STUDIES IN THE ETIOLOGY OF ROUP AND ALLIED DISEASES OF FOWLS

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THESIS

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INTRODUCTION.

Experimental work pertaining to the etiology of roup and allied conditions in fowls has occupied the attention of several investigators for a number of years. Roup, in its broadest sense, is not a new disease, but, rather one of the first to hamper the success of the poultry industry. The prevalence of this disease together with its mortality, of from 2 to 8[illegible] per cent of the birds affected, places it very nearly at the top of the list in importance so far as poultry diseases are concerned in Michigan.

Due to the wide variation in results obtained by the various investigators of this problem it would be impossible at the present time to classify roup with diseases like tuberculosis and fowl cholera, of which the causes are definitely known. This disease, therefore, must be placed in the same class as the so-called colds in the human family of which the etiology is not well understood.

The results obtained in my experiments indicate that a filtrable virus is the primary cause of these conditions and that other organisms are secondary invaders, of these a Pasteurella organism seems to occur most frequently.

REVIEW OF LITERATURE.

A study of the etiology of roup in fowls, according to Hausser (1), began in 1869, when Rivolta investigated an outbreak of the disease and recorded finding organisms which he believed to be protozoa.

In 1884 Loeffler (2) isolated a rod-shaped bacterium, belonging to the Pasteurella group, from pigeons affected with diphtheria. This he named Bact. diphtheriae columbarum.

The following year Moore (3) reported that an organism belonging to the aforementioned group was found in cases of roup but that he was unable to reproduce the disease with it.

Besson (4), in his text book, expresses the following opinions regarding the etiology of these conditions:

"Etiologically bird diphtheria is a totally different disease from human diphtheria." He also states that Guerin found a cocco-bacillus, belonging to the Pasteurella group, in the heart blood of infected birds: this organism on inoculation, however, gave rise to a fatal septicemia quite different from the naturally acquired disease.

By grinding up in normal saline solution the nictitating membrane of a fowl which had been infected with a thread dipped in an emulsion of a false membrane, it is stated that Bordet obtained an emulsion which produced in fowls the typical false membranes of bird diphtheria. When

this emulsion was sown on blood agar, the only visible growth consisted of a few colonies of adventitious organisms: but by scraping the agar where there was no visible growth with a platinum wire, and transferring the scrapings to a little drop of water and rubbing the mucous membrane of the mouth with the emulsion, false membranes were produced in a normal fowl. Serial cultures can also be obtained and occasionally extremely small colonies are visible. Under the microscope an emulsion of the cultures shows very large numbers of small granular dots generally collected together in masses. This organism and that of pleuro-pneumonia seem to be the smallest yet cultivated. The author states that according to Carnwath, this filtrable virus appears to be identical with that of molluscum contagiosum of birds. In an epizootic of diphtheria among birds investigated by him the virus produced indifferently molluscum contagiosum or diphtheria according as to whether the material was inoculated on the bucco-pharyngeal mucous membrane or on the comb.

G. Dean and Marshall are said to have recorded an outbreak of diptheria in the wood pigeon apparently due to a filtrable virus. By painting a filtered (Berkefeld filter) emulsion of a membrane from an infected bird on to the throat of doves they were able to reproduce the disease experimentally.

In 1915 Beach, Lothe, and Halpin (5) reported that from their investigations the high mortality in outbreaks of roup was due to a secondary invading organism somewhat similar to organisms of the Pasteurella group.

Brumley and Snook (6) on studying these conditions concluded that a filtrable virus was the primary invader that lowered the birds' resistance and thus prepared the tissues for secondary organisms. They claim that neither factor alone will cause the typical disease.

In 1917 Jackley (7) described an organism, belonging to the Pasteurella group, which he was able to isolate quite consistently from cases of roup. However, he found that the pathogenicity of this organism was not sufficient to produce the disease experimentally except by the contributory mechanical injury caused by suturing a thread soaked in a suspension of these organisms into the eyelid.

Kaupp (8) in 1918 isolated a chromogenic bacillus from a case of roup that showed some pathogenic properties for the fowl when injected into injured tissues as in intradermal inoculations.

In the same year Salmon (9) intimated that roup is strictly a contagious disease; that is, one which arises only, so far as known, by contagion from other diseased birds. He also stated that the nature of the virus is not known.

F. C. Harrison (10) in the second edition of Marshall's microbiology expresses the following opinions regarding the

etiology of roup:

"Roup, or fowl diphtheria, may be caused by a number of different organisms, among them the well known Ps. pyocyanea (green or blue pus organism), B. cacosmus and other species which give rise to a complex suppurative process. Sections of membranes from affected fowls show large numbers of pus cells, some regular masses, debris of epithelial cells and bacteria, and thus they differ from the diphtheritic membranes of man."

In 1920 Ward and Gallagher (11) stated that diphtheria and bird-pox are caused by a virus the nature of which is not well known. The organism has not been certainly identified microscopically and is so small that it will pass through a Berkefeld filter. However, it is stated that according to Marx and Sticker, it will not pass through a porcelain filter.

During the same year Beach (12) drew the following conclusions regarding the etiology of roup: As they exist in California, there does not appear to be any etiological relationship between chicken-pox and those pathological conditions of the nasal passages commonly designated as colds, roup, or swelled head.

Many so-called outbreaks of roup may in reality be a disease manifested by symptoms very similar to roup but due to nutritional factors.

There appears to be ample evidence that chicken-pox and those pathological conditions of the mucous membranes

of the mouth and eyes commonly designated as canker or avian diphtheria are etiologically identical.

Stafseth (13) reports that there is abundant evidence to show that chicken-pox and avian-diphtheria are caused by the same virus and that roup, which in its nature resembles colds in man and higher animals, is etiologically distinct from the former. Roup is not etiologically related to chicken-pox but may under certain conditions occur as an accompanying disease, is stated.

Weaver (14) was able to isolate a member of the Pasteurella group from naturally affected birds, he was also able to reproduce the disease with this organism in experimental birds in a high percentage of cases.

Van Ess and Martin (15) after a careful study report that the causative factor of roup is not known. They state that 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 disease producing functions in the same manner that the common colds of folks come about. It is further thought possible that the preponderance of certain bacterial species may cause the disease to present itself with special features.

Gwatkin (16) in 1924 states that membranes can be produced in the mouth and larynx of birds by introducing a filtrate obtained by passing the bloody exudate from the respiratory organs of birds that had died of the acute form of avian diphtheria through a Berkefeld filter so that this

is free from bacteria. He was also able to produce lesions of chicken-pox on combs of cockerels by using the same filtrate which led him to the conclusion that the causative agent of avian diphtheria and chicken-pox is a filtrable virus. Cheesy material plugging the larynx was also produced when ground and dried chicken-pox scabs were used in these places. He was able to demonstrate that the virus was not present in the spinal cord of affected birds. He found that the different organisms that he was able to isolate would not reproduce the lesions consistently.

Gwatkin does not believe that roup is caused by any specific organism but that if the resistance of birds is lowered any of the organisms associated with roup will cause the lesions.

May and Tittsler (17) describe a disease as tracheo-laryngitis which they believe to be an acute form of avian diphtheria. They state that a great deal of blood is found in the trachea in most cases. They claim that these conditions are predisposed by exposure.

Eriksen (18) states that the cause of roup is unknown. He says that poor ventilation, damp, tightly closed and filthy houses, crowding on roosts or in quarters are predisposing causes of great importance. He has found a member of the hemorrhagic - septicemia group quite uniformly. He further intimates that the virus of chicken-pox may be present as a complicating factor but that it

cannot be considered as the most important cause.

Verge (19) claims that whatever is the appearance of these infections; avian diphtheria, contagious epithelioma (chicken-pox) and oculo-nasal catarrh constitute a single morbid entity and are caused by the same filtrable virus.

Ludford and Findlay (20) maintain that epithelioma contagiosa is a misnomer, for they claim the disease has nothing in common either with the epitheliomata or with molluscum contagiosum in man. On the other hand it is claimed that there is considerable evidence to show that the virus of fowl-pox, if not identical with, is at least related to the virus of vaccinia. They have also demonstrated cell inclusions that help to support their views.

Nakamura (21) found a gram negative coccobacillus that was a non-motile, facultative aerobe and grew slowly, in early stages of avian diphtheria and by inoculation experiments found it to be pathogenic in a high percentage of cases.

SYMPTOMS, COURSE AND GROSS LESIONS OBSERVED IN THE DIFFERENT FORMS OF THE DISEASE.

It is difficult to give a definition that will satisfactorily describe these ailments. Therefore, I shall briefly describe the symptoms and lesions commonly observed in four more or less distinct forms of the disease. Any of these forms may or may not occur separately. Usually we find complications of two or more in the same individual at one time.

To avoid confusion regarding the terminology I shall use the term oculo-nasal roup or catarrh in referring to the inflammatory processes involving the nasal passages, eyes and adjacent sinuses. The term avian diphtheria shall be used in referring to those conditions manifested by the appearance of cankers, patches and pseudo-membranes in the larynx, bucco-pharyngeal mucous membranes and trachea. The term chicken-pox shall only refer to the wart-like scab formations that occur on the comb and wattles and rarely on other parts of the skin. Nutritional roup shall refer only to roup lesions referable to faulty nutrition, (12).

Oculo-nasal roup or catarrh is usually of a more or less chronic nature occurring most frequently in young adult birds, although birds of all ages are susceptible under proper conditions. It occurs most frequently during the fall and winter months. The condition starts with a

more or less viscous, but clear, discharge from the nostrils; this tends to dry on exposure to air and partially occludes the nostrils making it necessary for the bird to breathe through the mouth which often causes the darkened condition of the tongue spoken of as "pip." The anatomical arrangement of the ducts from the eyes to the nasopharynx in birds is such that they do not permit of sufficient drainage to take care of this discharge, thus it collects around the eyeball. In a few days this discharge becomes caseated and frequently yellow in color having a very offensive but characteristic odor. These accumulations may continue until the entire eyeball is destroyed. (Fig. 1).

Frequently both eyes become affected in this manner; under these circumstances the birds usually become totally blind and die of starvation. It is surprising to note that birds in this condition, with only one eye affected, will sometimes live for months without showing any marked systemic disturbances. On the other hand this condition frequently takes a rapid course and the bird becomes emaciated and dies in a short time, possibly from toxins absorbed from these diseased areas. Frequently birds recover from a mild attack and show varying degrees of immunity to subsequent exposure.

The form spoken of as avian diphtheria is usually of a more acute nature. It also occurs most frequently during the fall and winter months. It is usually rapid

in its onset. One may observe nothing wrong with the affected birds except that they may cough and sneeze a few times and occasionally gasp for breath. In very acute cases the birds may die inside of two days after the first symptoms appear. In these cases the larynx is usually filled with a cheesy exudate or membranes streaked with bloody mucous. In the majority of cases these birds die from suffocation. In other cases these inflammatory processes may extend down into the bronchi, resulting in an acute bronchitis. Occasionally we find only small membranes on the bucco-pharyngeal mucous membranes of a more chronic nature. (Fig. 2). Usually, however, this form is acute and results in a very high mortality.

Chicken-pox or epithelioma contagiosa avium is characterized by scab-like formations on the comb and wattles. (Fig. 3). Avian diphtheria has been referred to as an internal form of chicken-pox. Chicken-pox is very contagious and spreads rapidly in a flock. The disease, however, is of a chronic nature and a large number of affected birds recover and become immune to this form of the disease for varying lengths of time.

The form referred to as nutritional roup is characterized by white caseous material around the eyes. This material, however, lacks the peculiar odor associated with the infectious form. An enlargement of the orifices of the glands in the esophagus with considerable white exudate present is diagnostic. Deposits of urates are

found in kidneys and ureters. The cause of this condition is referable to faulty nutrition and should always be considered in differential diagnoses.

EXPERIMENTAL WORK

ORGANISMS IN EYES AND THROATS OF AFFECTED BIRDS.

The eyes and throats of twelve specimens having both eye and throat lesions were swabbed carefully with sterile cotton swabs. With these streaks were made on gentian-violet agar plates. The plates were incubated for 18 hours at 37° C and the predominating type of colony picked and transferred to blood serum agar slants. Transfers were made to five sugar solutions for fermentation reaction: namely: dextrose, lactose, maltose, sucrose and mannite. These sugars were used because we had them on hand for routine work. Hanging drops were made to study motility of the different organisms. Chart No.1 shows organisms and their reactions as found in twelve cases of naturally affected birds.

Seven of these birds were found to have in the eyes and throat lesions a bi-polar organism resembling Pasteurella avicida. In the remaining five, organisms resembling B. coli and Ps. pyocyanea were found.

The media used were made for the most part according to "Giltner's Manual". The gentian-violet agar was liver infusion agar containing gentian-violet in the proportion of about 1:10,000 and adjusted to a pH of 7.2 using brom-

thymol blue as indicator.

The blood serum agar was made by adding cow serum to liver infusion agar.

The sugar solutions were made up to contain 1 per cent of the different sugars used and 1 per cent Andrade's indicator added.

Swabs from eyes and throat lesions of birds affected with Roup and Avian diphtheria.	Growth on Gentian Violet Agar Plates	Blood Serum Agar Slants	Gram Stain	Rod	Coccus	Bi-polar	Motile	Gas	Dextrose	Lactose	Maltose	Sucrose	Mannite	Remarks
Bird #1	++	+++	-	Short-	+	-	-	+	-	-	+	+		Resembles Pasteurella avicida
#2	+++	+++	-	Long	-	-	+	+	++	++	++	++	++	Resembles B. coli
# 3	+++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida
#4	++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida
#5	++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida
#6	+++	+++	-	Long-	-	+	+	++	++	++	++	++		Resembles B. coli
#7	++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida
#8	++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida
#9	+++	+++	-	Long-	-	+	+	++	++	++	++	++		B. coli
#10	+++	+++	-	Long-	-	+	+	++	++	++	++	++		B. coli
#11	+++	+++	-	Long-	-	-	+	+	+	+	+	+		Unknown
#12	++	+++	-	Short-	+	-	-	+	-	-	+	+		P. avicida

++++ = Very numerous colonies
+++ = A few less colonies
++ = About 1/2 as many colonies
+ = acid
- = No acid nor gas
++ = Acid + gas

ORGANISMS FOUND IN THROATS OF NORMAL BIRDS.

To determine if the Pasteurella organism, encountered so frequently in birds affected with roup, was also present in the throats of normal birds the following experiment as indicated in Chart No. II was performed. It is noticed that in no case was the Pasteurella organism found. No attempt was made to identify the spreaders.

Chart No. II.

Swabs From Throats of Normal Birds.

Bird No.	Gentian Violet Agar	Blood Serum Agar Slants	Coccus or rod	Motile	Gram	Bi-polar	Gas	Dextrose	Lactose	Maltose	Sucrose	Mannite
1	+++	Spreader										
2	++	++	rod	++	-	-	+	+	+	+	+	+
3	+++	++	Cocco-Bacillus	-	+	-	-	+	-	+	+	+
4	-	Sterile										
5	+++	Spreader										
6	+++	+++	Rod	+	-	-	+	+	+	+	+	+
7	+++	Spreader										

ORGANISMS FOUND IN THROATS OF BIRDS SHOWING LESIONS OF CHICKEN-POX ON COMB AND WATTLES.

The throats of eleven birds were swabbed as indicated in Chart No. III and in no case was the Pasteurella organism found.

From these limited data it was temporarily concluded that the Pasteurella organism, resembling Pasteurella avicida, was of significance in bringing on lesions of roup and diphtheria, but data appearing elsewhere in this paper indicate that this organism is only of secondary importance.

CHARACTERISTICS OF PASTEURELLA ORGANISM.

The fermentation reactions using 1 per cent of the different sugars listed in Chart No. IV agrees with those of Pasteurella avicida.

Cross agglutination by using the serum of a rabbit immunized with this organism and a stock culture of Pasteurella avicida as antigen gives agglutination in a dilution of 1:200.

SWABS FROM THROATS OF BIRDS SHOWING LESIONS OF POX ON COMBS.

Chart No. III.

No. of Bird	Growth on Gentian Violet Agar	Blood Serum Agar Slants	Coccus or Rod Cocco-Bacillus	Motile	Gram	Bi-polar	Gas	Sucrose	Dextrose	Mannite	Lactose	Maltose
1	++	+++	Cocco-Bacillus	-	+	+	-	+	+	+	+	+
2	++	+++	Rod	++	-	-	+	+	+	+	+	+
3	++	Spreader +++										
4	++	+++	Cocco-Bacillus	-	+	+	-	+	+	+	+	+
5	++	Spreader +++	Rods & Cocci									
6	++	+++	Rod	+	-	-	+	+	+	+	+	+
7	++	+++	Cocco-Bacillus	-	+	+	-	+	+	+	-	+
8	++	+++	Short Rod	+	+	-	-	-	-	-	+	+
9	++	+++	Short Rod	+	+	-	-	+	+	+	+	+
10	++	+++	Short Rod	+	-	-	+	+	+	+	+	+
11	++	Spreader +++										

Chart IV.

FERMENTATION REACTIONS OF PASTEURELLA ORGANISM.

	24 hours		48 hours		7 days	
	Acid	Gas	Acid	Gas	Acid	Gas
Dextrose	+	-	+	-	+	-
Lactose	-	-	-	-	-	-
Maltose	-	-	-	-	-	-
Sucrose	+	-	+	-	+	-
Mannite	+	-	+	-	+	-
Levulose	-	-	+	-	+	-
Galactose	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-
Salicin	-	-	-	-	-	-
Inulin	-	-	-	-	-	-
Dulcite	-	-	-	-	-	-

PATHOGENICITY OF PASTEURELLA ORGANISM AND FILTRATE.

Injections of suspension of the Pasteurella organism in 0.85 per cent NaCL were made into the inner canthus of the eye on a large number of susceptible birds with only a very low percentage of infection occurring.

Thinking that a filtrable virus might play a part in the cause of these conditions, exudate from the trachea and larynx of a bird recently dead with typical lesions of avian diphtheria was ground up with 0.85 per cent NaCL and the suspension passed through a Berkefeld filter and this filtrate was used for injecting susceptible birds in the manner described above. A very high percentage of birds injected came down with typical lesions of the disease.

The filtrate in combination with the organism was also used for injection.

Knowing that it is very difficult to obtain birds that have not been exposed to some form of these conditions and have thus acquired some immunity, a rather detailed experiment was set up during July and August to test the pathogenicity of the Pasteurella organism and the filtrate.

Seventy-six birds, approximately three months of age, were purchased and placed in quarters that had been thoroughly cleaned and disinfected. These birds were kept in quarantine for a period of four weeks to see if any would come down with the disease. At the end of this period the birds were divided and placed in three pens.

One pen, or pen No. 1 according to Chart No. V, had 19 birds injected with the filtrate described elsewhere, 5 birds were left as controls; 10 of the injected birds or 52 + per cent showed typical lesions of roup and avian diphtheria. 8 birds died and 2 recovered. One control bird died showing extreme emaciation but no lesions of roup in any form.

Of the birds in pen No. 2 that were injected with the filtrate plus the Pasteurella organism only 38 per cent showed signs of infection. No control birds showed signs of illness.

Pen No. 3 were injected with the Pasteurella organism and only 11 per cent of the birds showed any signs of disease and these seemed to have a very acute form as all infected birds died. One control bird died but had no lesions of roup.

Chart No. V.

Pen No. 1 Injected with filtrate.	Pen No. 2 Injected with filtrate plus P. avicida.	Pen No. 3 Injected with P. avicida.
19 birds injected	21 birds injected	18 birds injected
5 controls	4 controls	3 controls
10 birds show lesions	8 birds show lesions	2 birds show lesions
8 dead	6 dead	2 dead
52,% infected	38,% infected	11,% infected
42,% dead	28,% dead	11,% dead
2 infected birds recovered without any treatment. One control bird died but had no lesions of Roup.	No control birds died or showed any lesions of Roup.	One control bird died but had no lesions of Roup.

From these data it would seem that the filtrate must contain a virus that is capable of producing lesions of roup and avian diphtheria in a very high percentage of cases.

Several attempts have been made at producing chicken-pox lesions on comb and wattles by using a filtrate obtained from eye and throat lesions, but in only one case was this accomplished successfully.

It would seem from this work that a filtrable virus is primarily responsible for lesions of roup and avian diphtheria and that other organisms like Pasteurella avicida are of secondary importance. It would also seem that the virus causing roup and avian diphtheria is different from that causing chicken-pox, but that the two may work together under certain conditions.

TECHNIQUE OF PREPARING FILTRATE.

The combs of susceptible cockerels were scarified and dried, ground chicken-pox scabs in 0.85 per cent saline suspension were rubbed in. Usually after two weeks one gets an abundance of ripe scabs. The birds were then anesthetized and the scabs removed and dried for a few days at room temperature. Later they were placed in a desiccator and then ground in a coffee mill; this powder was then triturated in a 0.85 per cent saline and filtered through filter paper.

This filtrate was then passed through a Berkefeld filter that had previously been tested to retain Victoria blue stain. The filtrate was always tested for growth on agar plates to be sure that all bacteria had been removed.

In using material from eyes and throat, deep seated lesions were removed; usually the mucous membranes and exudate were macerated in 0.85 per cent saline and the same procedure followed as with the pox lesions.

HISTO-PATHOLOGICAL WORK DEMONSTRATING CELL INCLUSIONS.

It has been known for a long time that several diseases caused by filtrable viruses are characterized and to some extent diagnosed by the appearance of cell inclusions. For examples, Negri's bodies in rabies, Guarnieri's bodies in variola and vaccinia, Mallory's bodies in scarlet fever and others might be cited. Just what these inclusions are has been a matter of speculation. They have been described as protozoa with complex life cycles. The term "Chlamydozoa", meaning cloak animal, has been used but none of these seem to be satisfactory explanations.

In studying the cytology of fowl-pox lesions one will notice definite degenerative changes in the cells. These first start by the appearance of one or more vacuoles in the cytoplasm of the cell; these may later coalesce to form one large body. These bodies or cell inclusions appear to be of a lipoidal nature as they reduce osmic acid and give

a black color that is easily recognized. (Figures 4, 5, & 6.)

TECHNIQUE OF FIXING AND PREPARING THE DIFFERENT TISSUES FOR HISTO-PATHOLOGICAL STUDIES.

A modified Schridde's method was used in preparing tissues as follows: Immediately after the bird was killed the comb was removed and placed in Orth's fluid which consists of potassium bichromate, water and formaldehyde. Tissues were left in this fluid at 55° C for 24 hours then they were placed in Muller's fluid, which has sodium sulphate in place of formaldehyde, for 24 hours, next in running water for 24 hours and then in 1 per cent Osmic acid 24 hours in the dark, then in running water another 12 hours. Dehydrating in 60, 80, 95 per cent and finally absolute alcohol. Chloroform was used in place of cedar oil for clearing before embedding in paraffin. Sections were examined both stained and unstained. Acid fuchsin and aniline water followed with an alcoholic solution of picric acid was used for staining. Sections were mounted in chloroform balsam.

Tissues were also fixed in Zenker's fluid and stained with eosin and methylene blue but the cell inclusions were not recognized and, seemingly, can best be brought out by the use of osmic acid.

DISCUSSION AND SUMMARY.

Bacteriological examinations revealed an organism closely related to if not identical with Pasteurella avicida in a high percentage of birds with lesions of avian diphtheria and ocular roup. The pathogenicity of this organism was never significant when inoculated into susceptible birds thus it must be considered of secondary importance.

The filtrate obtained from lesions of avian diphtheria and ocular roup was capable of reproducing lesions in a very high percentage of cases. In only one case, however, was filtrate from these lesions found to bring on lesions of chicken-pox on comb while pox lesions can be reproduced at will by using the virus from pox lesions as inoculum. This would suggest that two or more viruses play a part in these conditions.

Cell inclusions were demonstrated in lesions of fowl-pox on comb but not in diphtheritic lesions of larynx and trachea.

From this work it may be concluded that roup in its broadest sense, is caused by filtrable viruses either acting in definite combinations to bring on the various forms or different viruses acting singly to produce the different forms of the disease. The different organisms that are frequently found associated with the various forms must be considered of secondary importance so far as the actual etiology is concerned, but, may have considerable to do with the severity of the disease and the course of an outbreak.

EXPLANATION OF PLATES.

Fig. 1. Typical lesions of ocular roup.

Fig. 2. Membranes in mouth and throat typical of avian diphtheria.

Fig. 3. Fowl-pox lesions on comb.

Fig. 4. Micro-photograph of fowl-pox lesions on comb showing cell inclusions under low-power.

Fig. 5. Same as above with higher magnification of lesions revealing cell-inclusions stained with osmic acid.

Fig. 6. Same as above with still higher magnification.

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Figure 1.

Figure 2

Figure 3.

Figure 4.

Figure 5.

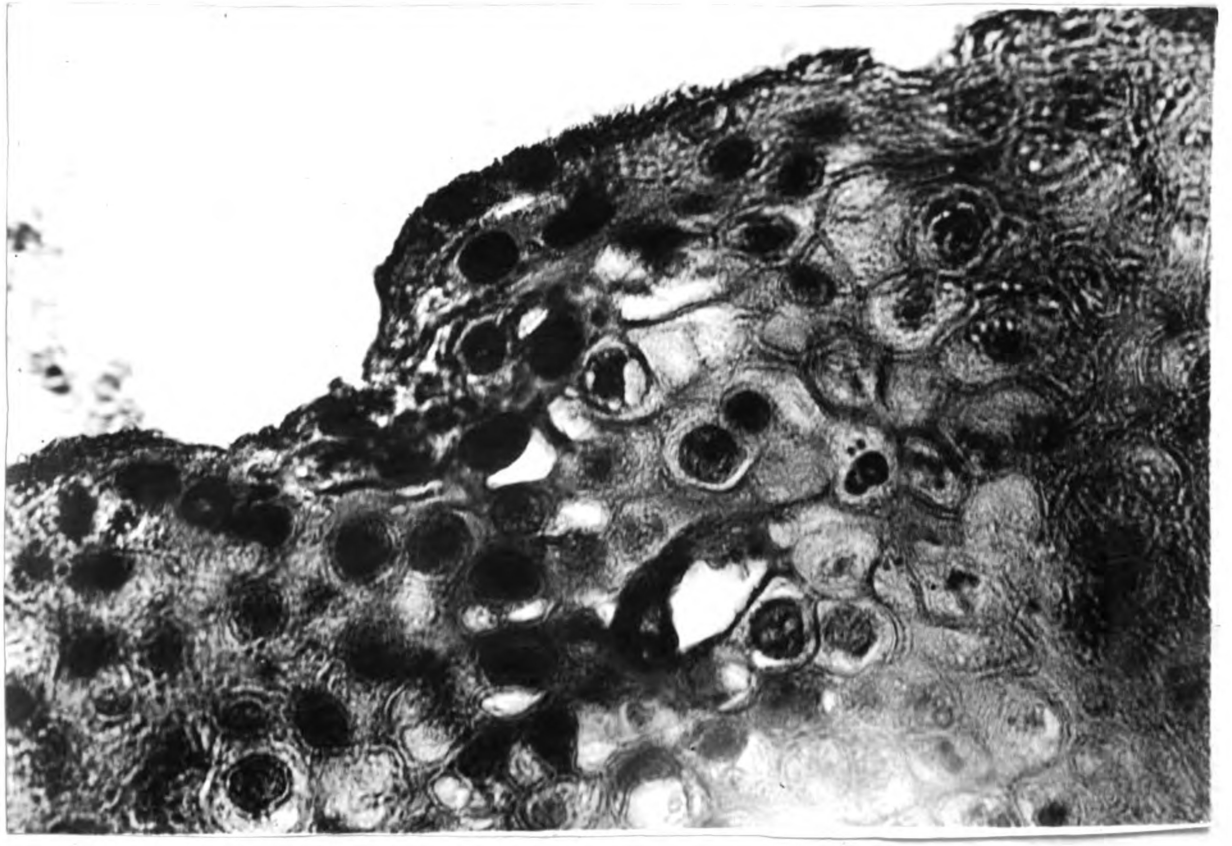

Figure 6.

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