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THE STUDY OF AN ORGANISM  
ISOLATED IN PURE CULTURE  
FROM DISEASED POULTRY

Thesis for Degree of M. S.

Helen J. Ellis

1925

THESIS

*Parasitology*  
*7/10/11*

SUPPLEMENTARY  
MATERIAL  
IN BACK OF BOOK

**THE STUDY OF AN ORGANISM ISOLATED  
IN PURE CULTURE FROM DISEASED POULTRY**

THE STUDY OF AN ORGANISM ISOLATED  
IN PURE CULTURE FROM DISEASED POULTRY

Thesis

Submitted to the Faculty of the Michigan  
State College of Agriculture and Applied Science  
in partial fulfillment of the requirements for  
the degree of Master of Science.

By

Helen J. Ellis

1925.

THESIS

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IN PURE CULTURE FROM DISEASED POULTRY.

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## I. INTRODUCTION

The incompleteness of knowledge of the cause of diseased conditions is well known. Poultry diseases have received much less study than those of other domestic animals and man. In only twenty percent of the cases coming to the attention of Michigan State College of Agriculture and Applied Science last year was positive diagnosis made. A variety of organisms are encountered, the significance of which is unknown. Among them is the organism here discussed. From July 1924 to May 1925, it was isolated in pure culture from thirty-five autopsies performed in this laboratory. As in no case any specific well known organism explaining the lesions could be discovered, it was thought worth while to make a thorough study of this organism and to attempt to show whether or not it is capable of producing pathogenic effects in birds.

## II. REVIEW OF LITERATURE

Monquet, in an article entitled "Deuxieme note sur la typhose aviare", in Bul. Soc. centr de Med. Vet., Par. 1922, 98, 65-70, tells of the isolation of a Gram-positive organism (single or diplo-), oval or round, isolated from blood, liver, spleen, bone marrow, peritoneal fluid of birds. It is impossible to tell from the description whether the organism is the same as the one here discussed.

### III. HISTORY OF CASES

It seems advisable, in the absence of historical background, to include reviews of the routine autopsies of the birds from which the organism studied was isolated, especially since there was great variety in the lesions reported. No effort is made to give the complete autopsy report, only positive pathological findings being listed. All organs and tissues not mentioned are to be considered normal.

361 - Legweakness;

Liver: small.

Heart: flabby, small hemorrhages on fat.

Duodenum: marked hemorrhagic inflammation.

364 - Liver: a few pinhead sized white spots.

Heart: flabby, chronic pericarditis.

Duodenum: chronic catarrhal inflammation.

367 - Liver: enlarged.

Gall bladder: much extended with thick bile.

Spleen: enlarged.

Proventriculous: hemorrhages around openings  
of glands.

Ovary: chronic lesions (tumor formation).

Mesentery: thickened with tissues like those  
found in ovary.

Peritonitis.

368 - Chronic suppurative peritonitis with adhesions.

Ovary: diseased, inactive, lesions suggesting  
early tumor formation.

371 - Lesions like 367 and 368, except that changes  
are more along the posterior part of  
the intestines.

Ovary: very large, like 367 and 368.

Liver: "nests" of necrotic foci.

372 - Liver: shows seven or eight circular necrotic  
spots suggesting blackhead lesions;  
however, with calcification.



Ovary: gelatinous degeneration.

Intestines: inflammation in upper half.

Cecum: wall thickened and resembling lesions seen  
in 367 and 368.

381 - Spleen: small and anemic.

Intestines: slight catarrhal enteritis.

419 - Retained yolk, size of hazelnut, caseous peritonitis.

Spleen: small.

Heart: flabby with minute hemorrhages in fat.

Intestines: catarrhal condition in upper part.

Cecum: areas of superficial necrosis.

420 - Legweakness.

Pancreas: much enlarged and hard.

Ovary: tumor formation.

Heart: flabby.

Intestines: catarrhal condition.

421 - Abscess on sternum.

Heart: sero-fibrous pericarditis and tumor in  
muscle.

Retained yolk.

Ovary: tumor formation.

Spleen: congested.

Rigth lung: small abscess.

Gizzard: ulcerations.

Intestines: general hemorrhagic inflammation.

422 - Ovary: poorly developed.

Oviduct appeared to be absent.

435 - Heart: serous pericarditis.

Intestines: hemorrhagic inflammation.

470 - Some legweakness, blindness and congestion in the middle part of the small intestines.

475 - Liver: enlarged, with a few small yellow spots; also hemorrhagic spots.

Spleen: somewhat enlarged, with nodules.

Kidneys: somewhat enlarged, yellowish grey in color.

Tubercle bacillus was demonstrated in spleen, liver and kidneys.

488 - Rabbit, inoculated for diagnostic purposes,

October 31, 1924, (died Nov. 11, 1924) with culture of the organism under discussion from autopsy 210, case 381. Peritonitis and marked pleurisy.

Liver: enlarged with yellowish grey spots, perhaps indicating chronic hepatitis.

Gall bladder: distended with bloody thickly coagulated bile.

Spleen: enlarged, swollen around edge.

Kidneys: right one showed a whitish area about 6 mm. in diameter, which on the out surface had shape of infarct.

Stomach: tore very easily.

Lungs: numerous hemorrhages.

Bronchi: edema

1. The first part of the report is a general introduction to the subject of the study. It discusses the importance of the study and the objectives of the research. It also mentions the scope of the study and the limitations of the study.

2. The second part of the report is a literature review. It discusses the previous studies on the subject of the study. It mentions the findings of the previous studies and the gaps in the knowledge.

3. The third part of the report is a description of the methodology used in the study. It mentions the research design, the data collection methods, and the data analysis methods.

4. The fourth part of the report is a presentation of the results of the study. It mentions the findings of the study and the statistical analysis of the data.

5. The fifth part of the report is a discussion of the results of the study. It discusses the implications of the findings and the limitations of the study.

6. The sixth part of the report is a conclusion. It summarizes the findings of the study and the implications of the findings.

7. The seventh part of the report is a list of references. It mentions the sources of the information used in the study.

8. The eighth part of the report is an appendix. It contains the raw data and the calculations used in the study.

9. The ninth part of the report is a list of figures and tables. It mentions the figures and tables used in the study.

10. The tenth part of the report is a list of abbreviations. It mentions the abbreviations used in the study.

496 - Some legweakness.

Liver: small yellowish grey spots, pin point to  
pinhead in size, over entire organ

Bones: brittle

Intestines: severe enteritis, croupous at middle  
portion.

The organism was isolated from liver and spleen.

B. pullorum was isolated from the heart.

B. sanguinarium from liver and heart.

505 - Legweakness, emaciated, had cold and retained yolk.

Spleen: small.

Intestines: inflamed.

507 - Legweakness, emaciated, many worms.

514 - Convulsions or spasms.

Liver: very light in color, large and fatty.

Heart: hemorrhages on fat.

519 - Liver: Necrotic spot, size of pinhead, surrounded by  
hemorrhagic area.

Spleen: small hematoma, size of pea

Heart: fibrinous pericarditis, with large hemorrhages.

Lungs: caseous pneumonia.

532 - Liver: small and anemic.

Spleen: very small

Heart: hemorrhages.

Proventriculus: many hemorrhages around openings  
of glands.

Intestines: hemorrhagic duodenitis.



534 - Liver: anemic.

Small retained yolk.

Larynx: hemorrhages.

540 - Liver and heart: congested.

541 - Liver: yellowed, slightly congested.

Spleen: small.

Intestines: slight enteritis in a few places.

543 - Liver: chronic necrotic lesions.

Spleen: small

Larynx: hemorrhages.

Pharynx and trachea: diphtheritic membrane.

545 - Heart: chronic pericarditis.

Spleen: small.

Larynx and trachea: congested.

557 - Intestines: inflamed.

584 - Died forty days after being injected with culture

from case 488 for laboratory diagnosis.

Emaciated.

Liver: enlarged, pale, few white nodular lesions.

Spleen: greatly enlarged, soft.

Heart: flabby pericarditis.

Kidneys: enlarged.

758 - All inner organs somewhat atrophied.

Heart: flabby.

Ovary: diseased.

Intestines: pronounced chronic inflammation.

763 - All organs rather small.

Ovary: diseased.

Retained yolk.



769 - Spleen: enlarged.

Liver: enlarged.

Intestines: catarrhal enteritis.

Retained yolk.

798 - Larynx: occluded with canker.

Heart: numerous hemorrhages on fat.

Liver: somewhat congested.

Spleen: mottled.

Intestines: hemorrhagic enteritis.

Lungs: pneumonia.

906 - Died thirty days after being injected with culture  
from case 763 for laboratory diagnosis.

Liver: enlarged, ruptured, mottled showing many  
small yellowish spots.

Spleen: enlarged, pale.

Intestines: same hemorrhages.

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Bird  
No.

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584



#### IV. METHODS OF INVESTIGATION AND RESULTS.

In studying the organism culturally the descriptive chart recommended by the Society of American Bacteriologists was used, with some additions. All strains were invigorated five days in broth before they were grown on the standard media and all experiments were performed at least three times in duplicate, with intervals of some weeks between the repetitions. Media were prepared according to instructions in Giltner's "Microbiology" unless otherwise specified.

#### A. Source and Isolation of Cultures.

The strains used in the culture study were isolated in the spring and early summer of 1924. Those used in serological studies and for injection, were isolated in the fall of 1924.

The method used in isolation was that used in the laboratory in the routine bacteriological diagnosis of chicken diseases. Organs of the chickens that are autopsied are plated out on beef infusion agar. Colonies are picked from the plates after twenty-four hour incubation, and are grown on beef infusion agar slants, (pH 6.8) for twenty-four hours. Durham fermentation tubes of one percent lactose, sucrose, dextrose, maltose and mannit with Andrade's indicator are inoculated from the slants and read in twenty-four hours, forty-eight hours, and at the end of a week. Microscopic examination is made of the organisms that do not produce acid in mannit but do in the other four sugars used.

The sources of cultures studied:

##### Culture 1006

This culture was isolated, May 8, 1924, from a hen brought in while still alive, by a local farmer. The bird was dumpy, and died in four days. The clinical symptoms noted were drooping of head and tail, sleepiness. The droppings were yellowish and watery in the early stages, greenish in later stages of the disease. The lesions observed were numerous petichial hemorrhages

in the heart muscle. The liver was yellowish. The colonies of the organism were small and white on beef infusion agar.

Cultures 955 and 956.

These cultures were isolated from two large and three small chicks sent in from Bangor, Michigan. The autopsy of the larger ones showed no abnormalities except a retained yolk in one. There were no lesions in the three small ones except that the liver of one was yellowish in color, and one had a retained yolk. The organisms were isolated from heart and liver.

Culture 1087

This culture was isolated from five small chicks sent in from Stockbridge, Michigan. Three had ochre colored livers, one had a large retained yolk, three had smaller retained yolks. The colonies of the organism were small and bluish on infusion agar. The culture was from a yolk.

Cultures 624 and 625

These cultures were isolated from two live hens and one dead one. One live one showed signs of legweakness. All were emaciated. One showed discolored liver and inactive abnormal ovary. The colonies were small and bluish white.

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## B. Morphology and Staining.

Vegetative Cells. Twenty-four hour cultures of beef infusion agar and nutrient broth were used in the study of cell forms. The organism seems to be a coccus. Many seem to be very short rods, but the coccus form predominates. Pleomorphism is not uncommon, spindle and club shapes being most often found. During the serological studies a rod with bipolarly staining bodies was encountered. Serum of injected animals had been mixed with a very dilute suspension of the organism, incubated for some hours, and a small amount of the mixture plated on agar. A smear of the colonies that grew was stained with dilute carbol fuchsin and showed a predominance of these forms with large deeply stained polar bodies. (photograph, page 1 ) No effort was made to explain these seeming inconsistencies. Such an attempt would involve an intense study of life cycles.

### Arrangement.

The organism is found in a great variety of groupings. There seems, However, to be a large proportion of diplococcus forms, the individuals showing a tendency to flatten on the adjacent sides.

### Size.

The average diameter of the organism is .97 microns with a few measuring one micron. There was very little variation.

### Staining characteristics.

The organism stains readily with carbol fuchsin, diluted one to ten with Loeffler's methylene blue, with aqueous



alcoholic solution of saffranin and Bismark brown. It is positive to Gram stain. It is not acid fast.

No capsules or spores were demonstrated.

### C. Cultural Characteristics.

Agar Slant. Beef infusion agar was used. When first isolated this organism grew well, appearing much like Bacterium pullorum. After it had been transferred two or three times it grew very poorly. On agar after several days incubation at 37°C the colonies were beaded, raised, glistening, smooth, and transparent. There was no chromogenesis, odor or gas formed. The color of the medium was not altered.

Gelatin Stab. The organism did not grow in gelatin. Cultures were incubated both at twenty and thirty seven degrees for three weeks. Due to the fact that it did not grow, its ability to liquify gelatin could not be determined.

Nutrient Broth. After repeated transfers in broth the growth became scarce or disappeared entirely. Broth made with chicken meat seemed to stimulate growth. This was made according to instructions for the preparation of nutrient broth in Giltner's "Microbiology", page 27, except that chicken meat was substituted for chopped beef. The medium was clouded, with no surface growth, sediment or odor.

Agar Plates. Beef infusion agar was used. Observations were made at the end of 24 hours incubation at 37°C. The colonies were small, circular, smooth, raised and entire. When examined under the microscope they appeared to be bursting at several points, with organisms exuding at these places. When first isolated the colonies were

about a millimeter in diameter and bluish white.

Gelatin Plates. No growth.

Potato Slant. The growth on potato medium was very poor. There was no chromogenesis nor any other change in the medium.

Blood plates. Two percent sterile sheep blood was added to beef infusion agar, plates were poured and allowed to harden. They were then streaked from a broth culture. Methemoglobin was formed except by culture 625, which failed to grow. The amount of growth was moderate.

Glucose agar. The organism grew fairly well on sugar agars, its appearance being much like that on beef infusion agar when first isolated.

#### D. Physiological Characteristics.

Sugar Fermentations. Sugar free broth containing one percent sugar, one-half percent sodium chloride, and one percent Andrade's indicator was used. The tubes were inoculated with one loop of twenty-four hour broth culture, and were read at the end of twenty-four and forty-eight hours, and seven days. The sugars used were inulin, dextrin, dextrose, dulcitol, levulose, sucrose, mannitol, rhamnose, adonitol, salicin, galactose, arabinose, maltose, xylose, raffinose, glycerin, and lactose. No gas was formed in any of these sugars. Acid was produced in dextrose, sucrose, maltose, levulose, lactose, and salicin, very slowly in raffinose and dextrin. Cultures 624 and 625 died before the studies were completed, but showed no inconsistencies in the fermentations in which they were used. It has been found that the fermentation of lactose is sometimes slow, in the routine laboratory work, in which peptone broth is used in making the sugars.

Litmus Milk Reaction. Acid reaction was produced in milk at the end of twenty-four hours incubation at 37°C. Coagulation was found in the lower part of the tubes in forty-eight hours and was complete in four days. A small amount of whey was extruded at the end of seven days. The litmus was reduced in sixteen hours. Re-oxidation took place to the extent of 10 percent in twenty-four hours and was complete in most cases at the end of seven days. Strains 625 and 626 were more rapid in all these processes than the other cultures. The first time the

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experiment was made, re-oxidation of the litmus had taken place only in the upper half of the tubes at the end of seven days.

Indol Production. For the indol test, seven-day cultures in Dunham's peptone solution were used, Ehrlich's method being followed. Indol was produced by all strains studied.

Nitrate Reduction. Four day old nitrate-peptone solutions incubated at 37°C were used, following the method explained in Giltner's "Microbiology", page 131. Presence of both ammonia and nitrites was demonstrated.

Hydrogen-sulphide Production. The organism was streaked on plates of agar, to which one gram of lead carbonate had been added. Blackening of the media (caused by the formation of lead sulfid) denotes the production of  $H_2S$ . Hydrogen sulfid was not produced by any of the strains studied.

Proteolytic enzymes. Neither Loeffler's coagulated blood serum medium, milk, nor gelatin showed the presence of proteolytic enzymes.

Oxygen Requirements. The organism grew on anaerobic plates as well as on aerobic plates of the same medium (beef infusion agar). Anaerobic conditions were obtained by the pyrogallio acid and NaOH method. (Giltner's "Microbiology", page 158).

Hydrogen-ion Requirements. The organism grew on agar varying in pH from 6.2 to 7.8.



Thermal Death Point. For detailed description of method used see Giltner's "Microbiology", page 198. This is Sternberg's method. In general, it consists of drawing a small amount of broth culture into a capillary tube, sealing the end, exposing the tube at the desired temperature a definite length of time, breaking off the ends of the tube and dropping it into broth. Growth or lack of growth is noted after twenty-four hours incubation. The organism died at 58°C in ten minutes.

### E. Serological Studies.

Bacteriolysins: Tests for the presence of bacteriolysins were made in a great variety of proportions of immune serum and suspension of organism, using the method explained in "A Laboratory Course in Serum Study", by Zinsser, Hopkins and Ottenburg, page 58. In general the method consists of incubating 1 cc. of 1-500 dilution of twenty-four hour broth culture of the organism, with varying amounts (.01 to .0001 cc) of immune serum. Two loopfulls of mixtures are added to tubes of agar that have been melted and cooled, and plates are poured. These are examined for colonies after twenty-four hours incubation.

The experiments were carried out with serum of guinea pigs that had been injected with live and attenuated organisms two months before. Serums of two control pigs were also used. Bacteriolysins were not demonstrated in any dilution. The autopsy of these guinea pigs seemed to show that the animals are not susceptible to the organism, in which case it is not surprising that the presence of the antibody could not be proven.

Agglutination tests were run, using the serum of guinea pigs injected with live and attenuated organisms, and controls (not injected); the serum of chickens injected with live organism, and with filtrates, and controls; on the serum of birds showing lameness; and on flocks in which there was no reason to suspect the presence of the organism.

The antigen used in making the agglutination tests was made by growing the organism forty-eight hours on dextrose agar in Kille flasks. Dextrose agar was used because the

organism grew much more abundantly on this medium. The growth was washed off with physiological salt solution to which five-tenths percent phenol was added, and diluted to a turbidity of tube number one of McFarland's nephelometer with the same solution. One cubic centimeter was placed in Wasserman tubes. .05, .025, .01 and .005 cubic centimeters of serum were generally used, making dilutions of 1-20, 1-40, 1-100, and 1-200. The tests were read after incubation at 37°C for twenty-four and forty-eight hours, and were recorded as follows: +, +, - according to degree of agglutination; C if there was cloudiness obscuring the test, U if hemolysis or presence of cells made the test unreliable.

Tests were first run using this antigen and serums reacting positive, negative and partial to the test for bacillary white diarrhea (B. pullorum antigen employed), in an effort to determine the specificity of the reaction. In every case they were negative. Later there was reason to doubt the dependability of this work, which was not very extensive. Several samples sent in for bacillary white diarrhea testing gave "partial" results, several experimental birds from the animal room also. It is possible that these birds were infected; however, it does not seem probable.

#### Guinea Pig Serums.

Guinea pigs were injected with live organism (5 cc. of the turbidity of number three of McFarland's nephelometer suspension) and repeatedly with attenuated organism. For



details see studies on pathogenicity. The results of all the agglutination tests using guinea pig serum were negative.

Chicken Serum.

The results of the agglutination tests that were made with serum of the chickens that were injected with the organism are given in the following table. For details of the injections see "Pathogenicity" page 25. The antigen used was a mixture of four strains.

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CROSS AGGLUTINATIONS

|                                                 | .05 | .025 | .01 | .005 |                                                 | .05 | .025 | .01 | .005  |
|-------------------------------------------------|-----|------|-----|------|-------------------------------------------------|-----|------|-----|-------|
| Chicken No. 34<br>Injected with<br>culture 584L |     |      |     |      | Chicken No. 42<br>Injected with<br>culture 488S |     |      |     |       |
| Antigen 488                                     | +   | +    | -   | -    | Antigen 488                                     | -   | -    | -   | -     |
| Antigen 584                                     | -   | -    | +   | +    | Antigen 584                                     | -   | -    | -   | -     |
| Antigen 475                                     | +   | +    | +   | -    | Antigen 475                                     | -   | -    | +   | -     |
| Chicken No. 97<br>Injected with<br>culture 475H |     |      |     |      | Chicken No. 8<br>Injected with<br>culture 505Y  |     |      |     |       |
| Antigen 488                                     | C   | C    | C   | C    | Antigen 488                                     | +   | +    | +   | Unfit |
| Antigen 584                                     | C   | C    | C   | C    | Antigen 584                                     | -   | -    | +   | "     |
| Antigen 475                                     | C   | C    | C   | C    | Antigen 475                                     | -   | -    | +   | +     |
| Chicken No. 91<br>Not injected                  |     |      |     |      | Chicken No. 1<br>Injected with<br>culture 488S  |     |      |     |       |
| Antigen 488                                     | +   | +    | +   | +    | Antigen 488                                     | C   | C    | C   | C     |
| Antigen 584                                     | +   | +    | +   | +    | Antigen 584                                     | C   | C    | C   | C     |
| Antigen 475                                     | +   | +    | -   | -    | Antigen 475                                     | C   | C    | C   | C     |
| Chicken No. 61<br>Not injected                  |     |      |     |      | Chicken No. 92<br>Injected with<br>culture 475H |     |      |     |       |
| Antigen 488                                     | +   | +    | +   | +    | Antigen 488                                     | +   | +    | +   | +     |
| Antigen 584                                     | +   | +    | +   | +    | Antigen 584                                     | +   | +    | -   | -     |
| Antigen 475                                     | +   | +    | +   | -    | Antigen 475                                     | +   | +    | +   | +     |
| Chicken No. 48<br>Not injected                  |     |      |     |      | Chicken No. 13<br>Injected with<br>Culture 584L |     |      |     |       |
| Antigen 488                                     | +   | +    | +   | +    | Antigen 488                                     | +   | -    | +   | +     |
| Antigen 584                                     | +   | +    | +   | +    | Antigen 584                                     | +   | +    | -   | Unfit |
| Antigen 475                                     | +   | +    | +   | +    | Antigen 475                                     | +   | +    | +   | +     |
| Chicken No. 45<br>Not injected                  |     |      |     |      | Chicken<br>Injected with<br>toxin               |     |      |     |       |
| Antigen 488                                     | +   | +    | +   | +    | Antigen 488                                     | +   | +    | +   | +     |
| Antigen 584                                     | +   | +    | +   | +    | Antigen 584                                     | +   | +    | +   | +     |
| Antigen 475                                     | +   | +    | +   | +    | Antigen 475                                     | +   | +    | +   | +     |

All these birds were kept together in one colony house.

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### F. Pathogenicity.

The chickens used in the study of the pathogenicity of this organism were injected January 23rd, 1924. Eight received 2 cc of twenty-four hour broth cultures of different strains, intraperitoneally, three received respectively one and one-half, three, and five cubic centimeters of filtrate of a five-day peptic digest broth culture of strain 584L.

The birds were killed March 30th and were autopsied two hours after they were killed. In some cases parasites were found, consisting chiefly of round worms, usually in small numbers.

Ovary, liver, heart and spleen, in most cases, were plated in an attempt to reisolate the organism. A large number of colonies were picked and run on sugars after growing twenty-four hours on agar slants. Microscopic examination was made. The organism was isolated from only six birds, 97, 14, 34, 1, 42, and 67.

Bird No. 1. Injected with strain No. 488S. Slightly emaciated.

Comb: somewhat pale.

Blood: not coagulated.

Liver: very friable.

Spleen: congested.

Ovaries: active, some ovules showing hemorrhagic condition, some contain cheesy material and are collapsed.

Kidneys: congested.

Large Intestines: small areas of hemorrhage.

Small Intestines: slight enteritis.

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Proventriculus: small hemorrhagic areas.

Pancreas: hemorrhagic, necrotic areas in upper portion.

Bird No. 8. Injected with strain 505Y. Fair condition.

Comb and wattles: anemic.

Liver: congested, very friable.

Ovaries: active and hemorrhagic.

Large intestines: hemorrhagic.

Bird No. 13. Injected with strain 584S. Extremely emaciated.

Liver: congested with necrotic foci, in general  
atrophic.

Heart: atrophied.

Spleen: atrophied, anemic.

Small Intestines: slight hemorrhagic enteritis.

Bird No. 14. Injected with strain 505Y. Slightly emaciated.

Comb: anemic.

Liver: friable, hemorrhagic, greyish discoloration  
similar to fatty degeneration.

Ovary: active, slightly hemorrhagic.

Intestines: few petechial hemorrhages.

Proventriculus: hemorrhagic areas at posterior part.

Bird No. 34. Injected with strain 584L. Fair condition.

Liver: congested, friable, degenerated areas.

Ovary: active, some ovules showing hemorrhagic  
condition, others collapsed and  
containing cheesy material.

Kidneys: slightly anemic.



Bird No. 42. Injected with strain 488S. Slightly emaciated.

Comb: pale

Liver: congested in streaks, otherwise pale; friable

Ovary: active, some ovules collapsed and contain  
cheesy material.

Bird No. 61. Not injected. Emaciated.

Liver: friable hemorrhagic; fatty and parenchymatous.  
degeneration.

Heart: subacute pericarditis.

Bird No. 67. Injected with 3 cc. of filtrate of peptic  
digest culture.

Liver: congested, showing a few necrotic foci, in  
general of friable consistency.

Heart: a few petechial hemorrhages on visceral  
part of pericardium.

Ovaries: inactive, some ovules collapsed, and  
containing cheesy material.

Small intestines: ecchymotic hemorrhages.

Proventriculus: few petechial hemorrhages.

Bird No. 92. Injected with strain 475H. Fair condition.

Comb: somewhat anemic.

Liver: congested, friable, showing numerous very  
small yellowish spots.

Heart: fatty degeneration of muscle.

Kidneys: show a greyish yellow discoloration  
suggesting small necrotic foci.

Gizzard: mucosa of reddish color in small spots.

Bird No. 97. Injected with strain 475H. Fair condition.

Wattles: somewhat anemic.

Liver: hyperemia and parenchymatous degeneration,  
friable.

Spleen: anemic

Ovaries: active, congested.

Pancreas: small spots of congestion.

Small Intestines: small hemorrhagic areas.

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The rabbits used in the study of the pathogenicity of the organism were injected, January 23, 1925, with two cubic centimeters of twenty-four hour broth culture, intravenously. Two of them were killed in April. The lesions were so slight that it was not considered necessary to kill the other two, especially since they showed no symptoms of disease.

Rabbit No. 166. Injected with strain 584L.

Liver: normal in size and color, but on surface are several whitish spots about the size of a pin head, or a little larger. They are of tough consistency, and some may be found on the out surface. They are seemingly of parasitic nature.

Heart: chronic pericarditis, probably due to bleeding.

Cecum: glandular part was enlarged and thickened.

Rabbit No. 149. Injected with strain No. 475H.

Liver: chronic lesions, consisting of a number of small nodules which are probably parasitic in nature.

Kidney (right): small pin point sized whitish spots.

Kidney (left): two small spots of the same nature.

Cecum: thickened, but not so much as 166. Worms.

The possibility that the organism loses virulence when grown on artificial media led to the injection of two more chickens. These two hens were each injected intramuscularly with 4 cc. of suspension from twenty-four hour agar cultures of a strain isolated seventy-two hours before. They were killed forty-four days after injection, and were autopsied at once.



Bird No. 100.

Liver: normal in size, and in general of normal color.

On the right lobe is a bluish discoloration on the capsule. The capsule is somewhat thickened at this point. Near the border of the left and right lobes are some spots that are not sharply circumscribed, of somewhat darker reddish brown color. On the border of the left lobe is a yellowish spot of the size of a pea, the consistency of which is a little tough. Near this spot but on the lower surface is a larger spot showing partly dark brown, partly a yellowish color. On the lower surface of the right lobe is a projection about the size of a pea, of dark red brown color, probably a hematoma.

Heart: chronic adhesive pericarditis, a few very small hemorrhages on the visceral part of the pericardium.

Ovary: some ovules quite normal, others collapsed and greyish yellow in color.

Slight peritonitis.

Bird No. 101.

Liver: peculiar yellowish discoloration, though of normal consistency. Probably due to fatty degeneration.

Heart: flabby, adhesive pericarditis.

Ovary: some ovules collapsed and of yellowish greyish discoloration.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial matters. The text suggests that organizations should implement robust systems to track every detail, from small expenses to major investments.

2. The second section focuses on the role of technology in modern record-keeping. It highlights how digital tools can streamline the process, reduce errors, and provide real-time access to data. The author argues that embracing technology is not just a convenience but a necessity for staying competitive in today's fast-paced environment.

3. The third part of the document addresses the challenges of data security and privacy. It notes that as more information is stored digitally, the risk of breaches and unauthorized access increases. The text provides several recommendations for safeguarding sensitive data, including regular security audits, employee training, and the use of encrypted communication channels.

4. The fourth section discusses the importance of regular reviews and audits. It states that periodic checks are crucial to ensure that records are up-to-date and accurate. The author suggests that organizations should establish a clear schedule for these reviews and assign specific responsibilities to designated personnel.

5. The fifth part of the document touches upon the legal implications of record-keeping. It mentions that certain industries are subject to strict regulations regarding data retention and access. The text advises organizations to consult with legal counsel to ensure they are fully compliant with all applicable laws and standards.

6. The sixth section explores the benefits of a well-maintained record system. It points out that accurate records can facilitate better decision-making, improve operational efficiency, and provide a clear history of past actions. The author concludes that investing in a solid record-keeping system is a long-term benefit for any organization.

7. The final part of the document offers some concluding thoughts and encourages organizations to take proactive steps to improve their record-keeping practices. It reiterates the key points made throughout the text and expresses confidence that these measures will lead to greater success and stability for the future.

On February 13th four guinea pigs were injected intraperitoneally with .5 cc of suspension of live organisms (turbidity 3) in .85 percent salt solution. Four pigs were injected with suspension of organism (turbidity of 2) that had been heated 30 minutes at 55°C for the purpose of attenuation. They were given four injections, at four day intervals, the size of the dose being doubled each time. The first injection was 1/2 cc., the last one 4 cc. Strain number 488 was used for all guinea pig injections.

Two control pigs were kept with the others. The entire group was killed March 9th. The lesions were slight and were found in the control pigs as well as in the injected ones. Therefore it is not considered worth while to include even a brief summary of the findings. The animals probably are not susceptible.

## V. DISCUSSION OF RESULTS.

As may be concluded from the data of a number of postmortems reported before, the results obtained were to a large extent inconsistent and inconclusive. Consequently it is not an easy matter to decide whether the organism in question represents a pathogenic parasite or a harmless saprophyte or perhaps an intermediate form.

The fact that the organism has been repeatedly isolated in pure culture from birds that died and from which no other organism known to be capable of causing disease was isolated, seems to suggest that we have to deal with an organism, the pathogenicity of which is at least highly probable. This probability is supported by the fact that as it may be seen from Table III a number of the birds infected experimentally with this organism showed more or less pronounced lesions and that from six out of ten so infected birds this organism could be reisolated.

The variation and in many cases the absence of lesions in a fairly large number of cases of the routine autopsies (Table I) as well as in the autopsies of a number of injected birds tends to show, however, that this organism may, under as yet unknown circumstances, be capable of causing changes in some of the organs of the host. If such favorable conditions are absent the organism seems to be harmless for either birds or rabbits. Guinea pigs are entirely resistant. Of special importance so far as the pathogenicity of this

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organism is concerned are the following cases:

Rabbit No. 488 (page 6 ) injected intravenously with culture freshly isolated by Dr. Stafseth. The rabbit died in twelve days, and many lesions were found. All the lesions noted have been found in chickens, though none of them are consistently found in all animals from which the organism had been isolated. Birds 906 and 584 (pages 9 and 8) also injected with freshly isolated strains are evidence also of pathogenicity. Both died within a comparatively short time, and many lesions were noted. The autopsies of birds 496 and 475 seem to point out that the presence of the organism is coincidental and the cause of the diseased condition. In these cases, though the organism was isolated, other organisms that are known to be capable of causing death were also isolated. It would not be possible to say which organism killed the bird.

Further the organism seems to be avirulent in most cases. This seems to be proved not only by its occasional pathogenic effects, but also by its cultural peculiarities its ability to grow on artificial media decreases very rapidly. It might be due to this low virulence that in some of the experimental infections (100 and 101) no lesions could be encountered and that even after heavy doses the organism very soon disappeared from the organism of the host.

The low virulence is indicated further by the nature of the lesions of different organs. These usually show



a chronic character though all organs and especially the blood may harbor large numbers of this organism (bacteraemia).

Whether or not the disease possibly caused by this organism is contagious, is a question of interest. The organism has been isolated from a number of birds of two flocks. Contagiousness might account for the finding of lesions in the control bird kept with those injected for the study of pathogenicity, also in the ones that were injected with filtrate of peptic digest broth. It might be the cause of the positive reactions to the agglutination test obtained with the serum of the birds that were kept in the animal room near those injected during the study of this organism.

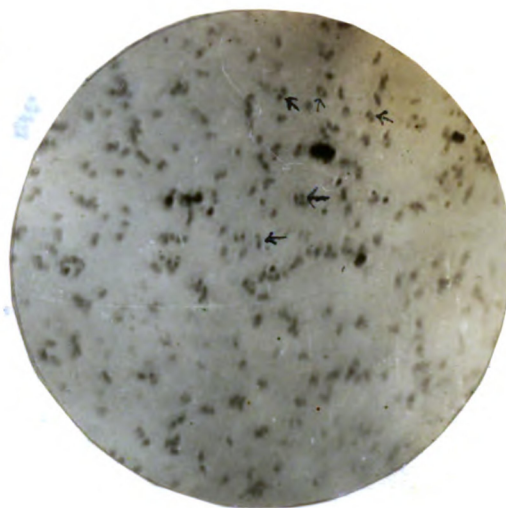
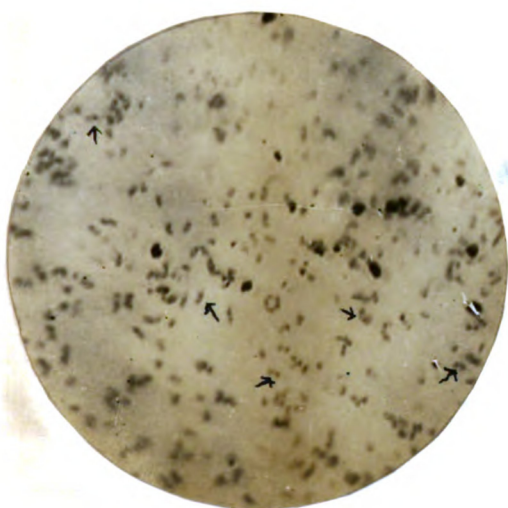
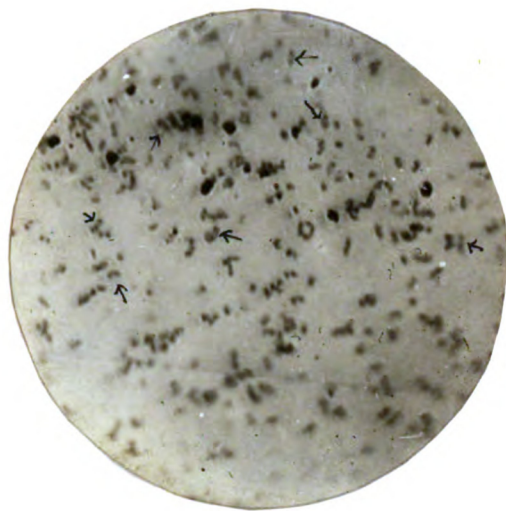
It is not known whether or not the organism produces toxin. The lesions found in the birds injected with filtrate of peptic digest cultures might have been caused by infection contracted from the bird injected with cultures, or the lesions of those injected with cultures may have been caused by toxins injected with the organism, since broth cultures were used. The two hens last injected were given suspensions from agar slants, but the evidence from lesions in these cases is not such as to warrant weight being given to it.

The writer has made no effort to classify the organism. Careful search has been made in Bergey's "Determinative Bacteriology", with the conclusion that the organism has

not been studied before. Characteristics about which there was no doubt, such as sugar fermentations, milk reaction, indol production, and Gram staining, were used as a basis of the search. The possibility that it is either a coccus or a rod was taken into consideration.

## VI. SUMMARY

1. The organism is a small coccus or cocco-bacillus, measuring about one micron, occurring most often in pairs, growing poorly on artificial media, producing methaemoglobin on blood plates, fermenting dextrose, sucrose, maltose, levulose, lactose, salacin, raffinose, and dextrin, with production of acid, but not gas, giving acid reaction in milk, with coagulation and reduction of litmus, producing indol, reducing nitrates to nitrites and ammonia. It is aerobic and facultative anaerobic. Its thermal death point is 58°C.
2. Under as yet unknown circumstances this organism may be pathogenic for chickens and rarely for rabbits.
3. In most cases its virulence is very low and then its occurrence in chickens seems to be without any significance.
4. If lesions are caused by this organism they are of chronic nature.
5. Infected birds apparently do not produce specific agglutinins.



Photographs, showing bi-polar bodies.



**Drawing of field, showing bi-polar bodies**



**Drawing showing characteristic groupings**



**Involution forms, (drawn on much higher scale**

## VII. ACKNOWLEDGMENT.

The writer wishes to acknowledge indebtedness to Dr. Ward Giltner, Dr. H. J. Stafseth, Dr. A. Kotlan, Mr. W. L. Mallmann and Miss Dorothy Yakeley for assistance and helpful suggestions received during the course of these investigations.

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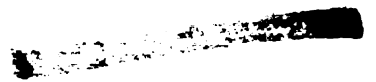
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