria and roup". Filtrates obtained from lesions in these conditions regularly reproduced the disease; symptoms of fowl pox on the comb were recorded to have occurred in a single fowl. Webster, Thomas and Hughes (1929 '105') produced experimental evidence that P. avisepticus may be localised to the head region, giving rise to symptoms of what they termed "roup-cold" cases, which cases may remain carriers of fowl cholera infections.

In contrast, Bakkar (1923 '2') cultivated from the diphtheritic membranes of a dead fowl a bacillus which he could not differentiate from B. diphtheriae. He also recorded the occurrence of lesions similar to those produced by the human bacillus in the guinea-pigs when they were inoculated with this organism. Similarly, Popov (1928 '72') has isolated an organism biologically and biochemically identical with that of Klebs-Loeffler's bacillus from birds that showed symptoms varying from simple Coryza to marked diphthereses involving the mouth and other parts. Anti-diphtheritic serum gave the fowls a protection against this organism.

Kaupp (1918 '51') has given an account of a chromogenic bacillus which he isolated from a case of "roup";

this organism was highly pathogenic to rabbits and showed some pathogenic properties for the tissues of the fowl.

Gwatkin (1923 '39') believed that the causal agent of contagious catarrh (roup) is unknown. In trying to define "roup", Van Es and Martin (1930 '93') said, "Our knowledge regarding the diseases included in the term roup is quite incomplete and hence the attempt to define roup can be regarded more or less tentative". They were cautious in giving a definition of the causal agent, but seemed inclined to believe that it was bacterial by saying "it is quite possible that under certain conditions to which the fowls are exposed, a number of bacteria normally present on the mucous membranes are induced to assume didease-producing functions in the same manner that common colds of folks come about". In this connection, it will be well to mention that Kurse (1914 '54') and Foster (1917 '31') have demonstrated that common colds in the man are due to a filterable virus.

Bushnell and Brandly (1924 '13') and later Bushnell and Hinshaw (1929 '14') admitted that Coryza (colds) is contagious in nature, and describe its causal agent as unknown. Neglected cases of cold lead to "roup", the aetiological agent of which is also unknown, but many bacteria normally present in the nasal chambers may

turn active. Crofton (1924 '16') appears to be the first worker to report that fowl diphtheria is caused by an influenzoid micro-bacillus, the pathogenicity of which was demonstrated by its antigenic powers and was confirmed by the reproduction of the disease in healthy hens. He also reported that vaccines prepared from this organism have effected a marked decrease in case mortality and case resistance.

Discussing "roup" Folley (1926 '30') classified the condition into (a) nutritional roup and (b) roup of an infectious nature, both of which he believed to be due to deficiency troubles resulting in a lowered resistance on the part of the bird; nevertheless, he thought the administration of bacterins for prophylactic and curative purposes had a marked effect in eradicating the disease. In the same year Verge '94' proclaimed that contagious epithelioma, diphtheria and roup of the fowl are due to a common cause - a filterable virus.

Stafseth (1928 '84') stated that "roup" is another name for "colds" and thought that it is caused by a virus. Later, in 1931 '81' he suggested that bacterins would be of some aid in controlling the disease. Reis (1930 '74') working with a limited number of cases of Coryza, reported that he was able to produce lesions of fowl pox in experimental fowls by inoculating them subcutaneously with purulent material taken from the

conjunctival sac of a fowl suffering from the disease. He cautiously agreed with Ward '106', Gallacher '107' and Reinhardt '108', who believed that Infectious Coryza in the fowl is a particular form of contagious epithelioma. Similarly, Medveczky (1933 '60') gave an account of his findings when examining forty-six cases of contagious Coryza in the fowl. These findings led this author to conclude that pure cases of Coryza were not infective; in thirty cases, he was able to demonstrate the presence of the causal agent of fowl cholera in the nasal passages. From eight other cases he reproduced lesions of fowl pox, while in the remaining cases, no evidence of the transmittability of the disease was produced either on cultural or on experimental inoculations. He attributed such cases to be due to chilling, avitaminosis or any factor that lowers the resistance of the birds.

While Beach and Freeborn (1930 '5') did not attribute the cause of "colds and roup" to any specific infection, Beach (1931 '4') supported the view that P. avisepticus may be of some aetiological significance in connection with the disease. Later, in 1936 Beach in collaboration with Schalm '79' stated that Infectious Coryza in the fowl is definitely caused by a haemophilic organism. These authors, in agreement with Elliot and

Lewis '27' gave the organism the name H. gallinarum and noted that it needed for its growth requirements both the X and the V- factors. It was also found by these authors that the organism lost its pathogenicity for fowls after a series of subcultivation: in such cases, the duration of the disease was stated to have reached to as short a period as two days, which period could be steadily increased by frequent animal passage. In agreement with Nelson '63' they found that the haemophilus-induced Coryza was regularly milder than the exudate-produced type. Inoculations with filtrate were reported to have been unsuccessful in reproducing the disease.

While De Blieck and Van Heelsburgen (1923 '18') admitted that roup in the fowl and fowl-pox were due to the same virus, De Blieck (1931 '19') differentiated between the two conditions and in contradiction to Schneider '81' who did not admit the infectivity of Coryza, De Blieck demonstrated the transmittability of Coryza to healthy fowls by various routes and could not define the causal agent, stating at the same time that the condition is not caused by a filterable virus. In continuation of this work, De Blieck in 1932 and 1935 '20' and '21' recovered from the nasal exudate of cases of Infectious Coryza a cocco-bacillus which he

named B. haemoglobinophilus gallinarum (B. coryzae gallinarum). Both the X- and the V- factors were needed by the organism for its growth. Its ability to reproduce the disease was diminished after thirty or forty passages, or even less. Filtrates from the original exudate failed to reproduce the disease. He also refuted the idea that a pasteurella organism could be the cause of the disease.

McGaughey (1932 '97') recorded an organism of the para-influenza group in the upper respiratory tract of fowls affected with Coryza. He encountered the organism in various other diseases and stated that members of the influenza group are not infrequently present in the upper respiratory passages of healthy fowls. He also noted that no definite opinion could be formed of the significance of these organisms in relation to Coryza. Dalling (1934 '17') suggested that the cause of infectious catarrh is in keeping with a virus infection, while Heidkamp (1934 '45') was convinced that the cause of Infectious Coryza was not yet known. In the same year, Lewis and Mueller '55' mentioned that the filtrate of the nasal exudate obtained from cases of the common cold in chickens was incapable of setting up the disease. In 1934, Elliot in collaboration with Lewis '27' recorded in cases of Infectious Coryza the

presence of a haemophilus that regularly reproduced the disease on intra-nasal inoculation into healthy chickens. They named this organism H. gallinarum. Erwin and Stewart (1935 '109') isolated H. gallinarum from cases of Coryza and reported that the disease was regularly reproduced as a result of inoculating this organism into healthy chickens. They reported that intraperitoneal and subcutaneous inoculation of the organism only rarely produced the disease in healthy chickens. This organism was regularly isolated from the facial oedematous swellings twenty-four hours after their appearance in the experimental cases. The workers found this method to be very convenient for diagnostic purposes of Infectious Coryza.

On investigating an outbreak of epidemic cold in chickens, Gibbs (1935 '34') was unable to demonstrate the presence of any haemophilus in the infective exudate. On the other hand, he demonstrated a filterable virus in the nasal exudate of infected fowls. Symptoms typical of Infectious Coryza were reproduced in susceptible subjects on inoculating them with the bacteria-free filtrate. He has also found that the Coryza virus particles were able to pass through acetic-collodion filters with pores of an estimated diameter of 120 m M and were retained by those possessing a calculated pore-size of

80 m M. In another article, Gibbs '35' stated that the presence of H. gallinarum in cases of Infectious Coryza was of secondary importance: the organism being responsible for rhinosinusitis and swollen-heads in this condition.

Investigating Infectious Coryza in the fowl, Nelson published a series of articles dealing with the condition from its various aspects. In 1933 '62', he stated that bacteriological examination of the nasal exudate obtained from artificially infected fowls revealed the presence of a haemophilic organism which he afterwards referred to as H. gallinarum. This worker, however, experienced some difficulty while trying to isolate the organism at the beginning of his investigation; later he succeeded in isolating the bacillus in tightly-sealed blood-agar plates. Inoculation of healthy subjects with this bacterium regularly reproduced the disease, whereas filtrates failed to infect the experimental subjects. In some cases, the filtrate was capable of setting up the disease after it was cultivated in fresh horse-blood at the base of an agar slant. In the same year, Nelson '63' described three forms of the disease, each of which had differences in the period of incubation and the duration of symptoms; another difference in the aetiology of these types was subsequently suggested

by this author. The types are:

1. Coryza of a rapid onset and short duration. This type, states Nelson, is caused by H. gallinarum (1936 '66').

2. Coryza of a slow onset and prolonged duration. In this type, the investigator states (1936 '67') that attempts to recover the haemophilic bacillus were unsuccessful. On the other hand, Gram-negative coccobacilliform bodies were regularly seen in the incubated filtrates obtained from such cases, which material was capable of setting up Coryza in healthy birds. These bodies were referred to later as the X- bacillus. They were non-filterable and resembled the elementary bodies of vaccinia and other virus diseases. Their growth was constantly maintained on chick embryos or in tissue cultures. No growth was encountered on nutrient media enriched with blood and serum; one strain, however, was found to show an adaptation to fluid blood in an agar medium after many generations in tissue culture (1939 '71'). Inoculation of the chick embryos or of tissue cultures reproduced Coryza of slow onset and prolonged duration similar to that type from which these bodies were isolated '69'. Although these bodies were successfully subcultivated on tissue cultures for 100 generations, Nelson '68' states that their growth is

not dependent upon the presence of living cells in these media: thus showing the bacterial nature of the cocco-bacilliform bodies.

3. Coryza of rapid onset and prolonged duration. Both H. gallinarum and the X- bacillus were incriminated by Nelson (1938 '70') to collaborate in inciting this type of the disease. Nasal exudate obtained from this condition regularly showed the presence of these two agents which depended upon each other for their pathogenicity. In this connection, he said "The characteristics of type 3 Coryza were reproduced by injecting a mixture of the two agents. The behaviour of each component was altered by the association, indicative of a synergistic relation".

In spite of the above, Nelson seems to maintain his earlier views '63' that type 3 is a basic form of the disease and the other two are mere variants.

Quoting Nelson in respect to the classification of Coryza in the fowl, Kessen (1936 '53') and Van Dorsen (1937 '92') considered that the disease exists in two types only i.e. Coryza of slow onset and Coryza of rapid onset, both of which do not differ aetiologically; Haemophilus coryzae was stated to be the causal agent.

Purchase '73' on investigating an outbreak of roup in Kenya, demonstrated the primary aetiological agent

of the condition was a virus capable of passing through a Seitz bacterial filter, and through a Chamberland L2 candle. He also reported the frequent appearance in the lesions of an organism which had the morphological and staining properties of P. avicida, but was at the same time less virulent to fowls.

EXPERIMENTAL INVESTIGATION.

Live cases and dead specimens of Infectious Coryza in the fowl were received from various parts of Scotland and England. Material collected from dead birds did not prove satisfactory for the purpose of this investigation; samples usually take about forty-eight hours or even more, to reach the laboratory. During this period, the infective reagent in the carcase seemed to undergo considerable deterioration, as a result of which the reproduction of the typical lesions of the disease is rendered difficult, or uncertain. One outbreak from Shropshire, in which the infected birds regularly exhibited lesions characteristic of an acute form of the disease, was brought to the writer's notice. Nasal exudate was collected from the heads of freshly-destroyed birds in which the clinical symptoms and post-mortem findings were identical to those described in earlier

chapters of this work.

To demonstrate the transmittability of the condition, the nasal exudate was suspended in normal saline solution and the suspension was instilled into the nose of each of two healthy chickens of ten weeks. To ensure the passage of the infective material into the nasal chambers, a few drops of the suspension were also instilled into the palatine clefts of these birds. The method of intranasal inoculation was as follows:-

The experimental subject was controlled by means of the left hand of an assistant, who with his right hand kept the head of the bird in position, putting his right thumb at the back of the head to prevent unnecessary movements. After pipetting off the suspension with a pasteur pipette, it was instilled drop by drop into the nares at intervals sufficient to allow the material to pass through the nasal chambers without interfering with respiration. This technique of intra-nasal inoculation has been adopted by the writer throughout his work, as it was found to be convenient for both the operator and the bird.

The above subjects developed symptoms similar to those observed in the natural cases from which the original material for this work was collected. The temperature remained normal in these birds throughout the

course of the disease.

To eliminate any doubt as to the probability that this condition may not be an independent disease, but a manifestation of fowl pox (as suggested by Reis '47' and Medveczky '60') the writer collected the nasal discharge from the artificially infected fowls and tested this in the following manner:

(a) part of the discharge was applied to the scarified combs of two healthy cockerels.

(b) similarly, the material was applied to the scarified area at the base of the tongue of each of two healthy chickens and

(c) on the feather follicles of one leg of each of two healthy birds after the feathers of the legs had been plucked off; the other leg in each case was kept as a control.

No lesions were observed in any of these cases after a period of observation of one month. Further, inoculation of fowl pox virus in these subjects produced pox lesions in these subjects, thus proving that the condition had no connection with fowl pox. The results of these tests are summarised in Table 2.

It should be pointed out that all the fowls used for experimental purposes in this work were of the Brown Leghorn breed, and were kindly supplied by the Poultry Department of the Institute of Animal Genetics,

King's Buildings, University of Edinburgh, where all these birds were hatched and reared under a strict quarantine system, and where no disease has been reported for many years. Save for the first forty birds supplied, all others were between the ages of six and ten weeks. The subjects were put under observation for a few days before they were used for experimental purposes.

TABLE 2.

Original material	2684 -- 2784	on the scarified combs	2488) 2448)	Negative "
		on the scarified buccal mucosa	2475) 2453)	Negative "
		on the bare surface of the leg	2487) 2486)	Negative "

Note: Subsequent inoculation of these birds with fowlpox virus proved positive, thus showing their susceptibility to this disease.

MODES OF INFECTION.

All workers on Infectious Coryza in the fowl have unanimously agreed that this disease could be transmitted from infected birds to healthy ones by intra-nasal inoculation and by contact. Nelson '64' has also reported that he was successful in transmitting the condition to healthy birds by ingestion and through other extra-nasal

routes e.g. intra-orbitally, intra-tracheally and intra-auditory. The disease was not transmitted on intra-cloacal inoculation. Purchase '73' has transmitted the disease by direct contact and ingestion. Schalm and Beach '76' noted that the causal agent of Coryza in the fowl was air-borne to at least a short distance; therefor the transmission of the disease by indirect contact was accomplished but did not take place readily.

During the course of his investigations, extending over two years, the writer has transmitted the disease by intranasal inoculating of the virulent exudate into susceptible chickens. To confirm the results reported by the different authors regarding the transmittability of the disease, the following experiments were carried out.

Extranasal Inoculations.

The author was able to reproduce Coryza in susceptible subjects by direct contact, ingestion, intra-tracheally, intra-conjunctivally and intra-auditory. Subcutaneous inoculation of the virulent exudate gave varying results.

1. Contact.

Fourteen healthy chickens, in varying sized groups, were put in contact with a number of infected fowls. All developed the disease with its characteristic

lesions after varying periods of incubation. One subject (3565), however, developed the disease after being exposed to infection for forty days, due to the fact that this particular bird was of adult age, and therefore it underwent a mild course of the disease. The condition in the chickens started with frequent sneezing and nasal discharge which was accompanied by conjunctivitis. Contrary to intra-nasal inoculation, sinusitis in such cases developed only after the disease had involved the conjunctival sac. The average period of incubation of the disease produced by direct contact was ten and a half days, as shown in Table 3.

TABLE 3.

No.of Birds	Incubation Period (Days)	Remarks.
3565	40	The disease ran a mild course.
156	26	The disease was acute, subject died after 15 days.
1501	17	Destroyed 3 days after appearance of symptoms.
2500	15	Destroyed. Course usual.
3077	3	Sinusitis lasted for a few days.
590	5	Course usual.
742	5	Post-mortem findings restricted to the presence of petechiae on the laryngeal mucosa.

Continued on Page 48.

TABLE 3 (contd.)

No.of Bird	Incubation Period (Days)	Remarks.
162	8	Both sides of the face were seriously involved.
144	5	Rattling breathing. On post-mortem catarrhal inflammation of trachea was observed.
745	5	Course and post-mortem appearances were usual.
554	4	Ditto.
773	4	On post-mortem, petechiae of the laryngeal mucosa and the presence of clear fluid in the pericardial sac were observed.
776	4	Course and post-mortem appearances were as usual.
782	4	Ditto.

2. Ingestion.

Nine healthy chickens were kept individually in separate cages, in order to obtain an accurate reading of the results and to avoid any contact between these birds. Virulent nasal exudate was mixed with their food, and as shown in Table 4, these subjects developed the disease after a period of incubation ranging between three and six days; the average being five days. In addition to the usual symptoms of the disease, four birds out

of these developed submaxillary oedema with complications in the wattles, but these swellings subsided after a few days. Post-mortem findings were similar to those encountered in cases infected by the nasal route. In a single case (2270) catarrhal inflammation of the trachea was observed.

TABLE 4.

No. of Bird	Incubation Period (Days)	Remarks.
1667	6	Post-mortem showed petechiae on the laryngeal mucosa and heart muscle.
1974	7	Submaxillary oedema with wattle complications.
1982	6	Ditto.
2270	4	Catarrhal inflammation of the trachea was observed on post-mortem.
2336	5	Submaxillary oedema involving the wattles. Yellow inspissated mass present in this region.
2368	7	Course and post-mortem appearances as usual.
2339	4	Unilateral sinusitis and ophthalmia.
3782	3	Oedema followed by the formation of a yellow inspissated mass in the submaxillary tissues
3734	3	Petechiae of the laryngeal mucosa and heart muscle along with yellow fluid in the pericardial sac.

3. Intra-tracheal Inoculation.

Four healthy chickens were used for this purpose. The feathers in the front of the neck were plucked out, to the extent that the upper part of the tracheal region was exposed. The virulent material employed in this experiment was collected from the trachea and nasal exudate of newly destroyed birds. The whole bulk was suspended in normal saline solution, and injected by means of a hypodermic syringe with a sharp, short, stout needle. .5 ml amounts were slowly pushed into the trachea of each bird. During the period of observation, the birds were daily examined, and the operator's hands were disinfected after inspecting each bird. Rattling breathing was heard in three of the subjects before the appearance of the nasal symptoms. One bird (2465) developed submaxillary oedema in addition to this. Catarrhal inflammation of the tracheal mucosa was seen on post-mortem examination of birds 2463, 2465 and 2466, when these birds were destroyed twenty-five days after the appearance of symptoms. Petechiae of the larynx were also observed. The abdominal air-sacs in all cases were found to contain yellow inspissated masses. Negative results were obtained on cultural

examination of this substance. The lungs were normal.

The results of this experiment are summarised in Table 5.

TABLE 5.

No.of Bird	Tracheal Symptoms	Nasal Discharge	Involvement of the Trachea on post-mortem.
2463	3rd day	5th day	Yes
2465	3rd day	6th day	Yes
2466	4th day	6th day	Yes
2485	----	7th day	Slight congestion only.

The average period of incubation was 6 days.

4. Intra-conjunctival Inoculation.

Fresh nasal exudate was suspended in normal saline solution and pipetted off with a pasteur-pipette. This was instilled into the conjunctival sac of each of nine healthy chickens. The lower eyelid of each chicken was pulled out in such a manner as to form a gap between the eyeball and the eyelid. A few drops of the virulent suspension were instilled into the gap thus formed, and afterwards the eyelids were closed and the inoculated eye was gently rubbed to allow distribution of the inoculum. This being done, the birds were put into separate cages to be examined daily. The disease

started with nasal symptoms at first; the course being similar to that produced by intra-nasal infection. Post-mortem appearances showed involvement of the larynx. Congestion of the mucous membrane of the upper part of the trachea was also observed in three cases (3184, 3376 and 2359). As shown in Table 6 below, the incubation period varied between three and five days, on an average of three and a half days.

TABLE 6.

No.of Bird	Incubation Period (Days)	Remarks.
3236	3	Sinusitis was observed on the 6th day.
3184	3	Bilateral involvement of the facial sinuses and eyes were observed on the 7th day. Congestion of the tracheal mucosa was observed on post-mortem.
2333	3	Post-mortem examination showed deep congestion of the laryngeal mucous membrane.
3376	4	Petechiae of the laryngeal mucosa and congestion of the tracheal lining was noted on post-mortem.
2351	3	Unilateral sinusitis and conjunctivitis.
2344	4	Petechiae of the laryngeal mucosa and the heart muscle along with the presence of clear fluid in the pericardial sac were noticed.

Continued on Page 53.

TABLE 6 (contd.)

No. of Bird	Incubation Period (Days)	Remarks
2359	5	Congestion of the mucous membranes of the larynx and trachea.
2380	3	Course and post-mortem lesions as usual.

5. Intra-auditory Inoculation.

After suspending the virulent nasal exudate in normal saline solution, a few drops of this suspension were instilled into the external auditory meatus of each of nine birds. The feathers covering the ears in these fowls were clipped off, thus exposing the external ear in order to facilitate the instillation of the inoculum into its inside. After the suspension was inoculated, the subject's head was kept still on the opposite side for a while with a view of allowing the inoculum to find its way through the ear channel. Three of the birds (3226, 3227 and 3204) showed a mild form of the disease which was manifested only by the presence of nasal symptoms, the duration of the disease in these cases lasting for less than a month. Immunity tests showed that these subjects were not immune for re-infection. In this case, however, the duration of the disease was shorter i.e. under twenty days. Although the duration of the disease

in the remaining six birds lasted for a longer period, the course was similarly mild. One bird (3201) in this lot was successfully re-infected later by subcutaneous inoculation, when the course of the disease on this occasion was of a short duration.

The period of incubation exhibited by the subjects in this experiment ranged between four and eleven days, the average being 6·25 days. This is summarised in Table 7.

TABLE 7.

No.of Bird	Incubation Period (days)	Remarks.
3226	6	A mild course of 25 days. Was re-infected.
3227	6	Mild course of 20 days. Was re-infected.
3204	6	A mild course of 27 days. Was re-infected.
3157	7	Course and post-mortem findings as usual.
3168	5	Ditto.
3146	7	Destroyed 17 days after the appearance of symptoms.
3201	11	Course lasted 65 days. Re-infected by subcutaneous inoculation.
3303	4	Congestion of the mucous membrane of the larynx and trachea.
3393	4	Temporary unilateral sinusitis. Yellow inspissated plugs found in the abdominal air-sacs.

6. Subcutaneous Inoculation.

Lewis and Mueller '55' concluded that subcutaneous and intra-muscular inoculation of virulent Coryza material did not produce the disease. Nelson '65' was able to reproduce symptoms in some cases on subcutaneous inoculation of the virulent material. Purchase '73' found that virulent roup material was fatal to healthy chickens on subcutaneous inoculation.

For the purpose of this experiment, twenty-three chickens of varying ages were inoculated subcutaneously with virulent nasal exudate at the rate of 1 ml per bird, in the pectoral region. Dullness was found to ensue soon after inoculation; listlessness and inappetancy resulting in rapid emaciation. Six fowls (3425, 3227, 3339, 3138, 2813 and 3047) of the twenty-three inoculated died as a result of general septicaemia at intervals ranging between two and twenty-two days. Death was preceded by unconsciousness and post-mortem examination revealed the presence of a yellow inspissated inflammatory exudate, and necrosis of the muscles at the seat of inoculation. Subcutaneous oedema and congestion extended to some distance around the necrosed area. Cultures prepared from these lesions showed the

presence of occasional Gram-positive cocci and coliform bacilli. The heart-blood was microscopically and culturally sterile.. Particular search for the presence of P. avicida reported by Purchase '73' to have been regularly found in the heart-blood, proved negative. Symptoms of Coryza were observed in one of these birds (3372) before death. Five survivors (3215, 3406, 3711, 2809 and 3201) also exhibited symptoms typical of the disease. Altogether, six birds out of the twenty-three tested, developed Coryza. The remainder gradually regained their normal condition before they were destroyed. In these cases, post-mortem examinations revealed thickening at the seat of inoculation, and necrosis was rarely observed. The inspissated inflammatory exudate, if present, was encapsulated with a thick, fibrous capsule. But for these lesions, the birds were otherwise healthy.

TABLE 8.

No.of Bird	Symptoms.	Remarks.
3425	Dullness	Died after 5 days with no nasal lesions.
3369	Dullness	Recovered. No nasal complications.
3372	Nasal lesions on 3rd day	Died after 5 days.

Continued on Page 57.

TABLE 8 (contd.)

No.of Bird	Symptoms.	Remarks.
3239	Dullness	Died after 5 days. No symptoms of Coryza.
3215	Nasal symptoms on 4th day.	Coryza lasted for 12 days. Recovered.
3138	Very dull	Died after 22 days. No nasal complications.
3412	Dull.	Destroyed after 36 days. No nasal complications.
2431	Unthriftiness	Ditto.
3413	Dullness	Ditto.
3647	Unthriftiness	Ditto.
3699	Ditto.	Ditto.
3639	Ditto.	Ditto.
3668	Nasal symptoms on 16th day.	Destroyed.
3433	Depression.	Destroyed after 25 days. No nasal complications.
3442	Ditto.	Ditto.
3406	Nasal discharge on 8th day.	Destroyed.
3711	Symptoms of Coryza on 8th day.	Destroyed.
3184	Unthriftiness.	Destroyed after 25 days. No nasal symptoms.
3087	Ditto.	Ditto.

Continued on Page 58.

TABLE 8 (contd.)

No.of Bird	Symptoms.	Remarks.
2809	Nasal symptoms on 5th day.	The subject survived a previous infection of the disease. No immunity.
2813	Very dull.	Died after 2 days.
3201	Nasal discharge on 9th day.	Already survived a previous infection. No immunity.
3047	Very dull.	Died after 6 days.

PATHOGENICITY OF THE DISEASE.

Workers on Infectious Coryza in America agreed that chickens up to four months old are the usual victims of the disease. In Europe, although De Blieck did not give any indication as to the age limits, it seems that he has been dealing with a young stock as shown by his illustrations. Doyle and Minett (1927 '24') related their experience of a single outbreak of roup and stated that subjects affected with the disease were usually two months old. Purchase '73' has reported his observations that chicks four to six weeks old were most susceptible to roup.

In the disease under consideration, the writer has found that chickens up to three months old are susceptible, adult ones being comparatively resistant. This is shown

by the fact that in the early part of this work, the author has experienced some difficulty in producing the disease in adult fowls; the disease was found to undergo a considerable reduction in its virulence, producing lesions in these subjects of a benign and temporary nature. The virulence of the disease was then enhanced by passage through six to ten weeks old chickens. These subjects served an excellent reservoir for the disease. Turkeys, as shown in Table 1, are as susceptible to the malady as the fowl. Ducks and pigeons had, on the other hand, a natural resistance.

Intra-nasal inoculation of virulent exudate did not seem to affect laboratory animals: 1 ml of a saline suspension of the exudate was instilled into the nostrils of each of two rabbits and two guinea-pigs. A few drops of the same suspension were also instilled intranasally into two white mice. No symptoms suggestive of the disease were exhibited by these subjects.

DISTRIBUTION OF THE CAUSAL AGENT IN THE BODY OF THE FOWL.

Purchase '73' tabulated his experimental findings with regard to the distribution of the causal agent of roup in the internal organs of the infected chicks.

The results indicated the presence of the virus of roup to a small extent in the blood, brain and liver. Experiments with the lungs gave negative results.

The writer's experience in this respect varied from the findings of Purchase. The internal organs of chickens artificially infected with the disease under investigation did not seem to contain the causal agent. As the nasal chambers are the predilection seat of the disease, it is consequently a fact that the exudate in the nose and mouth is infective. Similarly, any discharge from the eye, when this organ is involved, is also infective. The infectivity of the tracheal exudate, when present, was demonstrated by intra-nasally inoculating this substance into two healthy chickens, when these subjects exhibited symptoms of Coryza after an incubation period of four and six days. On the other hand, intra-nasal inoculation of an emulsion prepared with the yellow inspissated masses which were observed in the air-sacs of infected subjects, did not produce the disease in six healthy chickens. These birds readily contracted Coryza when they were later exposed to infection. Similarly, the pericardial fluid was innocuous to two healthy birds on intra-nasal inoculation. Negative results obtained on intra-nasal inoculation experiments with emulsions of the various internal

organs, indicated the absence of the causal agent of the disease from the blood, bone-marrow, spleen, liver, lung and brain. No immunity against the disease was exhibited by these subjects when they were later inoculated with virulent exudate.

The agents employed for the purpose of this experiment were obtained from fowls with severe symptoms of the disease. Table 9 summarises the results of this experiment.

TABLE 9.

Material Inoculated.	No. of Bird	Symptoms of the Disease.	Remarks.
Tracheal exudate	2744	4th day	
	2102	6th day	
Emulsion of plugs in air-sacs	220	-	Reacted to infection with virulent exudate.
	154	-	
	162	-	
	144	-	
	2796	-	
	2807	-	
Pericardial Fluid.	2559	-	
	2661	-	Ditto.
Emulsion of spleen, liver and bone-marrow.	2230	-	
	2377	-	Ditto.
Emulsion of lungs and brain.	1992	-	
	1970	-	Ditto.

Viability of the Causal Agent.

Little reference has been made by workers to the viability of the causal agent of Coryza, but it has been generally agreed that the virulent nasal exudate retained its infectivity for about seven days in the ice-chest. Schalm and Beach '79' stated that cages contaminated with virulent exudate ceased to be infective to healthy chickens in twenty-four hours after the infected fowls have been removed.

The writer's observation on experiments with virulent exudate kept at ice-box temperature showed that the material could retain its pathogenicity to fowls for as long as seventeen days: Only one subject out of two inoculated with this material was observed to show symptoms of the disease twenty-three days after inoculation; the other bird did not react. Two healthy chickens were inoculated with virulent exudate after it had been preserved for thirteen days in 50 per cent glycerin, and kept at ice-box temperature. Symptoms of Coryza were exhibited by one chicken twelve days after inoculation, whereas in the other bird the incubation period was nineteen days, after which period the subject showed a benign form of the disease accompanied by rapid emaciation. On destruction, the larynx of this subject was deeply congested, and in addition the abdominal and thoracic air-

sacs were found to be packed with yellow inspissated masses.

At room temperature, the virulent material was not viable after ten hours. Virulent exudate was sprinkled in an already-contaminated cage, and this was left unoccupied for ten hours. At the termination of this period, two healthy chicks were put inside this cage. Symptoms of Coryza appeared in these birds after twelve and fourteen days respectively. No positive results were obtained when healthy chickens were put into contaminated cages that were left unoccupied for forty-eight, twenty-four and eighteen hours. At the same time, no symptoms of the disease were produced after the inoculation of virulent exudate that had been left for fifty-two hours at room temperature.

Heating the virulent exudate at 60°C. for 30, 15 and 10 minutes rendered this material harmless to susceptible birds, whereas heating at the same temperature for 5 minutes did not seem to influence its virulence; two healthy chickens developed Coryza after being inoculated for three or four days with this material. Incubation of the virulent exudate for 10 hours at 37°C. destroyed its pathogenicity to chickens; incubation at the same temperature for 5 hours did not affect the pathogenicity of the exudate, and on inoculation into two

chickens, these subjects developed symptoms of Coryza after two and three days.

The infective exudate seemed to retain its pathogenicity for chickens after separate exposures to the action of •5% solution of carbolic acid and 1/2000 solution of patassium permanganate for 2 hours. The incubation period on this instance varied between three and four days.

Non-reactors to the above experiments were found to maintain their susceptibility to the disease when they were exposed to infection by contact or on intranasal inoculation. This fact indicates that no immunity was conferred on the birds as a result of inoculating them with the treated exudate.

BACTERIOLOGICAL INVESTIGATIONS.

Nelson '62' and '63', Lewis and Mueller '55' and others claimed that smears prepared from the nasal exudate of fowls infected with Coryza were found to constantly show on microscopical examination, the presence of Gram-negative cocco-bacilli, which were subsequently defined as a haemophilic organism.

In the disease under investigation, the writer was rarely able to detect cocco-bacilli in smear preparations.

In the first few days of the disease, microscopical examination regularly revealed the presence of Gram-negative rods suggestive of a coliform. Diphtheroids, Gram-positive cocci and yeast were also met with. In chronic cases, however, the inflamed nasal mucosa makes a suitable medium for the various saprophytes and other bacteria to adapt themselves to a state of existence in the nasal chambers, thus the bacterial flora of the nose is increased in the advanced stages of the disease.

While Crofton '16' regularly isolated a haemophilic organism from cases of "avian diphtheria", Purchase '73' did not report the isolation of such an organism; instead he reported a bi-polar staining organism to be always present in the nasal exudate and mouth lesions. On the other hand, unsuccessful attempts were made by Marcato (1936 '59') to isolate a haemophilic organism from a particular outbreak of Coryza in fowls. Beach and Schalm '7' and Delaplane and collaborators '22' advised for the successful isolation of H. gallinarum from the exudate, the incubation of the inoculated blood-agar plates under a partial atmosphere of 10% carbon dioxide. Nelson '63' stated that he succeeded in isolating the haemophilic organism from tightly-sealed plates which he designed for the purpose, whereas De Blieck '21' found that agar containing 25% defibrinated blood, and heated at 70°C. served as an

excellent medium for the isolation of B. haemoglobinophilus gallinarum from the nasal exudate. Using Fildes agar and blood-agar, McGaughey '97' was able to demonstrate a para-influenzoid organism in the trachea and throat region of fowls.

With the above facts in mind, the writer made it his aim to make a thorough search for the haemophilus in the cultures prepared from the nasal and other exudates taken from sixty cases examined at different stages of the disease. "Chocolate-agar", Fildes agar and Serum-agar plates were used for the purpose of this work. All these media were prepared according to Mackie and McCartney '58'.

In order to test the value of the various methods suggested for the isolation of the haemophilic bacteria, several plates were inoculated in each case. A loopful of the exudate was inoculated into the media in successive strokes, and the plates were incubated at 37^{o}C. for 24 - 48 hours; the edges of one "chocolate-agar" plate were tightly sealed with plasticine, the other being incubated under a pressure of 10% carbon dioxide, while the last one was incubated aerobically. When cultures were made from cases on the first or second day of the onset of the disease, they were found to exhibit differences in growth under the various conditions mentioned.

Plates incubated aerobically did not show, however, any haemophilus colonies, except on two occasions; other colonies were those of diphtheroids, coli and Gram-positive micrococci and Staphylococci. On the other hand, colonies similar to those of the influenza group in their growth characters were encountered in the plates that were sealed or those that were incubated in carbon dioxide. Out of the sixty cases examined, twelve specimens yielded colonies suggestive of the influenza growth after micro-aerophilic incubation. Two aerobically incubated plates, however, showed the presence of similar colonies. On Fildes agar, these colonies with the exception of three strains, appeared like flat, circular discs with sharp and round edges. The diameter varied in the individual colonies; usually about 1 mm. after 24 hours incubation, although bigger dimensions were seen when the plates were incubated for more than 24 hours. The colonies were colourless and translucent, with fine granulations in the centre. Generally, as described by Scott and McCartney '82', the consistency of these colonies resembled thin oil paint and was neither sticky nor watery, and they could be easily emulsified on the slide. Three of these strains exhibited some difference in their growth characters; being comparatively opaque,

moist and of a creamy colour with a tenacious consistency. They did not emulsify easily; forming small granular masses on the smears. Microscopical preparations from all the varieties of colonies showed Gram-negative cocco-bacilli singly distributed. Slender, long filaments were also met with, especially in old cultures (Fig.10). A tendency for deeper staining at either end was sometimes observed. Colonies with such a description were picked off to be tested for their growth capacity on various media. A colony was emulsified in 10 c.c. of sterile saline solution and a drop of this suspension was inoculated into plain agar and serum-agar slants, using a tube of Fildes agar as a control. After a period of incubation of 24 - 48 hours, these media were examined; the negative results obtained on serum-agar and plain agar were sufficient to indicate that the organism under consideration belongs to the influenza group, and thus was submitted for further tests as follows:

(a) Satellitism.

A colony of each of the strains under test was emulsified in 10 c.c. of sterile normal saline solution, and in this suspension a Staphylococcus colony was also emulsified. One drop of this mixture was inoculated

on a blood agar plate with a view to demonstrate the phenomenon of the satellisation; the growth of giant colonies of the haemophilus bacteria around colonies of cocci, which is a characteristic of certain species of the haemophilus organism. This was exhibited by all the strains tested. It was found also that none of them produced haemolysis in blood agar media.

(b) Growth Requirements.

The need for the accessory growth factors required by the organism under consideration was determined according to the method of Fildes (1923 '29') which showed that all the strains were alike in their dependence upon the accessory substance for their growth; all required the V- factor only as was observed by their ability to grow in the absence of the X- factor. A small amount of growth from Fildes agar was picked off at the end of a platinum needle in such a way as to avoid any accessary substance to be carried along with the inoculum. This inoculum was then emulsified in 10 c.c. of sterile normal saline solution, and a loopful of the suspension was inoculated into each of the following four peptone-water media which were enriched as follows: to two of these tubes ·1 c.c. of a 1% solution of haematin (B.D.H.) was added. To one of the tubes

containing haematin and to another containing peptone water only, 1 c.c. of yeast extract was added. This extract was prepared according to the method of Thjötta and Avery (1921 '89') and was filtered through a Berkefeld N candle, as suggested by Fildes '29'. After incubation for 24 - 48 hours, subcultures from each tube were made on "chocolate-agar" slopes; growth on subculture proved the multiplication of the organism in the test media. All strains tested grew well in the media containing the yeast extract and in those containing both haematin and yeast extract. No growth was encountered in the ordinary peptone-water media nor in those containing haematin alone. This clearly indicated that the organisms under test needed the V- factor only for their growth. These tests were repeated twice over a period of six months with no variation in the results. After a long course of subcultivation on Fildes agar, the organisms were observed to grow less profusely; the addition of yeast extract to the medium helped to restore the condition of the growth to its original. Addition of yeast extract to plain agar made this medium also suitable for the growth of the organisms under test.

(c) Biochemical Reactions.

Sugar fermentation tests of the haemophilus group

when carried out in peptone-water media were reported to give varying results due to the fact that this group has the property of forming acid in solutions containing protein, which acidity may be in some cases as great as that produced in the same solutions after the carbo-hydrates were added.

Yeast extract was added to ordinary carbo-hydrate media prepared with peptone-water; at the same time control tubes of yeast extract and peptone-water were also kept. The indicator used was •25% of a 1% solution of Neutral Red. In this test, one strain (A) was found after incubation for 10 days to produce acid from Maltose, Mannite and Inosite. No acidity was observed to occur in Saccharose, Laevulose, Lactose, Glucose, Dextrose, Galactose, Dulcite and Dextrin. Another strain (B) fermented Glucose and Raffinose only, while a third strain (C) fermented Raffinose, Lactose, Glucose and Galactose. The remainer of the strains, however, did not produce any change in the test sugar-media. No growth was encountered when the organisms were inoculated into Uschkinsky's protein-free medium and physiological saline solution, after these were enriched with Fildes peptic digest.

No indole was produced by any of the strains. This

test was carried out by adding Ehrlich's Rosindol reagent to cultures after 24 - 48 hours and 10 days. These negative results were confirmed by the absence of the pink colouration on strips of filter paper which had been soaked in a saturated solution of oxalic acid and which were suspended above the cultures. Nitrates were reduced to nitrites by all the strains after incubation for two or three days. Only three strains produced a slight acidity in Litmus milk.

(d) General Characters.

The organisms under consideration were Gram-negative non acid-fast, and non-motile cocco-bacilli. For the purpose of their primary isolation, these bacteria preferred a micro-aerophilic atmosphere which was not essentially required for their propagation on subculture. Mutation and degeneration forms were sometimes observed in old cultures. Cultures remained viable for as long as 10 days at room temperature. No growth was obtained on subcultivation of cultures after these were heated at 60°C for 5 minutes. In fluid media, growth was of a thin, uniform turbidity after 24 hours, later sedimentation at the bottom of the tube being observed.

(e) Pathogenicity.

As far as the writer's knowledge goes, no experiments

were carried out on laboratory animals to determine the pathogenicity of the haemophilic organisms reported in connection with Coryza in the fowl. McGaughey '97' however, has recorded that certain strains of the para-influenza organisms isolated by him were pathogenic to white mice: the invasive power varied in the individual strains.

De Blieck '21' and the American workers on Coryza stated that the power of invasion of B. haemoglobinophilus gallinarum and H. gallinarum (both of which according to Nelson are closely similar to, or identical to each other) is restricted to the upper respiratory passages of the fowl, even if the cultures were administered subcutaneously or intra-peritoneally '80' and '86'. In some cases, H. coryzae was reported to have been recovered from lung lesions in cases of Coryza (Van Dorsen 1927 '92'). This worker also claimed to have isolated the organism from the spleen of a chronic case that died of Coryza of "rapid onset" and this led him to suggest that Coryza in the fowl is a generalised condition. Gibbs '36' suggested that the haemophilic organisms associated with Coryza in the fowl may be responsible for the oedematous and other facial complications in these birds.

The pathogenicity of eight strains of the haemophilus

organism isolated by the writer were tested as follows:

1. Strains A, B, C, D, E, F, G and H were grown on "chocolate-agar" slopes for 24 hours, the growth from a slope of each was emulsified in 5 c.c. of normal saline solution, and was inoculated intra-peritoneally into two white mice at the rate of •25 c.c. each. The mice inoculated with strain E died after 7 days, those inoculated with G died after 12 days, while those inoculated with the remainder died after 24 hours. Post-mortem examination revealed the presence of a small amount of clear fluid in the chest cavity of some of the mice. The organism was recovered from the trachea, lungs, heart-blood, liver and the enlarged spleen and lymph glands, and from the kidneys of all the mice excepting those inoculated with strains E and G.
2. Suspensions of the above-mentioned strains were mixed together and inoculated: (a) intra-peritoneally into 4 guinea-pigs at the rate of •5 c.c. per animal. (b) two guinea-pigs were inoculated subcutaneously with •5 c.c. each, (c) two rabbits were inoculated intra-peritoneally with a dose of 1 c.c. each and (d) two rabbits were inoculated intravenously at the rate of 1 c.c. per animal.

These subjects remained healthy for a period of eight weeks.

3. •5 c.c. of a mixed suspension of these strains was inoculated intra-peritoneally into 4 pigeons. The subjects remained healthy for a period of observation of over 6 weeks.

4. Saline suspensions of the above mentioned strains were each inoculated into 2 fowls (about 12 weeks old) at the rate of 1 c.c. each, subcutaneously and intravenously. All these fowls were observed to be depressed for a few days after inoculation, but later they regained their healthy appearance. One chicken, however, inoculated intravenously with strain F, died after 10 days, when on post-mortem examination no external or internal lesions were observed. Cultures prepared from the different internal organs of this bird did not show the presence of any haemophilic organisms.

5. Saline suspension of the individual strains of the haemophilic bacteria as well as a mixture of them were intra-nasally inoculated into healthy chickens in order to test their reaction to such inoculation. Out of 23 chickens inoculated intra-nasally, 21 remained normal during a period of observation

of 5 weeks. The other 2, however, exhibited symptoms of emaciation. On post-mortem examination, the internal organs of these birds were found normal and cultural examination did not reveal the presence of any haemophilic organism.

Cultural examination of the tracheal exudate and suspensions of swabs from the throats of diseased birds showed the presence of the haemophilic organisms in these lesions. Cultures prepared from the pericardial fluid and from the yellow, solid masses found in the air-sacs in cases of Coryza were bacteriologically sterile.

As previously stated, the organisms of the pasteurella group were once incriminated as the inciting agents of "roup" in the fowl. Purchase '73' found that inoculation of susceptible animals viz. rabbits, pigeons, and fowls with exudate obtained from lesions of this disease, made easy the primary isolation of a pasteurella organism recorded by him; he found that subjects thus inoculated generally die between one and three days and the heart-blood of such subjects regularly yielded a pure culture of a bi-polar staining organism.

The author's experience in this respect is that a bi-polar staining organism was but on rare occasions (two instances) recovered by direct inoculation of serum

agar plates with the exudate. Attempts to recover the organism by animal inoculation were seldom successful due to the fact that the virulent exudate was found to be harmless to small animals and birds. Exudate obtained from the nose, eyes and infra-orbital sinuses of seriously affected chickens was inoculated as follows:

(a) 2 c.c. of the virulent exudate were inoculated subcutaneously into each of two rabbits and two guinea-pigs. No reaction on the part of these subjects was exhibited for as long as 4 - 6 weeks, when they were destroyed and cultures from the heart-blood and internal organs did not show any bacterial growth.

(b) •5 c.c. and •25 c.c. of virulent exudate were each inoculated subcutaneously into a pigeon. The subjects did not show any reaction to this inoculation when they were destroyed after 6 weeks. Cultures made from the heart and internal organs of these birds were bacteriologically sterile.

(c) •5 c.c. and •25 c.c. of exudate, each of which was inoculated intra-muscularly into the pectoral region of a pigeon, gave different results. One pigeon died the following day with lesions of septicaemia i.e. subcutaneous congestion on post-mortem

examination. No growth was obtained from cultures prepared from the heart-blood and internal organs of this pigeon. The other subject was in no way affected by this inoculation.

(d) 2 c.c. of the virulent exudate were inoculated intraperitoneally into a rabbit. This subject died after 2 days, and on post-mortem examination purulent peritonitis and subcutaneous congestion were observed. Smears prepared from the peritoneal exudate were found to show occasional Gram-negative bi-polar staining rods. Serum agar plates inoculated with the heart blood of this rabbit showed several colonies of a pasteurella organism. A second rabbit, inoculated with 1 c.c. of heart-blood from the first subject, did not react over a period of 8 weeks.

General Description of the Bi-polar Staining Organism.

The organism is a Gram-negative, non acid-fast and non-motile ovoid rod with rounded ends. The bi-polar staining character was distinctly seen when the organism was freshly isolated. On subcultivation on

artificial media this character was not observed after some time. The bacillus did not grow on the McConkey medium. On plain agar, the colonies appeared translucent and growth was moderate after 24 hours, with a butryous consistency gradually giving a greyish-yellow colouration. It was easily emulsified on the slide. Growth on Gelatin was confluent and resulted in partial liquefaction of the Gelatin stab. After 24 hours, slight turbidity was produced in broth, later this turbidity was increased resulting in a powdery and granular deposit. A thin surface pellicle was noticed at the top of liquid media; this broke easily on gentle shaking. No haemolysis was produced on blood-agar. On potato, scanty yellowish growth was observed, getting deeper in colour on long standing. Although the organism is aerobic, growth was unimpaired under micro-aerophilic incubation. Heating a suspension of the organism for 7 minutes at 60°C, did not yield any growth when inoculated on serum-agar or Loeffler's serum.

Biochemical Reactions.

Indole was not produced from cultures even after 15 days. Methyl red and Voges-Proskauer tests gave negative results. Ammonia was not produced before 12 days from peptone-water. Tested by Greiss-Ilosva method,

the organism reduced nitrates to nitrites. A very minute trace of hydrogen sulphide was produced: the test was performed by including between the cotton-wool plug and the tube of the medium, a strip of filter paper that had been soeaked in 10% solution of lead acetate. For this test, liver-extract agar was employed. Methylene Blue was incompletely reduced, giving a green colouration. Catalase test was positive after 24 hours. Acid was produced in Litmus milk after 3 days, and no clotting was observed. The organism fermented Mannite after 8 days, Saccharose and Sorbite after 6 days, Glucose, Aesculin and Maltose after 2 days, while Lactose, Laevulose, Inulin, Xylose, Salicin, Arabinose, Inosite and Dulcite were not fermented. No gas was produced in the process of fermentation, as will be observed in the following Table.

Staining:	Gram-negative and non acid-fast.
Motility:	Negative.
Indole:	Ditto.
M.R.:	Ditto.
V.P.:	Ditto.
Nitrate Reduction:	Positive.
Ammonia:	Positive. (after 12 days)

Hydrogen Sulphide:	A slight trace.
Methylene Blue Reduction:	Partially positive. (green colouration).
Catalase:	Positive.
Saccharose:	Acid and no gas.
Glucose:	Ditto.
Maltose:	Ditto.
Mannite:	Ditto.
Sorbite:	Ditto.
Aesculin:	Ditto.
Dulcite:	Negative.
Lactose:	Ditto.
Arabinose:	Ditto.
Inosite:	Ditto.
Xylose:	Ditto.
Laevulose:	Ditto.
Inulin:	Ditto.
Salicin:	Ditto.

Pathogenicity.

Boggero '9' reported that the bi-polar staining organism isolated by him was pathogenic to fowls, pigeons, ducks, rabbits and mice. Beach, Lothe and Halpin (1915 '8') stated that no ill-effects were produced by the organism they recovered, provided it was administered into

chickens on the unabraded membranes of the mouth, nose and eye, while subcutaneous inoculation and the application of cultures on to the abraded tissues produced fatal results in chickens and pigeons. The organism isolated, by Weaver '95' was reported to be very pathogenic to rabbits, guinea-pigs and pigeons on subcutaneous inoculation. Edwin and Johnston '26' reported a strain, the pathogenicity of which was of no significance on inoculation into fowls. Purchase '73' isolated a bi-polar staining organism that was highly pathogenic to laboratory animals and birds. He stated this organism hindered to a great extent the transmission experiments carried out in connection with roup due to its pathogenicity.

Save for its fatal effect on one rabbit, that was inoculated with virulent exudate, the bi-polar staining organism reported here by the writer was found to be harmless to pigeons, chickens, rabbits, guinea-pigs and mice on intra-peritoneal, intramuscular, intravenous and subcutaneous inoculations. Similarly, negative results were obtained on the intra-nasal inoculation of this organism into healthy chickens. The data of the experiments conducted in this connection are summarised as follows:

No.of Animals.	Dose.	Inoculation Route.	Result.
2 chickens	1 c.c. each	Intra-nasally	-
1 chicken	•5 c.c.	Subcutaneously	-
1 chicken	•5 c.c.	Intraperitoneally	-
2 pigeons	1 c.c. each	Intramuscularly	-
1 pigeon	•5 c.c.	Subcutaneously	-
1 pigeon	•75 c.c.	Intraperitoneally	-
1 rabbit	•75 c.c.	Subcutaneously	-
1 rabbit	•75 c.c.	Intraperitoneally	-
1 rabbit	•5 c.c.	Intravenously	-
1 rabbit	1 c.c.	Ditto.	-
2 guinea-pigs	1 c.c. each	Intraperitoneally	-
2 mice	•25 c.c. each.	Ditto.	-

Serological Characters.

To test the power of this organism for stimulating the production of antibodies in animals, growth on a big slope of liver-extract agar was emulsified in 3 c.c. of sterile normal saline solution. Gradually increasing doses of this living suspension were inoculated subcutaneously into a rabbit at 4 day intervals. After 5 inoculations of •1, •2, •5, •8 and 1•4 c.c., the rabbit

was bled and its serum was tested for the presence of agglutinins. Positive agglutination was encountered at a dilution of 1/320 only.

This organism was not recovered from the tracheal exudate, nor from any other lesion in affected cases.

Diphtheroids were among the bacteria frequently met with on bacteriological examination of the virulent exudate in cases of Coryza. They were recovered from 40 out of the 60 cases examined. These organisms were seen as Gram-positive non acid-fast, non-motile and rod-like pleomorphic organisms. The club-shaped swellings at the poles and the barred staining characteristics of this group were infrequently observed. Bacilli from 18 hour old cultures on solidified serum did not show segmentation when stained by Neisser's method. On solidified serum, growth starts as small, pin-point colonies, later becoming larger in size, when they appear as opaque, circular discs slightly convex, and with entire edges. In old cultures, the growth gives a dirty-yellow colouration and the solidified serum was observed to be slightly liquefied. Gelatin was also slightly liquefied. Agar was found to be an unfavourable medium for the growth of this organism. Haemolysis was not produced on blood-agar. Dextrose, Maltose and Saccharose were not attacked

by this organism. No ill-effects were produced in a rabbit and a guinea-pig that were inoculated subcutaneously into the flank region with •5 c.c. of saline suspension of a 24 hour old culture of the organism on solidified serum. Intra-nasal inoculation into healthy chickens did not produce any harmful effects: saline suspension of the organism mixed with suspensions of other bacteria recovered from the nasal exudate of infected fowls did not produce any harmful effects in 12 healthy chickens that were inoculated intra-nasally.

Escherichia coli (B. coli communis) has been frequently recovered from lesions caused by the disease, especially in the nasal and tracheal exudates. Although this organism is usually a normal inhabitant of the intestinal tract, most probably it migrated to the upper air-passages after the inflammation of their epithelial lining, and thus the organism accomplished a parasitic existence and added to the gravity of the disease. None of the workers on Coryza in the fowl has reported Escherichia coli to be of any pathogenic significance in this condition. This fact has also been confirmed by the writer, as on intra-nasal inoculation of this organism into healthy chickens, no symptoms suggestive of any pathological process were observed. Nevertheless, it

might be worth while to mention here that Marcato '59' reported Escherichia coli from three fowls that were supposed to be suffering from Infectious Coryza. He claimed that this organism could reproduce the disease with its typical lesions in healthy fowls. Although this worker has reported lesions of Vitamin A deficiency in the natural cases he dealt with, he does not seem to admit that the disease was Avitaminosis. This investigator supports his point of view by stating that Escherichia coli, normally present in the nasal passages, was induced under certain favourable conditions to maintain a pathogenic existence, thus causing Infectious Coryza in the fowl.

Staphylococcus albus and Staphylococcus citreus were found in about 25% of the cases examined, especially in the advanced stages of the disease. The organisms appeared as harmless parasites; this was shown by inoculating these organisms intra-nasally into healthy chickens. On one occasion, Pseudomonas aeruginosa was recovered from purulent panophthalmia in the eye of a badly-infected case. This organism was also found to be harmless on intra-nasal inoculation. Sarcina lutea has been very rarely met with in cultures, as this organism is normally free-living. It is most probable that it had

adapted itself to a saprophytic existence in the unhealthy nasal mucosa.

On rare occasions, non-haemolytic Streptococci were recovered from lesions of Coryza. Intra-nasal inoculation of these organisms into healthy chickens did not produce any ill-effects.

Bacteriological study of the tracheal exudate gave results approximate to those encountered on examination of the nasal discharge; no bi-polar organisms could be recovered. On a single occasion, the writer was able to demonstrate a spirochaete in the tracheal exudate (Fig. 11). These spirochaetes were found in microscopical preparations which were made from a thick coating of catarrhal exudate of the tracheal mucosa. The smears were stained with Giemsa stain, and this was diluted with twice its volume of distilled water, and the stain was left on the slide for over ten minutes. The spirochaetes appeared distinctly in the field. No intracellular forms of this organism were noticed. They have not been seen in the nasal or the mouth secretions as was reported by Gibbs '33'. The spirochaetes were not found in the examination of further specimens and consequently it was not possible to carry out further studies with a view to the identification of this organism. At the same time, the writer

would like to record his opinion that as the Spirochaetes were only found on one occasion, it is unlikely that they play an important part in the pathogenicity of the disease under study.

FILTRATION EXPERIMENTS.

As it was previously shown that the intra-nasal inoculation of all the bacteria isolated from the virulent exudate on culture did not produce the disease in healthy chickens, it was thought therefore that a filterable agent may be connected with the condition. In this respect Gibbs '34', '35' and '36' claimed to have achieved successful results after adopting an improved technique for the ultra-filtration of the Coryza virus, by which method he determined the iso-electric point of the smooth, virulent suspension of the Coryza exudate after having it triturated in distilled water with Pyrex glass powder. For the purpose of his filtration experiments, Purchase '73' was able to get a virulent filtrate with which he reproduced "roup" in healthy chickens. Trituration of the virulent exudate with sand was enough to liberate the virus bodies free in the suspension.

In the early part of this experiment, the writer

experienced a great difficulty in obtaining a filtrate capable of setting up the disease in susceptible birds. This difficulty was solely due to the inability to obtain a suitable suspension which was able to pass freely through the candles; the exudate being highly tenacious. The virulent exudate had been subjected to various methods of treatment in order to get the desired suspension.

One of these methods consisted of diluting the substance with normal saline solution or broth to the proportion of 1/50, gradually adding the diluent and constantly triturating the mixture till a uniform suspension was obtained. This suspension was left in the ice-box for a few hours, or overnight, when a great deal of the mucous debris settled at the bottom of the container, leaving the clear fluid part of the suspension free for filtration. Although filtrate capable of reproducing the disease in healthy chickens was produced in some instances, the results of this method of preparing the suspension were varied, and not of sufficient encouragement to justify the continuation of this method. Consequently, the more improvised method of triturating the exudate with sterile sand was resorted to, but with the same inconsistent results as experienced before. Later, it was thought that the method recommended by Gibbs to determine the iso-electric point of the virulent exudate before its

filtration, might improve the situation. Although some success was attained on this occasion, it was thought that a more efficient method with constant results could be obtained. Pieces of Pyrex glass were broken into small, coarse particles before the addition of the virulent exudate. After the thick mucus was thoroughly ground, the diluent was gradually run into the mortar, a little at a time, and the whole was thoroughly mixed. In this way, the exudate was brought to a final dilution of 1/50. The suspension was then centrifuged for 10 minutes at a speed of 2000 revs. per minute. The clear supernatent fluid was pipetted off, and the pH was adjusted to neutral. In order to remove the viscid substance from this fluid, it was coarsely filtered through paper pulp and sand (Mackie and McCartney '58'). After this treatment, the fluid was found to pass easily through the candles. Berkefeld and Chamberland candles and the Seitz E.K. asbestos pad were usually employed. Before use, each candle was thoroughly washed and tested for leakage by forcing air through them when they were submerged in water; candles permitting the escape of large air-bubbles were rejected. The candles selected were finally boiled to adjust their reaction before sterilisation. To control the efficiency of the candles and pads used, suspensions of B. prodigiosus at a moderate

opacity were added to the fluid material prior to filtration. Consequently the filtrate obtained was tested for the growth of the test organism: on each occasion amounts between •5 c.c. and 3 c.c. were inoculated on plain agar and "chocolate-agar" medium, and these were incubated for 24 - 48 hours. This test, however, was not applied in cases where the coarse type of the Berkefeld candles were employed.

This method of filtration was found to be more useful than the other methods mentioned above. Depending upon the type of the filter used, 20 c.c. of the virulent suspension took between 10 and 20 minutes to filter under ordinary tap-water pressure. Generally, the infective filtrates were observed to produce symptoms similar to those obtained on inoculation of the unfiltered exudate. Virulent material collected from positive reactors to infection by filtrate, was found to reproduce the condition in susceptible subjects both in the crude and the filtered form.

Both the Chamberland L3 and L5 candles were employed for the filtration of the virulent exudate. Fifty-four birds were inoculated with L3 filtrate; lesions of Coryza were observed in 38 of these subjects, developing a course of the disease similar to that induced by the inoculation of the crude exudate. The L5 candle was also

employed in order to ascertain if the causal agent could be permitted to pass through the small pores of this type of candle. For this purpose twelve healthy chickens were inoculated with Chamberland L5 filtrate, when 9 out of these subjects were found to exhibit symptoms of infection. The findings prove the possibility of the filterable agent to permeate through the pores of the Chamberland L5 candle.

The incubation period in this particular experiment varied between 2 and 20 days at an average of 6 days.

Of the Berkefeld varieties, both the N and the coarse candles were employed. Filtrate obtained through the coarse candle was inoculated into 41 healthy chickens. Of these subjects, 29 positively reacted to infection by this type of filtrate. The incubation period was in some cases as short as 2 days, whereas in others symptoms of the disease did not appear before 23 days. The causal agent of the disease was also found to pass through the Berkefeld N candle; this was demonstrated by inoculating the filtrate obtained from this candle into 12 healthy chickens, which subjects were found to show typical symptoms of the disease.

The results of experiments with the Seitz filtrates showed that the causal agent could pass through the E.K. pads of this bacterial type of filter. The Seitz filtrate

was inoculated into 41 chickens out of which 15 only were found to exhibit symptoms of Infectious Coryza after an incubation period of from 2 - 23 days.

In all, 160 birds were inoculated with the different grades of filtrates; 115 of these subjects developed typical lesions of Infectious Coryza and thus giving a positive result in approximately 72% of the subjects inoculated.

Experience has shown that the filtrate-induced Coryza is in no way different from that produced by inoculation of the unfiltered exudate; in both types the clinical symptoms and post-mortem findings did not differ macroscopically or microscopically (Figs. 12, 13). Sections made from the different lesions of both conditions did not show the presence of inclusion bodies when stained by Goodpasture's and Mann's methods of staining. The incubation period at the same time was not found to exhibit any appreciable difference under these circumstances.

The addition of the various strains of the haemophilus organism reported in this work did not seem to influence the virulence of the filtrate; inocuous filtrate, obtained on some occasions, was found to maintain its harmlessness to susceptible subjects after mixing it with suspensions of these organisms.

IMMUNITY:

Workers on Coryza in the fowl concluded that no lasting immunity is conferred on fowls recovered from the disease. According to De Blieck '21', the duration of the first infection influences the degree of immunity to a considerable extent - the longer the duration of the symptoms, the more solid the immunity. He found that the culture-induced Coryza results only in a partial immunity towards the exudate-induced type, while at the same time this immunity was sufficient to protect the experimental subjects against re-infection with cultures of B. haemoglobinophilus gallinarum. The duration of this type of immunity was found by Kessen '53' to be as short as 6 days.

Experiemts conducted by the writer in connection with immunity on fowls recovered from the filtrate-induced type of the disease and those recovered from that produced by the exudate, gave irregular results. In general, partial immunity was accomplished in such subjects; as solid immunity was encountered in fowls recovered from a very serious infection by filtrate, in other cases the resistance was manifested only by a prolonged period of incubation and a shorter course of the disease when these subjects were re-infected with the unfiltered exudate. Fowls that recovered from a natural infection or from

experimental inoculation with virulent exudate were found to exhibit varying results as above; no immunity was encountered in two subjects that were re-infected with crude exudate, 10 days after their recovery from an initial infection by the same type of material. On the other hand, chickens that recovered from a serious infection by exudate were found to be resistant to re-infection by the same material, 6 weeks after they had recovered from a previous infection.

The writer agrees with De Blieck that Infectious Coryza gives chickens an irregular immunity and this immunity depends mostly on the extent and the duration of symptoms in the initial infection.

DISCUSSION.

The disease investigated by the writer reported in this work was found to be identical with that described by the Continental and the American workers as INFECTIOUS CORYZA in the fowl. Marcato '59' appears to have mistaken Avitaminosis A for pure Infectious Coryza, as the description given by him is more in agreement with the former condition. The significance of Escherichia coli in relation to the disease reported by this investigator still lacks confirmation. It may be suggested that this organism may

have been a secondary invader which has only assumed its invasive character under the favourable circumstances produced by the pathological condition. The same may be said in connection with the Klebs-Loeffler's bacillus that was reported by Bakkar '2' and Popov '72' to produce lesions in the fowl similar to those observed in cases of Coryza; investigations carried out by Harrison and Streit '43' and Streit '87' and others proved beyond doubt the harmlessness of the Klebs-Loeffler's bacillus when inoculated into fowls.

While there already existed enough obscurity on the subject of Coryza, Reis '74' and Medveczky '60' seemed to formulate the theory that the condition is aetiologically connected with fowl pox. In this connection, it is well known that the instructive classification of roup by Dalling '17' suggesting four different entities, and the highly authenticated facts brought forth by the work of Doyle and Minett '24' regarding the nomenclature and aetiology of the various pathological conditions of the head of the fowl, are settled questions. The findings of Doyle and Minett '24' have also been repeated with particular reference to comb and mouth lesions by Kaura and Iyer '52'.

It seems that Jackley '47', '48' and '49', Bushnell '12', Nakamura '61' and Weaver and Mitchell '96'

were really dealing with outbreaks of fowl cholera, the only lesions of which were restricted to the head region, when in such conditions P. avicida is less invasive. As respiratory symptoms are frequently met with in the various stages of fowl cholera, the nasal type alone of this condition could therefore be easily mistaken for other specific respiratory troubles classified under such loose terms as "roup" and "diphtheria". In this connection, the writer wishes to stress the necessity for a more scrupulous system of terminology to be applied to the various diseases of the head region in the fowl, and the use of terms that are scientifically adequate and indicative of the specific infection without any confusion. Therefore the conditions described by the above workers as "roup" should on no account be classified as independent respiratory troubles, as they are in fact only symptoms of another specific disease.

In agreement with De Blieck, Nelson, Beach and Schalm, the writer finds that Infectious Coryza affects only fowls and turkeys; ducks and pigeons enjoy a natural resistance to the disease.

The writer was not able to confirm the findings of Nelson, Kessen, and Van Dorsen who reported that more than one type of the disease usually existed. The disease was successfully transmitted by extra-nasal inoculation

as previously mentioned, although subcutaneous inoculation did not always set up the disease. The conception of Schalm and Beach, that indirect contact infection may take place, was not confirmed.

During the course of his investigations, the writer was unable to isolate the haemophilic organism reported by the different workers to be the causal agent of Coryza in the fowl. Nevertheless, various strains of a different type of haemophilus organisms were isolated from the nose, throat, and trachea of some infected cases. The organism was also found to occur in lesions of the filtrate-induced forms of the disease. On intra-nasal inoculation, this organism was not found to produce any lesions in healthy chickens. It is closely related to that reported by McGaughey '97'.

On rare occasions, a bi-polar staining organism was isolated from the nasal exudate of infected fowls. This was found to be harmless to chickens, pigeons and other laboratory animals. Similar to the haemophilic organism reported above, this organism was of no significance in producing the disease.

Finally, in agreement with Gibbs and Purchase, the writer was able to reproduce the disease in healthy chickens on inoculating them with bacteria-free filtrate. The duration and seriousness of the disease thus induced were similar to those produced by the inoculation

of crude exudate. De Blieck, Nelson and others reported that the duration of the haemophilus-induced Coryza was always within the range of 5 weeks. In the writer's experience, no such difference was observed between the course of the filtrate-induced disease and that produced by the unfiltered exudate.

CONCLUSIONS.

Judging by the results of his investigations, the writer concludes that:

1. Infectious Coryza of the fowl is a specific disease caused by a filterable agent which was successfully passed through the pores of the Berkefeld V and N candles, the Chamberland L3 and L5 candles and through the pores of the sterilising pad of the Seitz filter. About 72% of the subjects inoculated with the bacteria-free filtrate exhibited symptoms of Coryza.
2. In certain cases, a haemophilic organism can be isolated from the various lesions of infected fowls. This organism differs in virulence and growth requirements from B. haemoglobinophilus coryzae gallinarum (De Blieck), H. gallinarum (the American group of workers) and H. coryzae (Kessen and Van Dorsen), which organisms were said to be the cause of fowl Coryza.

Due to its dependence upon the V- factor only for its growth, its inability to produce haemolysin on blood medium, and on account of its failure to produce indole and its restricted range of fermentation of sugars, especially Saccharose, this organism may be considered to belong to the para-influenza group (Topley and Wilson).

3. In rare cases, an avirulent bi-polar staining organism is met with in lesions of Coryza in the fowl.
4. Intra-nasal inoculation of suspension of the various bacteria isolated from the virulent exudate, does not produce any harmful effects in healthy chickens; thus indicating their insignificant importance in relation to the disease.
5. The causal agent of the disease is found to lose its virulence if left in the cage at room temperature for 18 hours, whereas it is observed to retain its pathogenicity for 17 days if kept at ice-box temperature. Incubation for 10 hours at 37°C. renders the virulent exudate harmless to susceptible birds. Heating at 60°C. for 5 minutes does not affect the virulence of the exudate. This material is found infective after its exposure to the action of •5% solution of carbolic acid, and to the action of 1/2000 solution of potassium permanganate for 2 hours. Inoculation of the virulent exudate after

being preserved for 12 days in 50% Glycerin at ice-box temperature, requires a long period of incubation before it can set up the disease in the inoculated subjects.

6. The disease exists only in one form, the incubation period of which is influenced by the indivual susceptibility of the subjects and the route of infection.

7. Young chickens and turkeys are the only birds susceptible to the disease. Recovered subjects may retain their susceptibility for another infection of the disease in their early life.

8. Transmission of the disease is easily accomplished by direct contact with infected birds, by ingestion, and by intra-conjunctival, intra-auditory and intra-tracheal routes.

A C K N O W L E D G M E N T.

This work has been carried out in the Department of Pathology, Bacteriology and Poultry Diseases, of the Royal (Dick) Veterinary College, Edinburgh.

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REFERENCES.

1. The Advisory Committee on Poultry Diseases(N.V.M.A.), Handbook on Poultry Diseases, 1937, pp 27,28 and 61.

2. Bakkar, M. 1923.
Avian Diphtheria and the Diphtheria Bacillus.
Abs. Bact. Vol. 7, p 185.

3. Barger, E.H. and Card L.E. 1938.
Diseases and Parasites of Poultry,pp 160,223 and 242.

4. Beach, J.R. 1931.
Fowl Pox and Coryza.
11th. Int. Vety. Cong. Vol.3, p 692.

5. Beach, J.R. and Freeborn, S.B. 1930.
Diseases and Parasites in Poultry in California.
Calif. Agric. Exten. Service, Circular 8,p 28.

6. Haring, Beach and Jaffa.
Special Pathology and Therapeutics of the Diseases of Domestic Animals, 1938. Vol.3, p 176.

7. Beach, J.R. and Schalm, O.W. 1936.
Studies of the Clinical Manifestations and Transmissability of Infectious Coryza of Chickens.
Poultry Science, Vol. 3, p 466.

8. Beach, B.A., Lothe, H. and Halpin, J.G., 1915.
An Outbreak of Roup and Fowl Pox in which the High Mortality was apparently caused by a Secondary Invader.
Jl. Inf. Dis. Vol. 14, p 554.

9. Boggero, A. 1911.
Observazioni Sulla difterite aviara.
C.F. Bakt. Vol. 50. ABT. I, p 243.

10. Booth, E.T. 1911.
Roup - Swelled head - or Diphtheria in Fowls.
Amer. Vet. Rev.,Vol. 40, p 503.

11. Blount, P.
Poultry Ailments, p. 186.

12. Bushnell, L.D., 1923.
The Relation between B. avisepticus Types isolated from Roup and Fowl Cholera.
Abs. Bact., vol. 7, p 30.

13. Bushnell, L.D. and Brandly, C.A., 1929.
Coryza (colds).
Kans. Agric. Coll. Bull. No. 247, p 34.

14. Bushnell, L.D. and Hinshaw, W.R., 1924.
Prevention and Control of Poultry Diseases.
Kans. State Agric. Coll. Circular 106, p 25.

15. Bushnell, L.D. and Patton, J.W. 1924.
The Use of Vaccines in Poultry Diseases.
Poult. Sci. vol. 4, p 65.

16. Crofton, W.M. 1924.
Diphtheria of Fowls: Its Cause, Prevention and Cure.
Jl. Path. and Bact., vol. 27, p 456.

17. Dalling, T. 1934.
Diseases of Poultry.
Agric. Progress vol. 11, p 22.

18. De Blieck, L. and Van Heelsburgen, T. 1923.
Vaccination against Roup and Fowl Pox in Chickens.
Abs. Bact., vol. 7, p 185.

19. De Blieck, L., 1931.
Fowl Pox and Coryza.
11th.Int. Vety. Cong., vol. I, p 424.

20. De Blieck, L, 1932.
A Haemoglobinophilic Bacterium as the Cause of Contagious Catarrh of the Fowl (Coryza infectiosa gallinarum).
Vet. Jour. vol. 88, p 9.

21. De Blieck, L. 1935.
Coryza Infectiosa Gallinarum.
12th. Int. Vety. Cong. vol. 3, p 161.

22. Delaplane, J.P., Erwin, L.E., and Stewart, H.O., 1935.
Studies of the Haemophilic Bacilli of Chickens.
Poult. Sci., vol. 14, p 312.

23. Doyle, L.P., 1929.
Roup and Chicken Pox.
Purdue Univ. Dept. of Agric. Extn. Leaflet, No. 147.

24. Doyle, T.M., and Minett, F.C., 1927.
Fowl Pox.
J. Comp. Path. and Therap., Vol. 40, p 247.

25. Eber, R.P., 1934.
Die durch Obduktion feststellbaren Geflügelkrankheiten. pp 126, 128 and 166.

26. Edwin, E. and Johnson, B., 1937.
Studies in the Etiology of Roup and Allied Diseases of Fowls.
Mich. State Coll. of Agric. - Agric.Ext. Sta. Tech. Bull. No. 85.

27. Elliot, C.P. and Lewis M.R., 1934.
A Haemophilic Bacterium as a Cause of Infectious Coryza in the Fowl.
Jl. Amer. Vet. Med. Assoc., Vol.84, p 878.

28. Falby, V., 1908.
Diphtérie Aviaire et Epithélioma Contagieux.
Ann. de Med. Vet., Vol. 57, p 69.

29. Fildes P., 1923.
The Classification of Haemophilic Bacteria based upon their Relation to Blood-pigment and to the "Vitamine" Factor.
Brit. Jl. of Exptl. Path., Vol. 4, p 265.

30. Folley, O.F., 1926.
Vaccination of Poultry for Roup.
N. Amer. Vet., Vol. 7, p 58.

31. Foster, G.B., 1917.
The Aetiology of Common Cold.
Jl. of Inf. Dis., Vol. 21, p 451.

32. Gallez, - see '37'.

33. Gibbs, C.S., 1931.
Saprophytic and Secondary Micro-organisms Occurring in the Respiratory Tracts of Domestic Fowls and Chickens in Health and in Disease.
Jl. of Bact., Vol. 21, p 97.

34. Gibbs, C.S., 1935. (a)
The Etiology of Epidemic Colds in Chickens,
Science Vol. 81. p 345.

35. Gibbs, C.S., 1935.(b)
Ultra-filtration Experiments with the Viruses of
Laryngotracheitis and Coryza of Chickens.
Jl. of Bact., Vol. 30, p 411.

36. Gibbs, C.S., 1935 (c)
Some Micro-organisms Complicating the Course of
Laryngotracheitis and Coryza.
Poult. Sci., Vol. 14, p 312.

37. Gratia and Liénaux, M.E., 1898.
Contribution à L'étude bactériologique de la
Diphtérie Aviaire.
Ann. de Méd. Vét., Vol. 47, p 401.

38. Guérin, M.C., 1901.
La Diphtérie Aviaire.
Ann. Inst. Past., vol. 47, p 941.

39. Gwatkin, R. 1924.
Contagious Nasal Catarrh in the Fowl (Roup).
Rep. of the Ont. Vety. Coll., p 61.

40. Hadley, P.B., 1909.
Roup of Fowls.
Expt. Sta. Rec., vol.21, p 387.

41. Hadley, F.B. and Beach B.A., 1913.
Controlling Chicken Pox, Sore head or Contagious
Epithelioma by Vaccination.
Amer. Vet. Rev., vol. 44, p 330.

42. Hardtwigk, 1936.
Purulent Panophthalmia in a Fowl affected with Coryza.
Tierärztl Rdsch., vol. 44, p 979.

43. Harrison, F.C. and Streit, H., 1903.
Roup of Fowls.
Amer. Vet. Rev., vol. 27, p 26.

44. Hauser, A. 1909.
Bacteriologische Untersuchungen über Geflügeldiphtherie.
C.F. Bakt., vol. 48, Abt.I, p 535.

45. Heidkamp, K. 1934.
Aetiology and the Recognition of Infectious Diseases of the Head of the Fowl.
Vet.Bull., vol. 5, p 232.

46. Hutyra, Marek and Manninger, 1938.
Special Pathology and Therapeutics of the Diseases of Domestic Animals, vol. 2, pp 453 and 511, and Vol. 3, p 172.

47. Jackley, J.G. 1917.
A Study of the Etiology of Roup.
Kansas St. Tech, Bull. No.4.

48. Jackley, J.G., 1918.
A Further Study of the Etiology of Roup in Fowls.
J. Amer. Vet.Med.Assoc. Vol. 52, p 853.

49. Jackley, J.G. 1922.
Anti-sera, Aggressins, and Prophylaxis with a Note on Roup in Birds.
Ibid. Vol.61, p 515.

50. Jowett, W. 1909.
Epithelioma Contagiosum.
J.Comp. Path. and Therap. Vol.22, p 23.

51. Kaupp, B.F. 1918.
A Chromogenic Bacillus from a Case of Roup.
Jl. of Inf. Dis., vol. 23, p 568.

52. Kaura R.L. and Iyer, S.G. 1936.
Studies on the Diphtheritic Form of Fowl Pox in India.
Indian Jour. of Vety. Science and Animal Husb., Vol.6, p 313.

53. Kessen, B.H., 1936.
Vergelijkend Onderzock Betreffende Haemophilus coryzae, Haemophilus influenzae En Andere Haemophiele Bacillen, p 100.

54. Kurse, W. 1914.
Die Erreger von Husten und Schnupfen.
Mün. Med. Wschr. vol. 61 Abt. 2, p 1547.

56. Lewis, M.R. and Mueller, E. 1934.
Studies on the Common Cold of Chickens.
J. Amer. Vet. Med. Assoc. vol. 84, p 759.

57. Loeffler, F. 1884.
Untersuchungen über die Bedeutung der Mikroorganismen für die Entschung der diphtherie beim menschen, bei der Taube under beim Kalbe.
Mitt. aus d Kais. Ges. vol. 2, p 482.

58. Mackie T.J. and McCartney, J.E. 1934.
An Introduction to Practical Bacteriology pp 105 and 483.

59. Marcato, A. 1936.
Fowl Coryza in Eritrea.
La Nuova Vet., vol. 14, p 235.

60. Medveczky, S. 1933.
Recherches sur l'etiologie de l'affection dite Coryza Contagieux des volailles.
Rev. Gen. Med. Vet. vol, 42, p 228.

61. Nakamura, N. 1925.
Experimental Studies on Avian Diphtheria.
Ann. Rep. of the Vety. Lab. Nishigaba, Tokio.
p 21.

62. Nelson, J.B. 1933 (a)
Studies on the Uncomplicated Coryza of the Domestic Fowl. I. the Isolation of a Bacillus which produces a Nasal Discharge.
Jl. Expt. Med. vol. 58, p 289.

63. Nelson, J.B. 1933 (b)
II. The Relation of the Bacillary Coryza to that produced by Exudate.
Ibid. vol. 58, p 297.

64. Nelson, J.B. 1935 (a)
III. The Effect of Extra-nasal Injection of Fowl Coryza Bacillus.
Ibid. vol. 61, p 351.

65. Nelson, J.B. 1935 (b).
IV. Susceptibility after Extra-nasal Injection of Fowl Coryza Bacillus.
Ibid. vol. 61, p 361.

66. Nelson, J.B. 1936 (a).
V. A Coryza of Slow onset.
Ibid. vol. 63, p 509.

67. Nelson, J.B. 1936 (b).
VI. Cocco-bacilliform Bodies in Birds Infected with the Coryza of Slow onset.
Ibid. vol. 63, p 515.

68. Nelson, J.B. 1936 (c).
VII. Cultivation of the Cocco-bacilliform Bodies in Fertile Eggs, and Tissue Cultures.
Ibid. vol. 64, p 749.

69. Nelson, J.B. 1936 (d).
VIII. The Infectivity of Foetal Membrane and Tissue Culture Suspensions of the Cocco-bacilliform Bodies.
Ibid. vol. 64, p 759.

70. Nelson, J.B., 1938.
IX. The Co-operative Action of Haemophilus gallinarum and the Cocco-bacilliform Bodies in the Coryza of Rapid Onset and Long Duration. Ibid. vol.67 p 847.

71. Nelson, J.B. 1939.
Growth of the Fowl Coryza Bodies in Tissue Culture, and in Blood Agar.
Ibid. vol. 69, p 199.

72. Popov, I.V., 1928.
Cases of Avian Diphtheria caused by Klebs-Loeffler's Bacillus.
Vet. Record vol. 8, p 882.

73. Purchase, H.S., 1939.
An Outbreak of Infectious Roup in Young Chickens.
Vet. Record vol. 51, p 3.

74. Reis, J. 1930.
Sur Les Relations Étiologiques Entre L'Épithélioma Contagieux Et Le Coryza Infectieux Des Oiseaux.
C.R. Soc. Biol. vol. 103, p 1335.

75. Rice, J.P. 1926.
Poultry Diseases: An Urgent Veterinary Problem.
A Paper presented to the Scottish Metropolitan Division of the N.V.M.A. of Great Britain and Ireland on November 3rd. 1926 p 16.

76. Reischauer, J.P. 1906.
Uber die Pocken der Vögel, ihre Bezeihungen zu den echten Pocken und ihre Erreger.
C.F.Bakt. (Orig.) vol. 40, ABT.I, pp 356, 474 and 653.

77. Salmon, D.E. 1913.
Important Poultry Diseases - Roup or Contagious Catarrh.
U.S.Dept. Agric. Farmer's Bull. No. 530 p 9.

78. Sawyer, C.E. and Worley, S.S. 1927.
Poultry Diseases in Washington- Colds and Roup.
Western Washington Expt. Station Popular Bull. No.5 p 37.

79. Schalm O.W. and Beach, J.R. 1936. (a)
Studies of Infectious Coryza of Chickens with Special Reference to its Etiology.
Poult. Sci. vol. 15, p 473.

80. Schalm, O.W. and Beach, J.R., 1936 (b)
Cultural Requirements of the Fowl Coryza Bacillus.
Vet.Bull. vol. 7 p 7.

81. Schneider - see 20.

82. Scott, W.M. and McCartney J.E. 1929.
The Influenza Group of Bacteria.
A System of Bact. vol. 2, p 326.

83. Seddon, H.R. 1914.
Oedema of the Wattles of Fowls due to an Organism of the Pasteurella Group.
Vet. Jour. vol. 21, p 24.

84. Stafseth, H.J. 1928.
Colds (Roup) - Diseases of Adult Poultry.
Mich. State Coll. Agric. Extn. Bull. No. 54 p 7.

85. Stafseth, H.J. 1931.
Fowl Pox Vaccination and Suggestions for Handling Roup.
Vet. Med. vol. 27, p 148.

86. Stevenson, see 43.

87. Streit, H. 1904.
Untersuchungen über die Geflügeldiphtherie.
Zchr. Hyg. u. Infektr. vol. 46, p 407.

88. Sweet, C.D. 1914.
A Study of Epithelioma Contagiosum of the Fowl.
Expt. Sta. Rec. vol. 30, p 884.

89. Thjötta, Th. and Avery, O.T. 1931.
Preparation of Extract of Yeast.
Jl. Exptl. Med. vol. 34, p 100.

90. Thomas, J.E. 1927.
A Disease of the Wattles of Fowls due to Infection with a Strain of the Fowl Cholera Organism of Apparently Low Virulence.
3rd. World's Poultry Congress, Ottawa, p 306.

91. Topley W. and Wilson, G. 1936.
Principles of Bacteriology and Immunity. p 621.

92. Van Dorsen, C.A. 1937.
Haemophiele bacillen in inwendige organen Van hoenders bij Coryza gallinarum met Korte incubatie.
Tijdschr. V. Diergeneesk. vol. 63, p 5.

93. Van Es, L. and Martin, H.M. 1923.
The more Important Poultry Diseases - Roup.
Univ. of Nebraska, Coll. Agric. Expt. station Bull. No. 195, p 41.

94. Verge, J. 1926.
Avian Diphtheria, Contagious Epithelioma and Occulonasal Roup of Fowls due to a Common Cause - A Filterable Virus.
N.Amer. Vet., Vol.7, p47.

95. Weaver, C.H. 1927.
A Few Facts Regarding Roup.
3rd. World's Poultry Congress, Ottawa, p 300.

96. Weaver, C.H. and Mitchell, C.A. 1924.
A Study of Roup in the Domestic Fowl from the Standpoint of Etiology.
Poult. Sci. vol. 4, p 9.

97. McGaughey, C.A. 1932.
Organisms of the B. influenzae Group in Fowls.
J. Comp. Path. and Therap. vol. 45, p 58.

98. Montague.
Veterinary Helminthology and Entomology, 1934, p 173.

99. Mönnig, H.O. 1934.
Veterinary Helminthology and Entomology, p 175.

100. Seifried and Schaff - see 25. p 166.

101. Moore - see 39, 48 and 73.

102. Colin - see 43 and 87.

103. Bordet - see 16 and 28.

104. Carnwath - see 28 and 48.

105. Webster, L.E., Pritchett, I.W. and Beaudett, F.R. 1929.
P. avisepticum infection of Poultry.
Biol. Abs. vol. 3, p 768.

106. Ward - see 47.
107. Gallacher - see 47.
108. - Reinhardt - see 47.

Fig. I.

The Subject is depressed with ruffled feathers and a swollen eye. The feathers of the hackle are matted together with discharge from the nose and eye.

Fig. 2.

The eyelids are glued together by the sticky, lachrymal discharge, giving the bird a swollen face.

Fig. 1.

Fig. 2.

Fig. 3.

Respiratory distress due to obstruction of the nasal chamber by mucus plugs; breathing through the open beak.

Fig. 4.

Facial oedema, particularly in the submaxillary region.

Fig. 5.

Oedema of the wattles in addition to other facial complications.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Cross section of the nose showing rhinitis manifested by inflammatory exudate, capillary engorgement and round-cell infiltration. - stained by Haematoxylin and Eosin (x 252).

Fig. 7.

Inflamed mucous lining of the nose showing round-cell infiltration, particularly in the sub-mucosa. Stained by Haematoxylin and Eosin (x 430).

Fig. 8.

A cross section of the face showing the presence of inspissated purulent substance in the facial sinuses and to a lesser extent in the nasal chamber of a diseased fowl (D). For comparison, a healthy head (H) has been put alongside to show the normal appearance of the facial sinuses and the nose in the chicken.

Fig. 6.

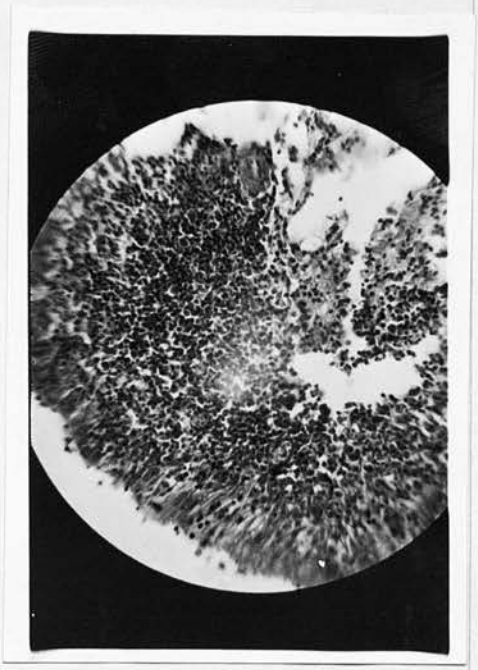

Fig. 7.

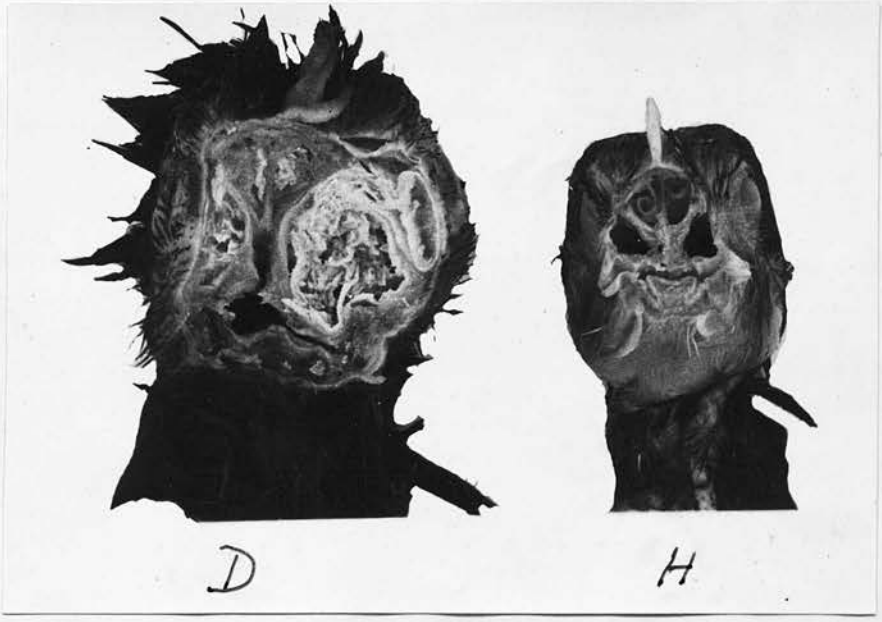

Fig. 8.

Fig. 9.

A section of the larynx showing round-cell infiltration in the laryngeal mucosa - stained by Haematoxylin and Eosin (x 430)

Fig. 10.

A smear preparation showing the cocco-bacillary appearance of the para-influenzoid organism reported in this article - stained by Gram's method (x 576)

Fig. 11.

A smear preparation showing the presence of numerous spirochaetes in the tracheal exudate of an infected fowl - stained by Leishman's stain. (x 950).

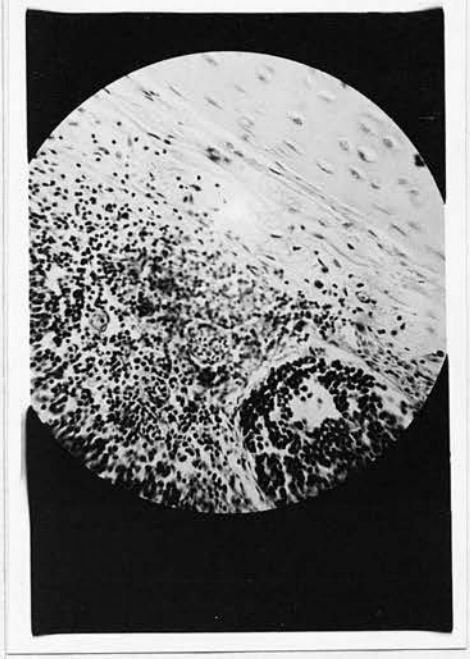

Fig. 9.

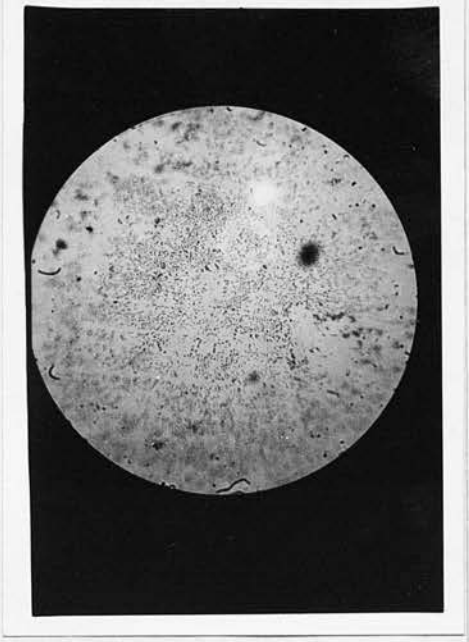

Fig. 10.

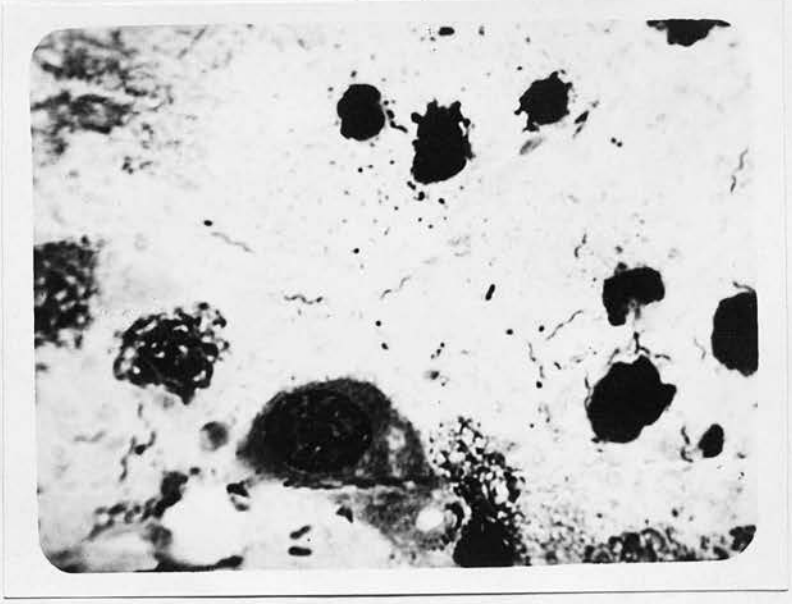

Fig. 11.

Fig. 13.

A chicken infected with bacteria-free filtrate exhibiting symptoms of Infectious Coryza similar to those produced by the inoculation of unfiltered exudate.

Fig. 13.

Petechiae of the laryngeal mucosa of infected chickens; in the case of (F), the infection was produced by the inoculation of bacteria-free filtrate, while in the case of (E), unfiltered exudate was employed. The mucous lining of the trachea in both cases is slightly congested.

Fig. 12.

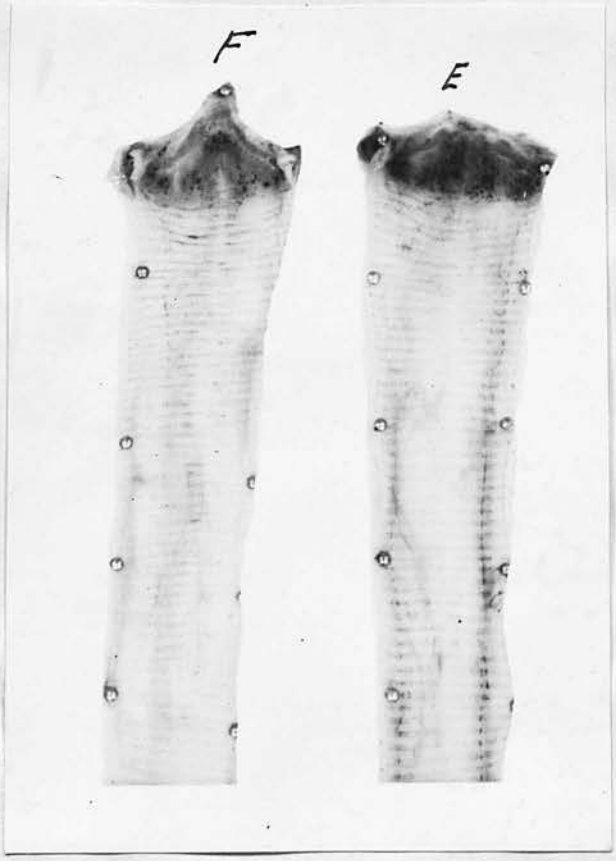

Fig. 13.