STUDIES ON BACILLARY WHITE DIARRHEA OF THE DOMESTIC FOWL

THESIS FOR DEGREE OF M. S.

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THESIS

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THESIS

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Introduction.

Domestic fowls of all types have up to the present received but comparatively scant notice from the scientific world. The embryologist, it is true, has had recourse to the egg for class-room work, for which purpose it is, indeed, by its abundance and cheapness well adapted. But these very qualities that recommend it to the embryologist have been largely responsible for the meagre attention bestowed by men in other branches of science on the egg and the hen that produces it. An individual fowl, except in rare cases, is worth but little, and the average farmer pays small attention to its loss. Hence, until the advent of the commercial poultry plant, the domestic fowl occupied a position in the economic world in no way comparable in importance with that occupied by such animals as the horse, cow, or pig. Lacking thus in strict money value, the domestic fowl has been studied more or less in a purely scientific spirit. Treatises on its anatomy and physiology are rare, and the treatment of its diseases is largely empirical. Of late years, however, the great expansion of the poultry industry has drawn attention rather forcibly to poultry diseases, and stimulated investigation in veterinary and bacteriological circles.

While old diseases such as tuberculosis and roup must be fought by the poultryman, others of later origin must be contended with as well. One of the most recent and destructive

of these is bacillary white diarrhea. It is not too much to say that this disease is appreciably crippling the poultry industry in not a few parts of the United States. Not only is the yearly money loss in young chicks great, but the destruction of so many well-bred birds hinders the development of strains desirable for utility or show purposes.

Considerable investigations have been carried on concerning bacillary white diarrhea, but there is room not only for work on some hitherto untouched phases of the disease, but also on corroboration of results already published. It has, therefore, been deemed wise to cover some ground more or less thoroughly gone over already, and to conduct newer experiments as opportunity offered.

Historical Resumé.

In 1900, as a result of high mortality from an unknown disease among very young chicks in certain Eastern sections, Rettger (1) started investigations and isolated an organism with which he was able to produce the disease by artificial inoculation. Studies on further outbreaks in succeeding years confirmed his earlier observations, viz.--- that the septicemia is due to a specific bacterium, usually affects chicks under four days old, causing drooping, a whitish discharge from the vent, loss of appetite, failure to grow, with death as a rule quickly supervening; autopsy showing the liver with congested patches or congested throughout; crop, lungs, kidney, and spleen apparently normal, unabsorbed yolk usually presenting abnormal color or

consistency. From chicks dying of the disease it is usually easy to isolate the casual organism, named by Rettger (2), in 1907, Bacterium pullorum. A valuable contribution to the subject was made shortly after by Rettger and Stoneburn (3) who established the fact that mature hens harbor the organism and lay infected eggs, the chicks from which, if they survive, in turn become carriers of the disease.

Working independently of these two investigators, Jones (4) corroborated some of their work. Results arrived at by Gage (5) about the same time were also corroborative in nature.

In 1912, Rettger (6) secured some encouraging results in the use of sour milk in lessening the mortality among young chicks exposed to infection.

With the knowledge that the nucleus of the disease lies in the ovaries of infected hens that are to all appearance normal, investigators naturally sought to perfect a method of detecting such carriers. Rettger (6) experimented with the egg test, as also did Gage (7), but the results were not gratifying. Neither was the agglutination test, first tried by Rettger (8), immediately successful. It was, however, later perfected by Jones (9). The value of the test has been well demonstrated, large numbers of hens reacting to the tests of various investigators having been found to exhibit typical ovarian symptoms of the disease on post-mortem examination.

It is unfortunate, but understandable enough, that practical poultrymen have failed to distinguish between bacillary white diarrhea, and more or less fatal ailments simulating, it is true, bacillary white diarrhea, but usually originating in im-

proper feeding. Serious losses among young chicks through errors in feeding have caused many poultrymen to ascribe bacillary white diarrhea as the cause, and to suspect healthy birds as chronic carriers of the disease. Such mistakes have hindered the gathering of reliable data on the extent of the disease. Moreover, such misconceptions are not confined to the ordinary poultryman alone. For example, when Morse (10) ascribed Coccidium tenellum as the cause of a diarrhetic outbreak which he investigated, it was assumed by Herzog (11) that this outbreak was identical with those epidemics studied by Rettger. Since Morse affirmed that the disease he investigated attacks chicks from two to five weeks in age, the two outbreaks clearly must have been different in character. The term "bacillary white diarrhea", which has come to be regarded by bacteriologists and veterinarians as the result of Bact. pullorum infection and nothing else, is accepted by others as covering a wide range of intestinal troubles, and its simplicity is more than counterbalanced by its lack of specific meaning.

The disease in the course of some fifteen years has made itself felt in probably every State (12), and has been observed in imported birds as well (13).

Morphology and Cultural Characteristics of Bact. pullorum.

Six different strains were studied. The following applies to all:-

Size: 1/2 micron by 2 1/2 microns on average.

Motility: No true motility, but Brownian movement pronounced.

Air Requirements: Facultative anaerobe.

Stains: Readily stained by usual methods. Gram negative. Not acid fast.

Spores: No spores have ever been observed.

Growth: Agar Slant,- most vigorous at 37°-38° C. Delicate, shining, never spreading far from path of inoculation; will grow about equally well on ordinary liver or glycerin agar; agar beginning to dry out favored. On agar plates colonies very delicate, round, translucent, sometimes difficult to detect.

Gelatin Stab: No liquefaction; slight granular growth along inoculation line.

Broth: Even turbidity in 24 hours. Sediment on longer incubation.

Egg medium (Lubenan's -14-) Rather scant to medium growth.

Indol production: None in Dunham's peptone solution.

Nitrates: Not reduced in nitrate peptone solution.

Fermenting power in sugar broths: The strains showed some slight differences, as indicated in the tables.

Dextrose Broth.

Strain.	Gas in closed arm.	Ratio of CO_2 to other gases.
A1	Trace.	Not tested.
A3	-	-
C	Trace.	Not tested.
W15Y	10%	1:2.3
R15Y	-	-

Maltose Broth.

Strain.	Gas in closed arm.	Ratio of CO_2 to other gases.
A1	-	-
A3	-	-
C	-	-
W15Y	-	-
R15Y	-	-

Saccharose Broth.

Strain.	Gas in closed arm.	Ratio of CO_2 to other gases.
A1	Trace.	Not tested.
A3	-	-
C	-	-
W15Y	-	-
R15Y	-	-

Lactose Broth.

Strain.	Gas in closed arm.	Ratio of CO_2 to other gases.
A1	15%	1:2.7
A3	-	-
C	10%	1:3.0
W15Y	-	-
R15Y	Trace.	Not tested.

Determination of Thermal Death Point of Bact. pullorum.

To determine the thermal death point of Bact. pullorum, pieces of glass tubing were drawn out into capillaries about 10 in. to 12 in. in length. Each capillary was flamed in the middle until it separated, and then flamed where it merged into the tube from which it was drawn. Thus, two capillary tubes were secured from one drawing, each about six inches long, with a bulging end and both ends sealed. The organisms to be tested were in 24 hour broth culture. The method of filling the capillaries was to break off the tip with a heated file, flame two or three times, and insert the tip in the broth culture after heating the bulging end slightly. The air contracting as it cooled in the thick end resulted in some of the broth culture being forced into the capillary. Every effort was made to secure capillaries of the same size and to draw the same volume of broth culture into each -- viz. 1 1/2 in. Each tube was taken from the broth culture before the heated air in the thick end had reached its limit of contraction, so that the lower end of the column of broth culture eventually stood at least 1/2 in. from the end of the tube. The tip was then sealed by flaming and the tube laid in a water bath at the desired temperature, the flame having been carefully regulated to maintain that temperature. At the end of ten minutes the tube was quickly withdrawn and immersed in cold 1:1000 $HgCl_2$ for three minutes. Handling with flamed forceps, it was then washed in sterile distilled water and immersed in 95% alcohol for about ten seconds. On withdrawal from the alcohol it dried quickly. The tip was

then broken with a flamed file on sterilized filter paper and the contents forced into a tube of broth by heating the bulging end. The broth was then incubated at 37°C. and read at the end of 48 hours.

The first tube was heated at 40°C. for 10 mns., the second at 42°C. for the same time, and the remaining tubes were heated in like manner, each one two degrees above the preceding. There were 16 tubes in all, the highest temperature employed being 70°C. See table.

Two thermometers were used in the water bath and the temperature was not allowed to vary more than a fraction of one degree Centigrade, while a tube was being heated.

Thermal Death Points of Bact. pullorum,

Strains W15 and C.

Temp.	Time.	Growth in plain bouillon.	
		W15	C
40° C.	10 mns.	+	+
42° "	"	+	+
44° "	"	+	+
46° "	"	+	+
48° "	"	+	+
50° "	"	+	+
52° "	"	+	+
54° "	"	+	+
56° "	"	+	+
58° "	"	-	+
60° "	"	-	-
62° "	"	-	-
64° "	"	-	-
66° "	"	-	+*
68° "	"	-	
70° "	"	-	-

*The characteristics of the growth in this tube indicated contamination, which was proved by microscopic examination and the agglutination test.

The slight difference in the resistance of the two strains cannot satisfactorily be explained. Agar slants were made from the last tubes of the two strains showing growth and the agglutination test run to make sure that the "end point" had not been obscured by some contaminating organism. Both growths proved to be pure cultures of Bact. pullorum. It is possible that the findings would have been reversed had another test been run, or that the inherent resistance of the C strain to heat is slightly greater than that of the W15 strain.

Resistance of Bact. pullorum to Some Common Disinfectants.

In view of the fact that the question as to what constitutes the best disinfectant for use in incubators and coops harboring Bact. pullorum frequently is asked, and also because no comparative tests of various disinfectants appear to have been made it was considered well worth while to run the following experiment:

1/10 c.c. of a vigorous broth culture of Bact. pullorum 24 hrs. old was put into 5 c.c. of disinfectant and mixed well. At the end of 30 seconds a loopful was removed and put into 5 c.c. of plain broth. At the end of one minute another loopful was removed and placed in a second tube of broth. This was repeated at 1/2 minute intervals for six minutes. The tubes of broth were then incubated at 37°C. for 48 hours and examined for growth.

The contents of all tubes were evenly mixed by gentle shaking and the usual precautions were taken to avoid contamination. See table for results.

Disinfectant	Time in minutes.											
	1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	5	5 1/2	6
1:1000 $HgCl_2$.	-	-	-	-	-	-	-	-	-	-	-	-
5% Phenol.	-	-	-	-	-	-	-	-	-	-	-	-
1% Phenol.	+	+	+	+	+	+	+	+	+	-	-	-
2% Liquor cresolis compositus.	-	-	-	-	-	-	-	-	-	-	-	-

The experiment indicates that Bact. pullorum yields readily enough to some common disinfectants when used in the dilutions generally recommended, and the disinfection of coops and incubators, as far as Bact. pullorum is concerned, should not prove a serious undertaking.

The Longevity of Bact. pullorum on Litter.

Three flasks of 250 c.c. capacity were filled about one quarter full with a mixture of equal parts of clover hay and wheat straw. The material was cut into lengths of approximately 1 cm. to facilitate removal of samples. The flasks were heated in flowing steam for one hour on four consecutive days, at the end of which time samples were carefully removed and added to tubes of plain broth. No growth resulted at the end of 72 hours incubation at 37° C.; the material was considered sterile.

Two 48 hr. agar slant cultures of Bact. pullorum were washed off with sterile physiological salt solution, the suspension -- about 8 c.c. in amount -- divided equally between two flasks and thoroughly mixed with the litter. The third flask,

or control, received 4 c.c. of sterile physiological salt solution. The flasks were kept in the laboratory and received diffused light during the day, but no direct sunlight. Samples were removed once a week and placed in bouillon, the resulting growths being examined in hanging drop and furnishing material for inoculation of agar slants. Growth on agar was carefully examined, and from time to time suspensions in physiological salt solution were made and agglutination tests run with positive sera.

At the time of taking samples, about 2 c.c. of sterile distilled water were added to each flask.

See table for results.

Date	Broth Culture, and Agar Streak Therefrom.		
	Flask 1.	Flask 2.	Flask 3. (Control)
Oct. 14, 1916	Characteristic of Bact. pullorum	Characteristic of Bact. pullorum	No growth
" 21 "	"	"	"
" 28 "	"	"	"
Nov. 4 "	"	"	"
" 11 "	"	"	"
" 19 "	"	"	"
" 26 "	"	"	"
Dec. 2 "	"	"	Contaminat B. subtili
" 9 "	"	"	No growth
" 16 "	"	"	"
" 23 "	"	"	"
Jan. 4, '17	"	"	"
" 11 "	"	"	"
" 18 "	"	"	"
" 25 "	"	"	"
Feb. 1 "	"	"	"
" 8 "	"	"	"
" 15 "	No growth	"	"
" 22 "	"	"	"
Mar. 1 "	"	No growth	"
" 8 "	"	Contaminated	"
Apr. 10 "	"	No growth	"
" 11 "	"	"	"
" 12 "	Contaminated	Contaminated	"

Agglutination Test.

Flask 1.			Flask 2.		
50	1:100	1:200	1:50	1:100	1:200
+	+	+	+	+	+
		Not run.			
		" "			
+	+	+	+	+	+
		Not run.			
+	+	+	+	+	+
		Not run.			
		" "			
+	+	+	+	+	+
		Not run.			
+	+	+	+	+	+
		Not run.			
+	+	+	+	+	+
		Not run.			
+	+	+	+	+	+
		Not run.			
			+	+	+

In the last inoculation, sterile broth was poured into the flasks in sufficient quantity to cover the litter.

As the dates show, the experiment was interrupted for fully a month at the end, but the results probably were not affected, since Bact. pullorum had not been isolated in the weeks immediately preceding. Period of uniform isolation = 16 weeks.

The results indicate that Bact. pullorum would persist for a considerable time on contaminated litter and emphasize the importance of thorough cleaning and disinfecting of coops and incubators that have harbored chickens suffering from white diarrhea. How the longevity of the organism would be affected by less artificial conditions -- e.g. by the presence of large numbers of other microorganisms -- is problematical. Experiments to determine this point would be valuable, but the obscuring of the delicate growth of Bact. pullorum by other organisms on agar would make its detection difficult.

The Agglutination Test as a Means of Detection of Carriers of Bact. pullorum.

Securing blood sample: The following method has been found convenient for drawing a sample of chicken blood.-- Tie the legs of the bird together and lay the fowl on a box or table of convenient height, on the right side, the head being next the operator. Lift the left wing and strip the feathers from the under side. Moisten the skin with 5% phenol and locate the vena ulnaris near the joint of the wing tip. With a very sharp

knife, at one cut if possible, slit the vena ulnaris longitudinally for about 1/8 inch. This will ordinarily give a suitable amount of blood -- viz. about 4 c.c. The wound is then moistened with phenol and the bird given its freedom. If the flow is poor, lay the bird on the other side and tap the right wing vein. Occasional excessive bleeding should be checked with a pledget of absorbent cotton. The blood should be collected in a sterile centrifuge tube clearly marked with the number of the bird, the clot broken with a sterile platinum needle and the tube set overnight in the ice-box to allow the serum to separate out. Centrifuging is not absolutely necessary unless it is desired to test immediately.

Samples drawn in this manner cause the bird no permanent harm, whatsoever. The wound heals very rapidly and the feathers are quickly restored.

Preparation of test fluid: Agar slants were heavily inoculated with strains of Bact. pullorum suitable for the test. The slants were incubated for 24 - 48 hours at 37° - 38° C. and the growths washed off in sterile .85% salt solution. The fluid was then filtered through sterile cotton to remove clumps, and sterile .85% salt solution was added until a distinct but not excessive turbidity resulted. (A turbidity corresponding to that in tube 2 of McFarlane's (15) nephelometer is suitable.) Enough phenol was then added to make .5%, and the fluid well shaken. It was then stored in brown bottles in the ice-box and would remain usable for a considerable length of time.

The Test: The serum was diluted with the test fluid to 1:50, 1:100 and 1:200. A dilution of 1:20 was at first used

as a base, but this was later abandoned and a suitable amount of serum added with a capillary pipette directly to the test fluid. Three tubes of about 5 c.c. capacity, containing 2 c.c. of the test fluid each, and correctly numbered, were placed horizontally in a step rack. To the first tube was added .4 c.c. of serum, to the second .2 c.c., and to the third .1 c.c. The tubes were then shaken and incubated at 37° for 24 hours. A positive test is marked by the organisms flaking together and settling down towards the bottom so that the supernatant liquid looks more or less clear. The reaction is often observable in 4 hours. It is well to run a control of 2 c.c. of test fluid minus serum and another of tubes to which known positive serum has been added.

Comparison of Strains for Agglutinability.

This experiment was conducted with a view to determine the best strain, or combination of strains, for the agglutination test. The test fluids were prepared as already outlined, the polyvalent fluid being a mixture of equal portions of the others, assembled before shaking.

Table I.

Test Fluid.	Serum.	Dilution.								
		1:50	1:100	1:200	1:300	1:400	1:500	1:600	1:700	1:800
A1	Positive M.A.C.55	+	+	+	+	+	+	+	+	+
A3	"	+	+	+	+	+	+	P	-	-
W15	"	+	+	+	+	+	+	+	+	+
R15	"	+	+	+	+	+	+	P	-	-
C	"	+	+	+	+	+	+	+	+	+
Polyvalent	"	+	+	+	+	+	+	+	+	+

Table 2.

Test Fluid.	Serum.	Dilution.							
		1:900	1:1000	1:1100	1:1200	1:1300	1:1400	1:1500	1:1600
A1	Positive 55	+	+	+	+	+	P	-	-
A3	"	-	-	-	-	-	-	-	-
W15	"	P	-	-	-	-	-	-	-
R15	"	-	-	-	-	-	-	-	-
C	"	+	+	+	P	-	-	-	-
Polyvalent	"	+	+	+	+	+	+	+	P

P = Partial agglutination.

In tables 3 and 4 the same antigen was used as in tables 1 and 2, the positive serum being from another bird.

Table 3.

Test Fluid.	Serum.	Dilution. 1:50	1:100	1:200	1:300	1:400	1:500	1:600	1:700	1:800
A1	Positive 35	+	+	+	+	+	+	+	+	+
A3	"	+	+	+	+	+	+	-	-	-
W15	"	+	+	+	+	+	+	+	+	+
R15	"	+	+	+	+	+	+	+	+	-
C	"	+	+	+	+	+	+	+	+	+
Polyvalent	"	+	+	+	+	+	+	+	+	+

Table 4.

Test Fluid.	Serum.	Dilution. 1:900	1:1000	1:1100	1:1200	1:1300	1:1400	1:1500	1:1600	1:1700
A1	Positive 35	+	+	P	-	-	-	-	-	-
A3	"	-	-	-	-	-	-	-	-	-
W15	"	+	+	+	+	+	-	-	-	-
R15	"	-	-	-	-	-	-	-	-	-
C	"	+	+	+	+	+	+	+	P	-
Polyvalent	"	+	+	+	+	+	+	+	P	P

The test indicated that any one of the strains was suitable for diagnostic work, since all gave satisfactory agglutinations beyond the 1:200 dilution. It is interesting to note that the polyvalent fluid was agglutinated in dilutions higher than those in which the individual strains of which it was composed were agglutinated. The seeming greater potency of one serum over another for a particular test fluid is also noteworthy. This is well brought out by a comparison of the agglutinations of test fluids W15 and A1. W15, with serum 35 in 1:1300 dilution, gave a positive agglutination; with serum 55, a positive result in a serum dilution of 1:900 only. A1, with serum 35 in 1:1000 dilution, gave a positive result; with serum 55 agglutination took place in as high a dilution as 1:1200.

In the routine work of testing the College flock, a polyvalent test fluid was invariably employed.

Specificity of the Reaction.

Since Bact. pullorum has been placed in the colon-typhi group, it was considered worth while to determine whether test fluids of the colon or typhoid bacilli would exhibit agglutination with known positive serum -- i.e. serum containing antibodies for Bact. pullorum. The dilutions used and results attained are shown in the tables.

Table I.

(Serum = positive 55)

Dilution.	Test Fluid.		
	Bact. pullorum.	B. typhosus.	B. coli.
1:50	+	-	-
1:100	+	-	-
1:200	+	-	-
1:300	+	-	-
1:400	+	-	-
1:500	+	-	-

Table 2.

(Serum = positive 35)

Dilution.	Test Fluid.		
	Bact. pullorum.	B. typhosus.	B. coli.
1:50	+	-	-
1:100	+	-	-
1:200	+	-	-
1:300	+	-	-
1:400	+	-	-
1:500	+	-	-

In addition, a test was run in the same dilutions with a serum containing no antibodies for Bact. pullorum by the agglutination test. In all dilutions, the readings were negative for both B. typhosus and B. coli.

The tests indicate that there is no "group" agglutination and that the agglutination test, as used for the detection of carriers of Bact. pullorum, is specific.

The Application of the Agglutination Test.

Some 300 mature birds of the College flock and of various breeds were tested. It was desired to find out as far as possible how closely post-mortem examinations of tested birds checked with the findings of the agglutination test, to get some idea of the percentage of reacting birds on a well-kept plant in this particular section, and to determine the convenience of the test when run on a somewhat large scale with all laboratory facilities.

From 6 to 18 percent of the birds tested gave positive reactions. The percentage of reactors was lowest among the heavier breeds, such as Wyandotts and Orphingtons, and highest among the Leghorns. Whether these results indicate that the Leghorn breed, by reason of its actively functioning ovaries, is more prone to carry Bact. pullorum than breeds that are less persistent layers, or whether the disparity is explainable on other grounds, is not known. It is worth stating that of 55 birds of the Leghorn breed comprising a well-kept private flock, 7 birds only, or about 12%, reacted to the test. Some male birds gave partial reactions, thus raising the question whether male birds can become carriers and, in turn, infect females.

It is a matter for regret that reacting birds were not segregated, wintered over and their eggs incubated. Some interesting and valuable data could thus have been secured. The

College flock, while not kept for commercial purposes, nevertheless could not be utilized even in greater part for experimentation on bacillary white diarrhea alone. The majority of the reacting birds were sold for the table and it was impossible to get complete data on the percentage of infected ovaries.

One hatch of some 200 eggs from infected hens yielded only 20% of chicks that grew to maturity, many chicks dying in the shell towards the close of the incubation period. This experiment was neither run nor supervised by the writer.

This year, 1917, the mortality among young chicks from bacillary white diarrhea has never assumed serious proportions. The management of the young chicks has been on a par with that of previous years and in all probability the weeding out of carriers in the Fall of 1916 has materially lessened the loss among newly hatched birds from bacillary white diarrhea.

The Complement Fixation Test in Diagnosis of Bact. pullorum infection.

The applicability of the complement fixation test to the detection of carriers of Bact. pullorum is considered not only of scientific interest, but it was realized that the test, if reliable, might prove very useful in clearing up doubtful reactions by the agglutination test.

Preparation of the Reagents:-

Antigen (1st method): Agar slants were inoculated and the growths washed off exactly as described in the preparation of test fluid for the agglutination test, except that the

turbidity of the suspension was adjusted to that of tube 4 in MacFarlane's (15) nephelometer.

Hemolysin: 2 c.c. of washed sheep's blood corpuscles were injected into the marginal ear-vein of a healthy rabbit four times on alternate days. A week after the last injection the animal was fastened back down on an animal board, the fur over the heart clipped short, the skin moistened with 5% phenol, the heart located, and a medium-sized needle that had been immersed in .5% phenol and flushed with sterile physiological salt solution just previously, inserted between the ribs, through the cardiac notch and into the left ventricle. About 30 c.c. of blood were drawn off, the needle withdrawn, the visible puncture phenolized and the animal given its freedom. The blood clot was then broken up and allowed to stand for 24 hrs. in the ice-box. At the end of this time the serum was poured off, heated to 56° C. for 1/2 hr. to destroy complement, enough phenol added to make .5%, and the hemolysin stored on ice until needed. In the test, 1/2 c.c. of the normal hemolysin was added to 49 1/2 c.c. of physiological salt solution, thus giving a 1% dilution.

Blood cells (sheep). These were secured by tapping the jugular vein of a healthy sheep with a medium-sized needle, after clipping the wool close, and moistening the skin with phenol. About 60 c.c. of blood were drawn at a time. The blood was collected in a stout flask containing sterile beads and shaken until defibrinated. It was then poured into centrifuge tubes and centrifuged smartly for 15 mns. The serum was then drawn off, physiological salt solution added to about the height occupied by the serum and the tubes, after shaking again

centrifuged for 15 mns. The salt solution was then pipetted off and a fresh quantity added. This process was repeated three times, the salt solution being pipetted off the last time and the cells stored in the ice-box in a securely stoppered bottle. The addition of a preservative was found to be unnecessary if the cells were all to be used within 10 days. In the test, 2 c.c. of blood cells were added to 98 c.c. of physiological salt solution, thus giving a 2% dilution, which made the test somewhat more easily read than when a 1% dilution was used, and introduced no undesirable factors.

Complement. The guinea pig was used as the source of complement and yielded so uniform a product that no experiments were tried with other animals. The method of drawing blood was identical with that described for the rabbit, except that a smaller needle was used. The blood -- about 10 c.c. in quantity -- was collected in a centrifuge tube, the clot loosened with a sterile platinum needle and the tube centrifuged for about 10 mns. The serum was then pipetted off, diluted to 20% with physiological salt solution and used at once. Complement kept over night was found to have lost much of its potency.

Positive serum. The method of drawing blood and preparing serum for the complement fixation test was the same as for the agglutination test already described.

Suspect and negative serum, when used, were prepared in the same manner as positive serum.

Complement Titration.

Reagents.	Tubes.						
	1	2	3	4	5	6	7
.85% NaCl solution	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
20% Complement	.02	.04	.06	.08	1.0	0.0	1.0
1% Hemolysin	.10	.10	.10	.10	.10	.10	0.0
2% Blood cells	.50	.50	.50	.50	.50	.50	.50
Readings after 1/2 hr. in 37° water bath.	-	+	+	+	+	-	-

+ = hemolysis. - = no hemolysis.

The titre of the complement was therefore .04, and this reagent was diluted so that .1 c.c. contained 1 1/2 times the titre - i.e. 4 c.c. of physiological salt solution were added to every 6 c.c. of complement.

Hemolysin Titration.

Reagents.	Tubes.							
	1	2	3	4	5	6	7	8
.85% NaCl solution	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c 1.5
20% Complement	.10	.10	.10	.10	.10	.10	.10	0.0
1% Hemolysin	.01	.02	.04	.06	.08	1.0	0.0	1.0
2% Blood cells	.50	.50	.50	.50	.50	.50	.50	.5
Readings after 1/2 hr. in 37° water bath.	-	+	+	+	+	+	-	-

+ = hemolysis. - = no hemolysis.

The titre of the hemolysin was therefore .02, and this reagent was diluted so that .1 c.c. contained 3 times the titre. Thus, 4 c.c. of physiological salt solution were added to every 6 c.c. of hemolysin.

Antigen Titration. No. I.

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Salt solution.	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
Positive serum.	.02	.02	.02	.02	.02	.02	.02	0.0	0.0	0.0	.02
Antigen.	.02	.05	.08	.10	.15	.20	.25	.15	.20	.25	0.0
Adjusted complement.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Incubated 1 1/2 hrs. in 37°C. water bath, then Hemolytic System added --

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Adjusted hemolysin.	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10
2% Blood cells.	.50	.50	.50	.50	.50	.50	.50	.50	.50	.50	.50
Readings a after 1/2 hr. in 37° water bath.	+	+	+	+	+	-	-	-	-	-	+

+ = hemolysis. - = no hemolysis.

These results were duplicated in a titration run immediately afterwards and indicated that the antigen possessed anti-complementary properties, since control tubes 8, 9, and 10 failed to show hemolysis. It was, therefore, decided to dilute the Antigen so that the controls might show hemolysis. Accordingly, the Antigen was diluted to correspond with tube 3 of MacFarlane's nephelometer, and titrated, using the same amounts.

Antigen Titration. No. 2.

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Salt solution.	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
Positive serum.	.02	.02	.02	.02	.02	.02	.02	0.0	0.0	0.0	.02
Antigen.	.02	.05	.08	.10	.15	.20	.25	.15	.20	.25	0.0
Adjusted complement.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Incubated 1 1/2 hrs. in 37° C. water bath, then Hemolytic System added --

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Adjusted hemolysin.	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10	c.c. .10
2% Blood cells.	.50	.50	.50	.50	.50	.50	.50	.50	.50	.50	.50
Readings after 1/2 hrs.' incubation in 37°C. water bath.	+	+	+	+	+	+	-	+	-	-	+

As will be seen from the table, this diluted Antigen gave as unsatisfactory results as before. It was decided to determine the anticomplementary dose of this Antigen and not to use an amount greater than one-half of this figure in titration. Accordingly, Antigen and complement were assembled as shown in the table, incubated for the usual period and the hemolytic system added and incubated as formerly --

Antigen Titration. No. 3.

Reagents.	Tubes.											
	1	2	3	4	5	6	7	8	9	10	11	12
Salt solution.	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
Adjusted complement.	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02
Antigen.	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.20	.21

Incubated 1 1/2 hrs. in 37°C. water bath and Hemolytic System added, as in Tables 1 and 2.

	1	2	3	4	5	6	7	8	9	10	11	12
Readings after 1/2 hrs. incubation.	+	+	+	+	+	+	+	-	-	-	-	-

The anticomplementary dose was thus determined to be .16 c.c. Since one-half of this amount had previously failed to prevent hemolysis in actual titration -- see Tables 1 and 2 -- there was no object in re-titrating, since hemolysis obviously would have taken place in all tubes.

This antigen clearly was worthless for complement fixation work. It was, however, prepared from a single strain of Bact. pullorum, and the possibility of securing better results by using a polyvalent antigen was recognized. (The necessity for polyvalent antigens in dealing with typhoid fever, glanders, etc., is emphasized by Kolmer -16-). The monovalent preparation was therefore discarded and an antigen composed of five different strains of Bact. pullorum prepared. This antigen was adjusted to correspond to tube 4 in MacFarlane's nephelometer.

Antigen Titration. No. 4.

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Salt solution.	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
Positive serum.	.02	.02	.02	.02	.02	.02	.02	0.C	0.C	0.C	.02
Antigen.	.02	.05	.08	.10	.15	.20	.25	.15	.20	.25	C.0
Adjusted complement.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Hemolytic System added in usual amounts after 1 1/2 hrs. incubation at 37° C. in water bath.

Readings 1/2 hr. after addition of Hemolytic System.	+	+	+	+	+	+	+	+	+	+	+

This titration was run in duplicate.

A preliminary titration, similar to that shown in Table 3, was run with the object of determining the anticomplementary dose. This factor, however, did not exist with the amounts used, nor did it appear in the actual titration. The first batch of antigen was prepared from growths grown on agar of very poor consistency and it is possible that minute particles of the medium were present in the antigen to an extent sufficient to give this reagent anticomplementary properties (17).

Though anticomplementary reaction was thus got rid of, the antigen apparently was of no value for the complement fixation test. Having been tried with two other sera that were strongly positive by the agglutination test with no better results, it was decided to emperiment with antigens of different preparation.

Plasmolyzed antigen. A polyvalent antigen was prepared as before, except that distilled water was used instead of physiological salt solution and the suspension heated for 1 hr. at 60°C. It was then shaken by machine for an hour more, after the addition of sterile beads. This heating and shaking were repeated on three consecutive days, at the end of which time the bacterial cells, as proved by microscopical examination, were efficiently ruptured. It was hoped that the bacterial amboceptors would be anchored by some substance in the bacterial cell that had diffused into the suspending fluid, or had become available by simple lysis of the cells.

This antigen was given a thorough trial, being run in duplicate with one positive serum, once with another positive serum, and once with a negative serum. The amounts used ran from .02 c.c. to .25 c.c., as usual.

In all tubes, with all sera, hemolysis took place.

Before the antigen was discarded, enough 20% salt solution was added to give .85%. The presence of electrolytes being necessary for agglutination, it was thought that, possibly, the absence of salts in the antigen was responsible for the failure of the reagent. On titrating, however, no better results were secured than before.

The uniform failure of all antigens containing suspen-

sions of the organisms pointed to the necessity of trying other types.

Filtrate from broth culture: Five bottles, each holding approximately 50 c.c. of plain broth, were inoculated with five different strains of Bact. pullorum and incubated at 37°C. for 72 hrs. The broth was then filtered through a Berkefeld filter by suction, the clear filtrate carbolized to .5%, shaken and put on ice.

This antigen was then titrated, the usual amounts of reagents being employed. The antigen thus ranged in amount from .02 c.c. to .25 c.c. Hemolysis occurred in all tubes except that containing .25 c.c. The set was put on ice over night and the blood cells settled out, there being no indication of hemolysis.

A titration with the same serum was now run, using large amounts of the antigen, other reagents being added as usual, as shown in the table.

Antigen Titration. No. 5.

Reagents.	Tubes.										
	1	2	3	4	5	6	7	8	9	10	11
Salt solution.	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5	c.c. 1.5
Positive serum.	.02	.02	.02	.02	.02	.02	.02	.00	.00	.00	.02
Antigen.	.1	.15	.20	.25	.30	.35	.40	.30	.35	.40	.00
Adjusted complement.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Incubated in 37°C. water bath for 1 1/2 hrs. and Hemolytic System added as usual.

	1	2	3	4	5	6	7	8	9	10	11
Readings after 1/2 hrs. incubation with Hemolytic System.	+	+	+	P	-	-	-	+	+	+	+

The titration was run a second time, results being duplicated.

So far, this antigen had yielded somewhat encouraging results. It was, however, realized that the antigen would have to meet several conditions before it could be pronounced successful

1. Yield negative results in an extended series of titrations with sera from normal birds.

2. Yield positive results in a similar series with sera from infected birds.
   (Normality or infection of birds to be determined by autopsy as well as by agglutination test).

3. Be capable of concentration so that handier amounts could be used. (Not absolutely necessary).

As a preliminary step, a titration was run using plain broth in place of antigen. The amounts employed were the same as in Table 5, thus ranging from .10 c.c. to .40 c.c. Other reagents, incubation period, and incubation temperature were as usual.

Complete hemolysis occurred in all tubes in 1/2 hr., proving that the fixation of the complement in the previous tests was not due to any substance originally present in the bouillon.

At this stage, complement fixation work was interrupted for the greater part of a week. Serum was then prepared from a blood sample from a vigorous fowl which had not reacted to the

agglutination test. As a precautionary measure, the test of this serum was run with that from the fowl whose serum had reacted positively in the previous tests. The amounts of reagents used were identical with those before employed, as shown in Table 5.

Both titrations showed hemolysis in all tubes.

Hemolysis in the tubes which had received positive serum was altogether unlooked for and it was thought that some error of technique had crept into the test. There was, also, a bare possibility of the serum no longer possessing antibodies (18). The titration was, therefore, re-run and an agglutination test set up. The titration showed the same results as before and the agglutination test showed distinct agglutination in all three dilutions at the end of 3 1/2 hours. An additional titration run with a different positive serum gave negative readings in all tubes.

There seemed to be but one interpretation to put on matters -- namely, that the antigen had lost the power of fixing amboceptor and complement which it had formerly, to a rather feeble extent, possessed. Another quantity was therefore prepared in a similar manner and at once tested out.

This antigen proved to be even feebler than the first, though the reactions when large quantities were used with positive serum were specific enough. A test with two negative sera gave negative readings, but the worth of the antigen disappeared at the end of three days, despite the fact that it was kept on ice in a securely stoppered brown bottle.

Following these experiments, various batches of antigens were prepared and tried with erratic results. Some were worthless, others acted in very large quantities only, while some gave clear-cut reactions in smaller amounts. Repeated testing bore out the

fact that no antigen would keep longer than four days. Whether such an antigen would lend itself to concentration or precipitation of the active principle (19) which would keep for some length of time could not be determined.

The results of some other experiments on complement fixation work in the detection of Bact. pullorum infection in fowls came to hand after the experiments before detailed had been completed. A limited number of tests, the antigen used being, presumably, a month-old broth culture of Bact. pullorum, was conducted at the U.S. Dept. of Agriculture (20) with uncertain results. The findings of Gage (21) are no more satisfactory.

The Intradermal Test.

The possibility of developing a test similar to the tuberculin test in cattle for the detection of carriers of Bact. pullorum easily suggested itself. From time to time, about 1/4 c.c. of bouillon cultures of Bact. pullorum of 24 hours' to 1 week's incubation, killed by heating to 60°C. for 1 hour, were injected into the wattles and combs of birds positive to the agglutination test. Negative reacting birds were similarly injected as controls. No satisfactory reactions being secured from these tests, it was decided to lengthen the period of incubation to at least one month and prepare a test fluid by the same method used for the production of tuberculin.

At this time a publication dealing with the subject was received from the Bureau of Animal Industry. The authors had secured good results from a bouillon culture incubated for one month, killed by heating to 60° C., phenolyzed, and kept on ice

for six months when it was used without further preparation. Filtration or concentration, according to their findings, did not seem to enhance the value of the fluid for diagnostic purposes.

The bouillon culture being incubated by the writer was accordingly kept for one month at 37°C., killed and phenolyzed as before mentioned, and set in the ice box. After 2 weeks' storage, about 1/4 c.c. were injected into the wattles and comb of a strongly positive chicken with negative results. The injections have been repeated at intervals of about two weeks to the date of writing. The test fluid is now two months old.

Three more cultures, all polyvalent like the first, but differing slightly as to strains involved, have also been employed without success.

The negative results secured may be due to lack of "ageing" of the test fluid. Just what effect this could have is not clear. There is also the possibility of the strains employed being unsatisfactory.

The intradermal test for the detection of Bact. pullorum carriers may be regarded as a new and interesting study for the pathogenic bacteriologist. The real worth of the test can only be gauged after a considerable period of time when any difficulties incidental to the preparation of the test fluid as now made may be justly appreciated, or obviated by the adoption of other methods, and a correct estimate of the convenience and reliability of the test in field work arrived at.

The Infection of Mature Fowls.

An important consideration in the Bact. pullorum carrier problem is whether healthy, mature fowls can become infected and, if so, in what manner infection occurs. The researches of Rettger (22) indicate that infection follows prolonged contact with diseased birds, as well as contact with contaminated litter, and the eating of contaminated food. The question seemed to the writer important enough to warrant experimentation. Changes in the College flock made since testing rendered the supply of reactors very low and it was decided to postpone a "contact" experiment for the time being. Twelve fowls of mixed breeds were, however, put in a roomy pen for a feed and litter infection experiment. These were tested immediately by the agglutination test and found negative. Ten days later they were again tested, with the same result. Beginning in the middle of January 1917, about 50 c.c. of a 48 hour bouillon culture of Bact. pullorum were daily scattered over the litter and into the feeding trough and drinking water. The birds were tested weekly, the pen cleaned out whenever it became dirty enough to possibly injure the health of the birds, and the attendant exercised the precautions, such as changing boots, etc., usual in a test of this kind.

The results of the experiment are embodied in the appended table.

Final Agglutination Test on June 1, 1917.

Bird No.	Breed.	Agglutination Test.		
		1:50	1:100	1:200
D 22	Leghorn	-	-	-
D 617	Brd. Rock	-	-	-
C 835	" "	-	-	-
C 364	" "	+	P	-
D 607	" "	-	-	-
D 936	" "	-	-	-
D 641	R. I. Red	-	-	-
D 658	"	-	-	-
D 663	"	+	+	+
D 651	"	+	+	+

Of the 12 birds, one died; another was used as a control in the intradermal test.

Fowls D 663 and D 651 first gave a positive reaction in the beginning of May. C 364 reacted positively towards the end of May.

The experiment was interrupted during the month of March.

A noteworthy feature is the apparently rapid formation of antibodies after infection. All three reactors were clearly negative the week before their serum gave a positive reaction, which in the case of two then showed in all three dilutions.

On post-mortem examination, all three fowls showed typical ovarian infection with Bact. pullorum.

During the first three months of the experiment the weather was unusually cold, the temperature of the pen frequently approaching Zero Fah. While the health of the birds did not suffer, owing to the pen being well ventilated and dry, quicker results would, perhaps, have been secured had it been possible to delay the experiment until warmer weather.

The experiment corroborates the findings of Rettger (23)--namely, that grown fowls may become infected with Bact. pullorum through the eating of contaminated food. Too much care cannot, therefore, be taken to keep down the spread of bacillary white diarrhea in this way. The custom prevalent among some poultrymen of feeding eggs that have failed to hatch to their fowls is specially to be condemned. Even the small pieces of egg shells that cover the incubator trays after hatching should be disposed of in a fashion to preclude the possibility of their being eaten by fowls. With dead embryos, they should either be burnt or buried deep with quicklime. The practice of darkening the incubator as a hatch comes off, to prevent the young chicks from pecking at shell fragments, etc., is undoubtedly sound.

Pathogenicity of Bact. pullorum for Experimental Animals.

This phase of the study of Bact. pullorum, owing to rather serious losses among the laboratory animals and the inadvisability of introducing fresh stock until the trouble had been checked, did not receive the emphasis originally intended.

Four guinea pigs were fed about 30 c.c. of a 48 hour broth culture of Bact. pullorum four or five times a week. The

culture was mixed with their drinking water and food. The experiment extended without interruption from April 19 to June 6, the sera being tested from time to time for agglutinins. At no period was a positive reaction secured by the agglutination test in dilutions of 1:50, 1:100 and 1:200. On June 6, the guinea pigs were killed. All organs of all animals appeared normal on post-mortem examination.

The test was not far-reaching enough to warrant the drawing of any positive conclusions. It is realized that a larger number of animals should have been fed over a considerably longer period. More recently isolated strains than those employed might have proved more desirable.

The researches of Gage (24), who found that Bact. pullorum is toxic for guinea pigs, rabbits, and rats, are extremely interesting. This investigator raises the question as to whether eggs infected with Bact. pullorum are dangerous for human consumption.

The Resistance of Bact. pullorum in Infected Eggs to Cooking.

Fresh eggs were immersed for three minutes in 1:1000 $HgCl_2$ and 50% alcohol. The blunt ends were then flamed and each egg inoculated with about 1/10 c.c. of Bact. pullorum in physiological salt solution. Precautions were taken to keep syringe and needles sterile. The punctures were filled with collodion and the eggs incubated for periods ranging from 24 to 72 hours. They were then immersed for varying periods in boiling water at 99°C.,

being placed in a dish containing cold 50% alcohol immediately after removal. The yolk was then emptied into a Novy jar containing about 40 c.c. of sterile bouillon and incubated for 48 hours. At the end of this period agar streaks were made, the growths thereon examined microscopically, and agglutination tests run.

Contaminations were not infrequently encountered, but the results of the experiments may be briefly summarized as follows:

In no case was a culture of Bact. pullorum recovered, directly or indirectly, from any egg that had been boiled for more than 1 1/2 mns. Little difficulty was experienced in recovering the organism from eggs that had been boiled 1 minute.

The condition of the eggs after 1 1/2 mns. boiling is what would be described as "soft boiled".

Experiments with eggs differently cooked were not completed.

In view of the fact that eggs naturally infected with Bact. pullorum contain but few organisms, and since the organisms appear to be killed in the process of cooking eggs to a "soft boiled" condition, the writer is inclined to the view that, even assuming the toxicity of Bact. pullorum for man, the naturally infected eggs undoubtedly on the market do not constitute an appreciable danger to public health.

Additional Remarks.

Some interesting and important phases of the study of Bact. pullorum intended to be touched upon had, for lack of time,

to be left alone. The writer refers particularly to the role of the male bird in the spread of Bact. pullorum infection. Partial agglutinations in low dilutions have been observed from two male birds of the College flock. Whether infection becomes localized in the reproductive organs of the male bird which, in turn, can infect females is a question which, to the best of the writer's knowledge, has never been investigated and offers scope for interesting experimentation.

The resistance of Bact. pullorum in infected eggs to cooking was intended as a preliminary step in a feeding experiment involving several of the staff of this Dept.

The problem of preparing an antigen suitable for use in the Complement Fixation Test could perhaps be solved by making an extract of infected ovaries, or even of normal fowl tissue, such as the liver or heart. The failure of all antigens employed up to this time is rather striking and stimulates further research.

While infection with Bact. pullorum must impair the egg-laying qualities of a fowl, an infected bird may, to all appearances, appear perfectly healthy. The writer had two reactors under almost daily observation for one year and never could detect any signs in the behavior of the birds that would indicate infection. In the College flock, also, where sickly birds are at once segregated, 12% gave positive reactions.

The writer estimates that the total time necessary to draw a sample of blood from a fowl under ordinary conditions is under 5 mns. Thus, at least 12 birds can be bled per hour. The actual time to set up an agglutination test, everything being ready, is very small, but the preparation of glassware, serum, test-fluid,

etc., consumes time more difficult to measure. With blood samples sent into the laboratory, probably 100 samples could be run daily; if the operator had to collect his own samples, the number would, of course, be much smaller.

Summary and Conclusions.

1. Bacillary white diarrhea has obtained a foothold in many of the commercial poultry plants in at least the Southern part of Michigan. The extent to which the disease has invaded ordinary farm flocks in uncertain, but is not, on the whole, serious.

2. Bact. pullorum will probably persist for a considerable time on litter, but yields readily enough to the common disinfectants.

3. No satisfactory Complement Fixation Test has been devised to detect carriers of Bact. pullorum.

4. The Intradermal Test has not been used sufficiently for its value to be determined.

5. The Agglutination Test is undoubtedly of value in the detection of Bact. pullorum carriers and much good has been accomplished through its employment. (See Report 6, 1917, of Storrs Exp. Stn., to hand at this writing.)

6. Mature fowls may become dangerous carriers of Bact. pullorum from eating food contaminated with this organism.

7. There is no evidence to show that a carrier ceases to become such except, possibly, in extremely rare cases. Holding over infected birds in the hope that they may free themselves from infection is, therefore, futile.

8. That any breed of fowl is more susceptible than another to bacillary white diarrhea has never been proved.

9. There is no preparation that will safeguard young chicks from white diarrhea if they are exposed to infection. If a chick has Bact. pullorum in the unabsorbed yolk, nothing it may be given can possibly reach the organisms; if the chick has eaten food contaminated with Bact. pullorum, nothing that could kill the organisms in the alimentary canal will leave the chick alive. The weeding out of all mature carriers, the proper disposal of dead embryos, and thorough disinfection of coops and incubators is the only way to combat the disease.

10. There is evidence that Bact. pullorum, fed in liberal quantities over a considerable period of time, is actually toxic for laboratory animals.

11. The toxicity of Bact. pullorum for man is uncertain. In any case, the thorough cooking of eggs probably would prove an efficient safeguard.

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Tube 1 = 99 parts 1% H_2SO_4 to 1 part 1% $BaCl_2$
" 2 = 98 " " " " 2 parts " "
" 3 = 97 " " " " 3 " " "
Etc.

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PLATES SHOWING TYPICAL MISSHAPEN EGGS

IN CHICKEN OVARIES INFECTED

WITH BACT.PULLORUM.

Infected eggs are usually greenish in color, are relatively hard, and lack the elasticity of normal deutoplasm.