# THE VALUE OF THE AGGLUTINATION TEST IN THE CONTROL OF BACILLARY WHITE DIARRHEA OF THE DOMESTIC FOWL

by

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# I - INTRODUCTION

Bacillary white diarrhea is an acute infectious disease of young chicks associated with high mortality and, as a rule, affecting the chicks a few days after hatching. In adult birds the disease is usually chronic but occasionally it may be acute. "While greater attention has been drawn to the disease because of losses to chicks, there are probably also heavy losses of adults and losses due to low productivity and low fertility and hatchability of infected eggs" (Bushnell et. al.).

The causal organism of bacillary white diarrhea infection is Salmonella pullorum (Bergey's classification), a member of the coli-typhoid group. It was first isolated by Rettger (1899) from the ovaries of infected fowls, dead embryos, chicks, etc. He and his associates worked out the life history of the parasite, and showed its localization in the ovary of the "carrier" hen and its transmission through the egg.

The rapid spread of the disease during recent years has created a serious problem for the poultry industry. It is apparently present in all parts of the world, and is said to be responsible for more losses in young chicks than any other one poultry disease.

In 1913 Jones demonstrated that infected fowls that

harbor the disease can be detected by means of a biologic test known as "The Macroscopic Agglutination Test for Bacillary White Diarrhea". This test which is based on the same principle as that employed in the diagnosis of contagious abortion in cattle and typhoid and paratyphoid in man, is considered highly efficient by many investigators and the only practicable method now known for controlling the disease. In the opinion of Rettger, "the value of the macroscopic agglutination test can no longer be doubted. It furnishes us with a reliable, inexpensive, and practical method of diagnosing ovarian infection in breeding stock, and no efforts should be spared in applying it and ridding breeding flocks of permanent carriers of Bacterium pullorum."

During the past few years the value of the agglutination test as a means of detecting and controlling bacillary white diarrhea infection in adult birds and young chicks has been questioned. Dissatisfaction has been expressed regarding the different methods and technique employed by various investigators and the different results obtained from testing the same blood sera in different laboratories. The need for "considerably more intensive research work in order to standardize the agglutination test", and of further "critical investigation work bearing on the efficiency of the test" has been emphasized. Not only has the accuracy of the agglutination test been doubted, but there is also doubt whether there was much difference among "reacting and

non-reacting hens as regards the hatchability of the eggs and livability of the chicks". Also great variation in the reactions obtained from birds tested at different times have been reported by several investigators. This factor more than anything else, has made poultrymen doubt the value and efficiency of the agglutination test for bacillary white diarrhea in the domestic fowl.

The present investigation was therefore undertaken to make a restudy of the whole problem of bacillary white diarrhea infection in young chicks and adult birds, and in particular to determine the value of the agglutination test as a means of diagnosing and controlling bacillary white diarrhea infection in the domestic fowl.

Except in the review of literature, Bergey's 1923 (57) nomenclature of bacteria will be used throughout this paper.

## II- REVIEW OF LITERATURE

In 1899 Rettger (1) observed that in a flock of 17 chicks 82 per cent succumbed of a disease, named by him "white diarrhea". Of the chicks in a flock studied in 1900 (2), 87.5 per cent died, while the disease killed about 80 per cent on two adjoining farms.

Rettger and Harvey (3) and Rettger(4) reported work with other epizootics of the disease and the recovery of a specific organism, which Rettger named Bacterium pullorum.

Rettger and his associates (5) worked out the life history of the parasite, and showed its localization in the ovary of the "carrier" hen and its transmission through the egg.

Gage (6) and Jones (7, 8) published results confirming the work reported by Rettger. Since that date numerous investigations have been published from all parts of this country and in a few cases in Europe reporting heavy losses due to this disease.

Jones (9) demonstrated that the agglutination test was of great value in detecting fowl harboring Bacterium pullorum. He recommended dilutions of the serum of 1:50, 1:100, and 1:200 for this purpose. He also correlated the agglutination titre with per cent of actual infection. "The blood serum of all infected fowls agglutinated at a dilution of 1:100, and 82.3 per cent agglutinated at a dilution of 1:500. Certain individuals gave a positive reaction with serum dilutions of 1:800, 1:1000 and 1:2000."

Gage, Paige, and Hylan (10) concluded that the examination of eggs for the presence of Bacterium pullorum gave results that were too irregular to be of practical value in the diagnosis of this disease.

Rettger, Kirkpatrick and Jones (11) issued a report summarizing their work on the use of the agglutination test.

Rettger and his associates (12) issued a sixth report in which they state that a single agglutination test and the elimination of the reactors is not an absolute guaranty that the flock has been entirely rid of ovarian infection.

Rettger, Kirkpatrick and Card (13) reported on an examination of over 21,000 hens by means of the agglutination test. They state that one of the greatest obstacles to permanent removal of all sources of infection from a flock by a single agglutination test is the condition of progressive infection from bird to bird. Maturing and adult hens are susceptible to infection from without. The spread in an adult flock may be very slow or stationary or may spread to involve 20 to 25 per cent of the entire flock within 12 to 15 months.

Gage and Flint (14) of Massachusetts, have issued several reports on the testing work of the Massachusetts Agricultural Experiment Station. They have devised three testing plans for the control of diseased flocks in that state.

Beaudette, Bushnell and Payne (15) reported on the relation of Bacterium pullorum infection to the hatchability of eggs. The losses in fertility and hatchability for 1922 were 19.05 per cent, and for 1923, 31.6 per cent.

Beaudette (16) published a review of the literature

concerning bacillary white diarrhea of fowls. He states that a positive agglutination test does not indicate infected ovaries in all cases, as the infection may be localized elsewhere in the body. Furthermore, young fowls may retain the agglutinins, but not the infection,from having had the disease as chicks.

Bushnell, Hinshaw and Payne (17) issued a technical bulletin on the subject of "Bacillary White Diarrhea in Fowls". They concluded that the agglutination test is most reliable for detecting carriers of the disease and recommend a two tube test of a low and high dilution.

Hinshaw, Upp and Moore (18) have made an important observation on the transmission of bacillary white diarrhea in incubators. They showed (1) "That bacillary white diarrhea was disseminated by artificially infected chick-down, placed in a forced air-draught type incubator, (2) That chicks hatching in the compartment opposite to the one in which infected down was spread did not suffer as high a per cent mortality as chicks hatching in the infected compartment, (3) That similar results were obtained by infecting the down of hatching chicks and exposing healthy chicks to the down which might be carried from the infected chicks to the non-infected.

Ward and Gallager (19) reported on the value of the

intradermal test for detection of carriers of Bacterium pullorum.

Sherago and Benson (20) concluded that the intradermal test gives results so inconsistent that it is practically worthless as a diagnostic agent for Bacterium pullorum infection in adult fowls.

Graham and Tunncliff (21) reported on the comparison of the agglutination test and pullorin test. They state that the variation in the results of the two tests represents a serious discrepancy which may be accounted for in part through one of several uncontrollable factors of error entering into the comparisons of this character. However, their results suggest the possible value of pullorin in the diagnosis of this disease.

Bushnell (22) in a comparative study of serologic and pullorin tests for bacillary white diarrhea concluded that "the pullorin test, in its present stage of development, can not be recommended to replace the present agglutination methods for the diagnosis of bacillary white diarrhea." However, it is a promising field for further research in an attempt to cheapen the method.

Bushnell and Brandly (23) made a study of several types of pullorin which they described as follows: the term "Ppt" refers to a dried precipitated pullorin which was suspended in a sterile salt solution immediately before use. "Cell"

refers to a cellular suspension in sterile salt solution (the organisms were killed either by heat or chemicals). "Ecto" refers to a dissociation product which is obtained in the washings from the cell. "Digest" is a pullorin obtained by breaking a heavy washed-cell sediment with N/10 NaOH for a few minutes and diluting ten times with sterile salt solution and sterilized by heat or chemicals and used fresh. (It must be used the same day it is prepared to obtain good results). The average association coefficient (determined according to Yule's method) for the four pullorins was 0.49, 0.67, 0.76, and 0.75 respectively; this is not a close enough correlation. The pullorin test therefore can not be recommended as a substitute for either the rapid or tube agglutination test. In samples tested by the rapid and tube agglutination test, the authors reported complete agreement.

Huddleson and Carlson (24) have modified the agglutinations technique for infectious abortion in cattle by introducing a rapid slide method.

Runnels, Coon, Farley and Thorp (25) developed a rapid method of testing the blood serum of fowls, based on the technique of Huddleson and Carlson. On 5000 blood samples tested by the rapid and tube agglutination test, the authors reported complete agreement.

Bushnell and Brandley (26) after several months of rather intensive study of the tube and slide agglutination

test reported that in only 3.6 per cent of the tests (5029 tests, of which 978 or 19.2% were positive) was there a disagreement. "This disagreement was due to the fact that 87 of the tests which were positive to the tube method were negative to the slide method. There were also 95 tests positive to the slide method which were negative to the tube method. In many cases these tests were repeated the next day with similar results. This leads to the belief that these differences are not due to avoidable technical difficulties, but to differences in actual reaction." These authors recommend a dilution of 1:25 for both the rapid and test tube methods.

Hitchner (27) reported on the presence of a fatty substance in the blood sera of fowl which affected the agglutination test. He found that fasting the birds before bleeding for the test reduced the amount of this substance in the serum and made the test more reliable. However, starving the birds from 36 to 48 hours caused the birds to fall off in production.

Mathews (28) found that the addition of two cc. of a two per cent sodium hydroxide solution to 100 cc. of Eberthella sanguinarium antigen did not influence its agglutinability and eliminated 95 per cent of the "cloudy tests" when added just before setting up the tests.

Mallman (29) repeated the work of Mathews and agreed

with him that when Bacterium sanguinarium is used for antigen, the alkali should be added only to the antigen used for testing cloudy samples, due to the lowered sensitivity of the antigen. On the other hand, the addition of one cc. N/1 NaOH to 100 cc. of Bacterium pullorum antigen tends to increase the sensitivity of the antigen. Mallman, therefore, recommends for routine agglutination tests Bacterium pullorum antigen containing 1 cc. of N/1 NaOH in 100 cc. of antigen, since it is slightly more sensitive than the usual antigen and has the added advantage of entirely eliminating cloudy reactions.

Hinshaw and Dunlap (30) isolated from atypical agglutination tests (in low dilutions) a gram positive coccus. "Smears made from the agglutination tubes showing such reactions consistently showed large numbers of a gram positive coccus. The control tube containing only antigen was never found to be contaminated with the organism, which was apparently growing in the tubes containing serum and antigen, even though the antigen was preserved with 0.3 to 0.5 per cent phenol. The contaminating organism was never found in tubes containing above 1-80 dilutions of blood serum." The authors showed that the normal habitat of this organism was the skin of birds, and that it could be isolated in pure culture from samples of chick blood serum that have been bled from the wing. To overcome the

difficulty they suggest that Martin's method of withdrawing blood samples, or disinfecting the skin with lysol or incubating the serum antigen mixtures at temperature above 45°C may be of value.

Erickson (31) tested 15 hens showing positive reactions for a period of 12 months, his results showing a fluctuation, varying from positive to negative during this period.

Gwatkin (32) made observations on the consistency of the reaction, testing the blood of 11 reactors from April to October, and his results showed that, with one exception, the results were constant.

Doyle (33) tested 14 carriers monthly for 11 months, the results indicating a fluctuation of the test, 21 per cent ceasing to react during the period, 35 per cent showed increasing reaction, 35 per cent decreased, and there was no change noted in 1 per cent.

Beach, Halpin and Lampman (34) found that there was great variation in the reactions obtained from birds tested at different times. They presented a table showing the reactions obtained from testing 63 hens from one to ten times in thirteen months. Eliminating four that were tested only once, the results were consistent in 28 of the birds and contradictory in 31.

J. R. Beach (35) presented the results of the first twelve of a series of at least twenty four monthly

agglutination tests of the same fowls for the detection of Bacterium pullorum infections. He showed that "adult fowls with well established ovarian infection with Bacterium pullorum may not always react to the agglutination test. A fowl that has reacted to an agglutination test may not react to subsequent tests even though it is still infected with Bacterium pullorum. A positive reaction to the agglutination test may be considered as a highly accurate indication of Bacterium pullorum infection. A negative reaction to a test, however, appears to less accurately indicate freedom from Bacterium pullorum infection, either recently acquired or of long standing."

Doyle (36) stated that "fluctuations in titre are of great importance in the case of infected cocks." In the routine testing of cocks instead of the usual 1:25 dilution he recommends the use of 1:15 dilution as indicative of positive infection.

Kaupp and Dearstyne (37) in a Technical Bulletin on bacillary white diarrhea state in the summary that "in flocks of reactors studied, the agglutination test would remove 90 per cent of the carriers if applied at any time in one flock of 29 reacting birds held 14 months; it would have removed 98 per cent of the carriers in an infected flock of 21 birds studied for six months, if applied at any time, and would have removed 98 per cent of the carriers of avian typhoid in a flock of 50 birds studied six months,

if applied at any time during that period."

Dalling, Mason and Gordon (38) reported that " a considerable variation in the agglutination titre of fowls' serum may occur from time to time, and there is evidence that a strongly positive hen whose ovary contains Bacterium pullorum may at times show a negative agglutination reaction".

Gwatkin (32) reported that out of one hundred and two ovaries examined by bacteriological methods, 94 or 92.2 per cent were positive and five negative for Salmonella pullora while the presence or absence of the organism could not be determined in three samples on account of the plates being overgraven with spore forming bacteria.

Doyle (36) made a bacteriological examination of 42 reacting hens, and from 37 Bacterium pullorum was recovered, giving a percentage of 88. The five negative cases occurred at the beginning of the work, and the failures were probably due to faulty technique; a slight modification was made and since then the organism has been recovered from every case. These figures prove conclusively the great value of the agglutination test in the detection of carrier birds.

Kaupp and Dearstyne (37) reported that they isolated Bacterium pullorum from 70.3 per cent of the hens and 57.4 per cent of the pullets reacting to the agglutination test. The heart structure of two of five males examined showed the presence of the organism.

Doyle reports (36) that out of thirteen naturally infected cock birds, the Bacterium pullorum has been isolated from the testes, spleen, gall bladder wall and the heart muscle.

Olney and Bederke (39) tested two flocks, numbering in all about 3500 birds. Of the 3500 fowls tested, 450 or 12.8 per cent were found to react to the agglutination test. One hundred and twelve birds were tested a second time, with the result that 89 reacted and 23 were negative. Two hundred and nine birds that reacted to the agglutination test were bacteriologically examined. Of these 121 were found to be positive infection carriers, Salmonella pullorum having been isolated from the ovaries. Of the 88 birds remaining no cultures were obtained. Seventy-five birds showing a negative reaction to the first agglutination test were used as controls. On the second test one was found to be a positive reactor, while Salmonella pullorum was isolated from three of the seventy five. Post-mortem examinations revealed that five birds had gross lesion of the ovaries.

In the above experiments a serum dilution of 1-50 was used. All reactions complete and incomplete were reported as positive on the ground that the flock is of far greater importance than a few individual birds. In each case the flock was given the benefit of any questionable reactions. All reacting fowls were immediately removed from the flock.

Bushnell and Brandly (23) reported on the variation in the testing of 59 birds of various ages which were kept over a period of eight months and tested five different times. The results indicate that there was a tendency for the titre to decrease rather than to increase. Four birds have developed a completely negative reaction.

Of the 59 birds autopsied 40 or 68.1 per cent showed visible lesions of bacillary white diarrhea. From 25 of these Salmonella pullorum was isolated in pure culture, from five Salmonella gallinarum was isolated, from two a staphylococcus, from one a culture Pasteurella avicida, from one a colon type, and from six no culture was obtained. Four birds which showed necrotic areas in the liver and normal ova gave cultures of colon organisms. In the remaining twelve birds the authors were unable to find lesions which would indicate the presence of bacillary white diarrhea,nor could they isolate Salmonella pullorum from the liver or ovaries. It is impossible to state whether the birds were immune,or carriers of the organism, in some part of the body.

From the work previously published the authors have shown "that the dilution of 1:20 will detect more reactors than higher dilutions. They believe that there is no typical agglutination in this dilution with serum of normal birds. Therefore, in the 1:20 dilution it signifies either part or present infection and the bird should be removed

from the flock. On the basis of this dilution the reactions in the flock previously referred to remained positive in 93.2 per cent. With most series of tests it should be possible to obtain results which approximate this figure."

Rettger (40) expressed the opinion that "our greatest need to-day in devising ways and means for obtaining the most out of an accepted diagnostic method is to establish a system of training and supervision of blood testing personnel whereby the persons who qualify for the work obtain a thorough grounding in the principles governing the test and intensive drill in conducting it according to those principles."

"The agglutination test, as conducted at the Storrs Experiment Station, Connecticut, consists of a 1:50 and 1:100 serum antigen dilution for every blood sample. With complete eradication of pullorum disease in flocks as our chief, and in fact only real goal, we believe that official dilutions lower than 1:50 (certainly as low as 1:25) will lead us into difficulties in that there will be frequent non-specific reactions,and make it extremely difficult to establish and maintain negative (accredited) flocks. The use of 1:25 as an unofficial socalled finding dilution can not meet serious objection, except as an added expense."

At the annual meeting of the United States Live Stock Sanitary Association held December first, second and third, 1926, the Committee on Poultry Diseases (41) submitted

tentative recommendations for the control of bacillary white diarrhea. In brief the recommendations are as follows: "The antigen suspension should be prepared by washing a 48 hour, agar culture with a physiologic salt solution containing 0.5 per cent phenol. Before it is used for the test, it should be diluted with a similar solution of one-half strength mentioned until the suspension corresponds in density to that shown in the 0.75-1.00 tube of McFarland's nephelometer.

The use of sodium hydrate as a means to remove cloudiness from the serum is permissible provided that not more than 2 cc. of a two per cent solution is added to 100 cc. of the antigen suspension, immediately before use.

When a one-tube test is used, a serum dilution of 1-25 is deemed acceptable. The use of incubation is preferred, but there is no valid objection to keeping the tubes at room temperature, if the final reading is not made before the termination of the 24 hour period.

In conclusion, your committee recommends that the United States Live Stock Sanitary Association address itself to a representative group of workers engaged in bacillary white diarrhea investigation and to request that a limited number of flocks be tested in accordance with the standards here tentatively proposed and that the results be placed at the disposal of the organization, accompanied by

such comments as may be relevant to the subject so that a definite opinion may be formed as the practical value of the proposed testing technique."

At the annual meeting of the same organization held November 30, December first and second, the Committee on Poultry Diseases (42) reported that the Agricultural Experiment Station laboratories of Illinois, Michigan, Nebraska, Virginia, and Kansas, have "studied the causes for variations in interpretations of results by different laboratories in the testing of samples of sera from the same birds. The sole object of the work was to find methods of improving rather than to condemn, the agglutination test." However, "due to the fact that the work completed to date can be considered only preliminary, the results are withheld for further checking."

As a result of the surveys made this past year, your Committee believes that efforts should be concentrated on the improvement of test fluids, getting more accurate field results of the efficiency of the present methods of control of bacillary white diarrhea, and continued effort toward standardization of terms, regulatory measures and technique.

At the annual meeting of the same organization held December fifth, sixth and seventh, 1928, the Committee on Poultry Diseases (43) stated: (1) That a program of research, elimination of reacting breeding fowls, and the application of sanitary measures are regarded as fundamental in the

control of bacillary white diarrhea, (2) Persons conducting agglutination tests should be approved by proper state officials, (3) It is recommended that the agglutination test be considered as the official test, the rapid or slide test being permissible, and that official testing be conducted on a uniform basis. It is suggested that a dilution of 1:25 be given a trial by those using higher dilutions and that a standard antigen be made available through proper channels to safeguard the testing work. (4) The outstanding need in the control of bacillary white diarrhea is a uniform method of procedure." Under the section of "Standardization of Tests" the following statement appears: "Some laboratories regard a dilution of 1:100 as diagnostic, while others employ a dilution of 1:50, 1:40, 1:25, or 1:10. Seven states use dilutions of 1:80 to 1:100, eight states, 1:50 and nine states, 1:25 or less. The macroscopic agglutination test is employed in a majority of laboratories, while a limited number are checking the rapid or slide test as suggested by Runnells and co-workers.

Beach and Merrick (44) with a view of throwing more light on the accuracy of the test undertook the following experiment: "Thirty-eight hens,which were being used in investigational work, were bled from the wing vein. The blood serum was allowed to separate. Some of the serum from each hen was then placed in each of six small tubes,

one of which was sent to veterinary laboratories in Kansas, Virginia, Minnesota, New York and New Hampshire. The sixth sample was tested at the Wisconsin laboratory." When the reports of the six laboratories were compiled, it was surprisingly discovered that there was a wide disagreement in final results. In only five cases out of the 38 were the reports identical. Twenty-three days later, 14 birds of the same flock, with 12 other hens were bled and the serum sent to the same laboratories. The results checked in only 14 out of the 26 cases and there was disagreement in the 12 remaining tests. Fourteen of the samples were duplicates of the first group. Many of the hens which, as a result of the first test, were supposed to be healthy were, according to the later reports, infected with the disease.

Edwards and Hull (45) reported on "the accuracy of the agglutination test in the diagnosis of bacillary white diarrhea". They conducted tests in a manner similar to those of Beach and Merric. The sera of 24 birds were divided into eight equal parts. Seven portions of each serum were placed in small bottles preserved by the addition of crystals of thymol. The eighth portion was used to set up agglutination tests in the authors laboratory. Seven other laboratories participated in the test. A table is introduced showing that the results of these tests were very uniform. "If the majority report be taken as correct in

each instance the agreement of the eight laboratories for the 24 samples is 96.1 per cent". The writers conclude that these results demonstrate that the agglutination test for the detection of bacillary white diarrhea is an accurate method of diagnosis.

Hinshaw (46) reported on the results of New England Conference of Laboratory Workers in bacillary white diarrhea control held at Amherst, Massachusetts, April 24, 25 and 26, 1928. The purpose of the conference was "To obtain cooperation between the six New England states, to attempt standardization of laboratory methods and equipment and for the development of better fellowship among the laboratory workers of this section".

Outstanding among the accomplishments of the group was the decision to use the same cultures for making antigens during the 1928-29 testing season; a tentative agreement to accept 1:50 as the masimum dilution;with all typical reactions, partial or complete, to be considered positive. Lower dilutions were left optional, as was also the autopsy of doubtful reactors. It was generally agreed that no bird shall be recommended for accreditation which does not give a negative reaction in the 1:50 dilution; tests are incubated for at least 24 hours at 37° C.

A portion of the time,at this conference, was devoted to the actual laboratory work of setting up agglutination

tests with antigen brought from each laboratory, and sera furnished by the Maine and Massachusetts laboratories. Comparisons as to pH value, turbidity, etc. were made of each antigen used, and much time as was available was given to studying the minor discrepancies which occurred. It might be well to mention that all the discrepancies which occurred were due to individual differences in interpretations of results." The following problems were agreed upon for further intensive investigation: (1) Use of NaOH in antigen to prevent "cloudy" reactions, (2) Proper age at which pullets can be tested, (3) Use of the "rapid method" for salvaging hemolyzed and "cloudy" serum samples, (4) Proper dilutions of serum antigen mixtures to consider diagnostic based on results of autopsy and bacteriological examination.

## III - A CRITICAL EXAMINATION OF THE AGGLUTINATION TEST

A careful examination of present day methods of conducting agglutination tests discloses the fact that there is no general agreement as to what dilution should be accepted as indicating infection. The question "what agglutination titre constitutes a "carrier", has aroused among investigators more disagreement and discussion than any other single factor affecting the accuracy of the test.

In establishing a titre of diagnostic value due consideration must be given to the presence of normal

agglutinins in the blood of all healthy poultry. Rebraissier (47) states that "the normal agglutination titre in healthy chickens is 1:10, and in the application of the agglutination test for this infection it would not be advisable to use a titre that was not well above this range."

To determine the probable range of normal agglutinins Gwatkin (48) examined four negative flocks, comprising 214 birds. Twenty samples showed some agglutination in dilutions of 1:10 and five of these showed slight agglutination in 1:25. The remaining 194 showed no agglutination in any dilution. Birds giving the highest reactions in three of the four flocks were found free from Salmonella pullora on bacteriological examination.

Beaudette (49) considers it quite possible that the agglutination produced in low serum dilutions (1:40) is due to the persistence of agglutinins after the infection has failed to become localized, or that it may indicate the beginning of infection in the case of mature birds.

Doyle (33) does not believe that there are any normal agglutinins for the Bacterium pullorum in the serum of healthy fowls.

Besides normal agglutinins, due considerations must be given to interagglutinability between Salmonella pullora and other intestinal organism. Gage (50) and Rebrassier (47) have shown that serum from Eberthella sanguinaria has a high

agglutination titre for Salmonella pullora antigen. Since other intestinal organisms show interagglutination with Salmonella pullora, it would be advisable not to use a titre of less than 1:100, in the opinion of Rebrassier.

Another important fact affecting the degree of dilution most suitable for diagnostic purposes is the so-called pseudo-agglutination phenomenon. This constitutes the greatest difficulty in the agglutination testing of fowls. The undesirable condition is favored by improperly titrated antigen, an excess of phenol and probably by other unknown factors.

A summary of so-called diagnostic dilutions used by various prominent investigators is herewith presented:

| | | | | | |
|---|---|---|---|---|---|
| Graham of Illinois | uses a | dilution | of | 1:10 | |
| Matthews of Indiana | " | " | " | 1:10 | |
| Brummett of Cornell | " | " | " | 1:30 | |
| Newson of Colorado | " | " | " | 1:60 | |
| Beach of California | " | " | " | 1:50 | and 1:100 |
| Bushnell of Kansas | " | " | " | 1:20 | and 1:80 |
| Beaudette of N.Jersey | " | " | " | 1:80 | and 1:100 |
| Rice of Ireland | " | " | " | 1:50 | |
| Gwatkin of Ontario | " | " | " | 1:50 | |
| Bransfield of Mass. | " | " | " | 1:100 | |
| Rebrassier of Ohio | " | " | " | 1:100 | |
| de Bleak of Holland | " | " | " | 1:100 | |
| Kalkus of Washington | " | " | " | 1:100 | |

The question naturally arises, "why should such a variety of opinion exist?" The literature on the agglutination test for Salmonella pullora reveals one striking variation of technique, namely the turbidity and the "factors" associated with the antigen used. In reporting

the different dilutions employed, the writers have neglected either to record the concentration of the antigen used, or to emphasize the importance of dilution and concentration of antigen as two interdependent factors. Herein, perhaps, lies the underlying cause of apparent disagreement between different investigators. To wit, the following concentrations of antigen are reported:

| | |
|---|---|
| Rice | - 0.5 on the McFarland nephelometer |
| Matthews | - 3.0 " " " " |
| Fitch | - 1.5 " " " " |
| Bushnell | - 1.0 " " " " |
| U.S.D.of Agriculture | - 0.5 " " " " |
| Gwatkin | - 5.6 cm. on the Gates nephelometer |

Beaudette (49) uses a very diluted antigen, believing that there is less opportunity for false reaction to take place when a test fluid of only slight turbidity is used. Those who are familiar with the McFarland nephelometer will readily realize the range of concentration between turbidity number three and number one and a very dilute antigen. Beaudette remarks that the question of a proper dilution depends a great deal upon the turbidity of the test fluid. If the test fluid is sufficiently turbid it might be possible to use 1:10 dilution of serum. On the other hand, if the test fluid is only of slight turbidity, a correspondingly high dilution of serum must be used. A similar opinion is held by Graham and Tunnicliff (21), "in view of the fact that the diagnostic dilutions in the agglutination test may be largely dependent on the sensitivity of the antigen

it is recognized that variations in antigen might easily influence the dilution to be employed in different laboratories."

The writer has failed to find any substantial literature dealing with the subject of the ratio that exists between the serum and concentration of Salmonella pullorum antigen, when a titre of a serum is reached. However, very specific information on this important subject is furnished by Mathews (51) who bases his data on Bacillus abortus (alcaligenes) antigen, standardized to correspond with three definite turbidities. Antigen 1 was diluted to correspond to a turbidity of 3 with McFarland's nephelometer. A definite amount of this antigen was diluted with an equal amount of phenolized salt solution and designated antigen 2. A definite amount of antigen 2 was diluted with an equal amount of phenolized solution and designated as antigen 3. He records the fact that there exists a definite ratio between the amount of serum and the number of organisms in the antigen when the titre of a serum is reached. The titre of a serum, on the border line between specific agglutination and normal agglutinating limits, with antigen turbidity 1 was 1:25. The titre of the same serum against antigen 2 was 1:50; and against antigen 3 was 1:100, this giving 3 titers for the same serum, but the ratio of serum to organisms in all 3 cases was the same since antigen 2

contained only one-half as many organisms as antigen 1, and antigen 3, one-half as many organisms as antigen 2. Mathews concludes that as the turbidity of an antigen decreases, its agglutinability increases.

Matthews also reported that when the turbidity of an antigen was decreased one-half, its agglutinability was doubled in 65% of all positive reactions. However, in 30% of the positive reactions, the agglutinability was more than doubled; i.e. the same serum that had a titre of 1:500 diluted with antigen 3 had a titre of 1:2500 with antigen 2; and one of 1:7500 with antigen 1. Another serum had a titre of 1:50 dilution with antigen 3, 1:200 with antigen 2, and 1:1000 with antigen 1. From these two instances it will be readily seen that an agglutination reaction is not only dependent on the number of organisms present in the suspension (turbidity or concentration of antigen) but on certain "factors" associated with the organisms. It is these "factors", when everything else is taken into consideration that determine what Weaver (52) calls the "antigenic value" of an antigen. We need not concern ourselves at the present time with what these factors are, but just to record the fact that they need to be considered when the agglutinating properties of an antigen are determined in the laboratory before being released for routine testing.

It is a well known fact that the agglutinating properties of Salmonella pullora vary considerably, not only between strains, but within individual strains. The number of strains used by various workers is presented below:

| | | | |
|---|---|---|---|
| May | uses | a | single strain |
| Mathews | " | " | " |
| Mallman | " | " | " |
| Beaudette | " | " | eight strains |
| Bransfield | " | " | four " |
| Bushnell | " | " | six to ten strains |
| Gwatkin | " | " | three strains |

The degree with which 46 strains of Salmonella pullora antigen agglutinate identical sera has been shown by Mallman (53) to vary from titres of 1:5120 to 1:40 with the best and poorest antigens respectively. The intervening antigens showed agglutination titres falling within the range of these two extremes. Appraising the fact that numerous strains of Salmonella pullora are used by various experiment stations,which must needs vary in agglutinating properties, it would be well to recognize the fact that no true conparison can be made between a serum antigen titre employed by one laboratory as against a serum antigen titre employed by another laboratory, without first taking into consideration the antigenic value of the antigens used.

Mallman (53) has also shown that the titre of an antigen is not constant with all sera tested. He recommends that only antigens of high agglutinability and constant titres (or nearly so on all sera tested) should be selected

for agglutination purposes. To guard against variations among different lots of antigens prepared from the same strains and under similar conditions, Bushnell tests each lot of antigen with known positive serum against antigens previously proved efficient. Gwatkin (32) in setting up a series of tests, concurrently sets up positive and negative controls. "To the positive control tube 0.02 cc. of known positive serum is added. Antigen alone constitutes the negative control tube". In interpreting the test, the negative and positive control are read along with the rest of the tests, and always show zero and complete agglutination respectively." If anyv variations from the normal (zero or complete agglutination) should occur, they ought to be taken into account when interpreting the rest of the tests. The United States Department of Agriculture recommends that controls should be set up in every agglutination trial. First a tube containing antigen only; second, antigen plus non-positive fowl serum in dilutions of 1:100 and 1:50. Measures like these help to keep a continuous check of the antigens used and maintain a uniform serum antigen titre.

Having found under experimental conditions the diagnostic dilution that gives the best results, the practical value of such a finding would need to be carefully checked with carefully controlled field trials and post mortem

examination of positive and negative birds. Only when the two show close agreement would it be proper to conclude that the dilution and the conditions surrounding the dilution are the ones to be adopted as a standard in routine testing.

To recapitulate, the question of what agglutination titre constitutes a positive reaction is of prime consideration, and is dependent upon several factors; (1) The proportion of serum to antigen, (2) The turbidity of the antigen, (3) The agglutinating properties of the strains used, (4) The "antigenic value" of each batch of antigen, (5) The correlation between laboratory and field findings.

## IV - TECHNIQUE OF MAKING THE AGGLUTINATION TEST

Preliminary experiments to determine the most suitable methods for conducting routine agglutination tests were begun by the writer at the Western Washington Experiment Station, Puyallup, Washington, and were continued at the University of British Columbia, from January 1, 1927 to August 1927. The following problems were under intensive investigation:

(1) Preservatives for antigens:
  (a) Antigen without a preservative
  (b) Antigen without a preservative but heated for one hour at 55° C
  (c) Antigen with the addition of .1% formalin
  (d) Antigen with the addition of .5% and .1% cresol

(e) Antigen with the addition of .1% tri-cresol
(2) The effect of NaOH on pseudo-agglutination
(3) The pH value of antigens
(4) Strains of Salmonella pullorum suitable for agglutination work
(5) One strain of Salmonella pullorum, vs several strains
(6) Freshly isolated cultures compared with old cultures
(7) Saline emulsions of antigen compared with broth cultures
(8) Comparison of different lots of antigens
(9) A comparison of 1:25, 1:50 and 1:100 serum antigen dilutions

As a result of this work and further investigations the following technique was adopted in the fall of 1927 for the routine testing of commercial breeding flocks:

(1) Preparation of antigen -

(a) Media: 1.5% plain nutrient agar. pH 7.0 - 7.2

(b) Cultures: 4 strains of Salmonella pullorum originally obtained from the Massachusetts Agricultural Experimental Station and used in agglutination work for the past 4-5 years

(c) Incubation: cultures incubated for 48 hours at 37° C

(d) Physiological salt solution: distilled water plus .85% gm. of C.P. NaCl

(e) Phenol: 5 cc. to 1000 cc. of physiological salt solution

(f) Antigen: the growth is washed off with phenolized physiological salt solution and is known as stock antigen

The stock antigen is diluted 1 part of antigen to about 15 parts of physiological salt solution (no phenol) and is known as standardized

antigen. The turbidity of this standardized antigen is adjusted to 0.5 turbidity on the McFarland nephelometer. Each lot of antigen is tested against known positive and negative serum.

Previous to making the dilutions with the serum, 1 cc. of a 1% NaOH solution is added to each 100 cc. of the standardized antigen.

(2) Serum -

Only clear sera are used. Contaminated blood samples or hemolyzed blood samples are discarded and a retest called for.

(3) Dilutions -

To make a 1:50 and 1:100 dilution to 0.04 cc. and 0.02 cc. of serum, 2 cc. of the standardized antigen are added respectively.

(4) Incubation -

Tests are incubated for 24 hours at 37° C and held for 24 hours at room temperature.

(5) Recording of reactions - symbols

+++, ++, +, -.

+++, ++ are considered as positive re-

+, - are considered as negative.

(6) Diagnosis -

A reactor is a bird whose serum agglutinates Salmonella pullora antigen in a dilution of 1:50 showing either a +++ or ++ reaction.

## V - BACILLARY WHITE DIARRHEA INFECTION IN YOUNG CHICKS

From the first of March 1927 to the first of September, 1927, 41* flock owners had submitted specimens for post-mortem examination, and 110 chicks were subjected to bacteriological, microscopical or such examinations as were required. In Table 1 a summary is given of findings of these examinations. It will be seen from this table that bacillary white diarrhea infection was more prevalent than any other chick disease in the flocks included in this investigation.

From the fifteen flocks showing indications of bacillary white diarrhea infection 43 chicks, from the ages of one week to six weeks, were bacteriologically examined. In the older chicks distinct lesions were present in the lungs and heart and in several chicks the pericardial cavity was filled with a semi-liquid exudate. In the case of two flocks in which the symptoms and history of the outbreak appeared to suggest bacillary white diarrhea infection, the organism was not isolated but, in these particular cases, only 4 chicks were submitted for examination. From

*The flocks from which chicks were examined were in part identical with those from which blood samples were taken.

Table 1. Distribution of chick diseases by flocks (Post-mortem examination)

Condition or disease.

| | B.W.D. | Chilling | Weaklings | Worms | Coccidiosis | Faulty Feeding | No Diagnosis | Putrid | Total |
|---|---|---|---|---|---|---|---|---|---|
| Number of flocks | 15* | 1 | 3 | 3 | 8 | 7 | 2 | 2 | 41 |
| Percentage | 36.58 | 2.43 | 7.31 | 7.31 | 19.51 | 17.07 | 4.87 | 4.87 | 100 |

* Two of these flocks showed indications of bacillary white diarrhea infection, but the organism of Salmonella pullorum was not isolated from the chicks submitted for examination.

the remaining 13 flocks Salmonella pullorum was isolated in pure culture from 21 out of 43 chicks examined. The mortality for the 15 flocks in which bacillary white diarrhea was suspected ranged in most cases around 50 per cent, but in one case the entire flock of 50 chicks died. Another case was particularly interesting in that of 300 chicks bought from one breeder, 35 per cent died, while of 200 chicks purchased from another breeder only 3 per cent died, conditions of rearing being identical. In another case, one breeder reported in three successive hatches, 57.5 per cent, 77.4 per cent and 71.2 per cent mortality, up to three weeks of age.

## VI - BACILLARY WHITE DIARRHEA INFECTION IN ADULT FOWLS

From the first of March, 1927, to the first of March, 1928, 41 flocks, involving 15,327 birds, were subjected to the agglutination test. Of this number of birds 1,745 or 11.3 per cent reacted positive to the test, (Table 2.) Of the 13,817 females, 1,703 or 12.4 per cent, and of the 1,510 males, 42 or 2.7 per cent, reacted positive. These figures are in close agreement with those reported by Rettger, Kirkpatrick and Jones (11) and Gwatkin (32) on approximately the same number of birds. Of the 41 flocks tested, 16 flocks, or 39 per cent, did not show any indications of bacillary white diarrhea infection. The total

Table 2. Distribution of Bacillary White Diarrhea infection by Breeds

| Breed | Flocks | NUMBER OF BIRDS Males | Females | Total | Negative | Doubtful | Positive Males | Positive Females | Per cent Positive | Not Tested |
|---|---|---|---|---|---|---|---|---|---|---|
| S.C.W.Leghorns | 19* | 566 | 7220 | 7786 | 6566 | 39 | 17 | 1111 | 14.4 | 53 |
| | 6" | 407 | 1637 | 2044 | 2028 | 3 | 0 | 0 | 0 | 13 |
| | 25 | 973 | 8857 | 9830 | 8594 | 42 | 17 | 1111 | 11.4 | 66 |
| W.Wyandottes | 4* | 8 | 731 | 739 | 585 | 3 | 0 | 147 | 18.6 | 4 |
| | 3" | 18 | 253 | 271 | 252 | 0 | 0 | 0 | 0 | 19 |
| | 7 | 26 | 984 | 1010 | 837 | 3 | 0 | 147 | 13.2 | 23 |
| R. I. Reds | 3* | 26 | 512 | 538 | 339 | 0 | 4 | 144 | 26.7 | 1 |
| | 1" | 4 | 24 | 28 | 28 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 30 | 536 | 566 | 417 | 0 | 4 | 144 | 26.1 | 1 |
| B. P. Rocks | 2* | 15 | 399 | 414 | 305 | 0 | 2 | 107 | 26.3 | 0 |
| | 1" | 8 | 70 | 78 | 70 | 0 | 0 | 0 | 0 | 8 |
| | 3 | 23 | 469 | 492 | 375 | 0 | 2 | 107 | 22.1 | 8 |
| Miscellaneous | 4* | 380 | 1728 | 2108 | 1887 | 7 | 19 | 194 | 10.1 | 1 |
| | 5" | 78 | 1243 | 1321 | 1303 | 0 | 0 | 0 | 0 | 18 |
| | 9 | 458 | 2971 | 3429 | 3190 | 7 | 19 | 194 | 6.2 | 19 |
| Total All Breeds Positive | 25* | 905 | 10590 | 11585 | 9732 | 49 | 42 | 1703 | 14.7 | 59 |
| Total All Breeds Negative | 16" | 515 | 3227 | 3742 | 3681 | 3 | 0 | 0 | 0 | 58 |
| Grand Total All Breeds | 41 | 1510 | 13817 | 15327 | 13413 | 52 | 42 | 1703 | 11.3 | 117 |

*Positive flocks grouped together.
"Negative flocks grouped together.

number of birds included under the heading of negative flocks was 3,742. In the 25 positive flocks, or 61 per cent of all flocks tested, the percentage of infection varied from less than 1 per cent to 80.4 per cent. In all 11,585 birds were included in the positive flocks. In Table 2 data are presented on all the tested flocks. The birds are grouped according to breeds, sex and reaction to the agglutination test. S. C. W. Leghorns constituted the largest group, White Wyandottes ranked second, R. I. Reds third, and B. P. Rocks fourth. Under the heading of miscellaneous breeds flocks were included in which two, three or more breeds were kept, in which it was not possible to segregate the data for the different breeds, as well as a few small flocks of Black Orpingtons, Light Sussex, Anconas, Black Minorcas and Brown Leghorns.

The percentage of infection in the different breeds (Table 2) varied from 26.1 per cent to 6.2 per cent. R. I. Reds showed the largest percentage of infected birds, next came B.P. Rocks, White Wyandottes, and S. C. W. Leghorns, and the miscellaneous breeds. Since in the miscellaneous group heavy and light breeds are both grouped together and a large proportion of S. C. W. Leghorns were included, no particular significance should be attached to the low degree of infection recorded in this group. Also the various percentages of infection in each breed can

scarcely be taken as indicative of the average degree of infection in the particular breeds, since the number of birds varied from 492 B. P. Rocks to 9,830 S.C.W. Leghorns, there being only three flocks of the former and 25 flocks of the latter breed respectively. Had an equal or proportionate number of birds and flocks been tested in each breed a better idea would have been obtained of the actual percentages of infection for each breed. The data herein reported agree with those of Rettger (12), Beaudette (49), and Kaupp and Dearstyne (37) who have reported a higher percentage of infection in the heavy breeds than in the light breeds.

A detailed examination of the data summarized in Table 2 reveals the following facts:

All breeds for which data were secured in this investigation were found to be susceptible to bacillary white diarrhea infection. In the case of infected flocks where two, three or four breeds were kept on a farm and the chicks raised together, "carrier" birds were found in each breed. Special interest is attached to a flock in which eight breeds are kept, namely, S. C. W. Leghorns, B. P. Rocks, White Wyandottes, R. I. Reds, Light Sussex, Black Orpingtons, Black Minorcas and Anconas. Infected birds were found in each of these breeds. In two flocks comprising two breeds and in one flock comprising three breeds,

infected birds were found in each breed. On three farms where two breeds were kept, none of the birds in either breed reacted to the test. In spite of the fact that the heavy breeds showed a larger percentage of reactors than the light breeds, the degree of infection in different flocks was found to vary irrespective of the breed; for instance in one flock of White Wyandottes 43.6 per cent of the birds reacted to the test, while in three other flocks of the same breed 11.5 and 1.5 and 1.2 per cent respectively of the birds reacted to the test. On the other hand 3 flocks of White Wyandottes did not have a single reactor. The extreme variation in the degree of infection that may exist within a breed is indicated by the following figures which are a part of the information used in preparing Table 2.

S. C. W. Leghorns

| Flock number | 20 | 7 | 17 | 22 | 6 |
|---|---|---|---|---|---|
| Per cent of infection | 1.2 | 1.5 | 15.2 | 44.9 | 80.4 |

Proprotionately fewer males than females were found to be infected. In one flock of 220 cockerels tested 12, or 4.9 per cent reacted to the test. Out of 394 pullets in the same flock 50 pullets, or 12.6 per cent, reacted. The highest degree of infection in males reported in this investigation was 25 per cent. Several flocks that were infected did not reveal a single reacting male by the

agglutination test.

The largest flock in which infected birds were found consisted of 1,753 birds, of which 14.7 per cent were infected. The largest flock in which not a single reactor was found consisted of 1,516 birds.

It is interesting to note that pedigreed birds with high official records and birds selected as good speciments of the breed are found among the reactors. This emphasizes the fact that it is not possible to identify reactors by their appearance nor to identify hens by their record. This agrees with data discussed later which shows that while reactors lay, on the average, fewer eggs than non-reactors, some of the reactors lay very well. A further interesting point brought out by the data on which Table 2 is based is the occurrence of reactors on supposedly well managed poultry plants. This may indicate that the disease can not be eradicated by such measures as are usually implied by "good" management.

### (a) Routes of Infection

The following data, obtained in connection with bacillary white diarrhea testing, demonstrate the insidious nature of the disease, and indicate what may occur in the case of flocks where the disease is present, if adequate measures of control are not promptly adopted.

In one instance, involving 400 birds tested out of a total of 1500 birds in the flock, there was found to be 80.4 per cent of infection. Losses due to heavy mortality in adult birds, poor hatchability and especially poor livability of chicks, have forced the owner of this flock to go out of business.

In another case, an acute outbreak of the disease occurred in the spring of 1926, causing considerable loss of valuable breeding stock. Of 339 birds that survived the outbreak, 51.6 per cent reacted to the test. Of the pullets entered in Record of Performance in the year 1926-27, 27.4 per cent reacted, and of 201 pullets entered in 1927-28, 21.3 per cent reacted to the test.

In another flock (No. 22) in which reactors were found to the extent of 4 per cent last year, the percentage this year had increased to 44.9. There are good reasons for believing that such a rapid increase of infection in this flock was due to the fact that raw, infertile eggs from infected birds were fed to the adult birds.

That infection can be brought in through the purchase of day-old chicks from infected flocks is shown in the case of Flocks 16, 27 and 39. In Flock 16 three separate tests were made. One lot of 139 birds tested September 24th, 1927, showed 7.9 per cent reactors. A second lot of 139 birds tested December first showed 7.1 per cent reactors.

A third lot of 139 birds tested December 10th showed 26.8 per cent. This lot of birds, although raised and kept on the same farm as the previous two lots, was purchased as day-old chicks from Flock 39, in which heavy infection was suspected. Flock 27, consisting of 136 birds, showed 43.6 per cent reactors. It was traced through the purchase of day-old chicks, to Flock 39. In these two cases infection was traced to one and the same flock. The owner of Flock 39 disposed of the breed in question, but a test made on another breed on this farm showed 6.2 per cent infection.

The following case merits special attention. The disease was apparently introduced into this flock through the purchase of breeding stock, which was subsequently shown to have been infected with bacillary white diarrhea. Over three hundred birds, involving four breeds, were tested in the fall of 1925 and 1926. Not a single reactor was found. In the spring of 1927 losses in chicks were traced to bacillary white diarrhea. Following a retest of all the birds on the place, reactors were found in two breeds recently purchased from an outside source that had not been previously tested. Since chicks hatched from the various breeds were brooded together, infection took place among all the breeds, with the result that when the chicks matured, over 12 per cent of the pullets reacted to the test.

## VII - REPEATED AGGLUTINATION TESTS

### (a) Retests of Commercial Breeding Flocks

In Table 3 data are presented on flocks, random samples of which were tested at intervals of six to twelve months. Only a small number of birds that were tested the first time (with the exception of Flock 8) were included in the second test. The most important observation to be drawn from this table is that in cases where the first sample in the flock yielded negative results, the second sample, taken some months later, also gave negative results, except where infection was known to have been introduced from outside sources in the interval. The occurrence of a single reactor among 751 negative birds in Flock 28 suggests group agglutination rather than Salmonella pullorum infection.

Flocks in which a small percentage of infected birds were found on the first test showed approximately the same percentage of infection (on different birds) in the second test. Flock 22 mentioned above is an exception to this rule. In this case, raw infertile eggs from the incubators were fed to the pullet flock, and apparently the large increase in the percentage of reactors was due to this factor.

Table 4 shows the results of retests on the remaining birds in a flock, after the reactors were removed. The data show that the agglutination test is highly effective

Table 3. Detail of flocks tested at an interval of 6-12 months.

| Flock | FIRST TEST No.of Birds Tested | FIRST TEST No. of Reactors | FIRST TEST Per cent Reactors | SECOND TEST No.of Birds Tested | SECOND TEST No. of Reactors | SECOND TEST Per cent Reactors |
|---|---|---|---|---|---|---|
| 1 | 50 | 2 | 4.0 | 348 | 13 | 3.17 |
| 4 | 34 | 0 | 0 | 236 | 0 | 0 |
| 5 | 133 | 0 | 0 | 467 | 0 | 0 |
| 7 | 110 | 3 | 2.7 | 407 | 5 | 1.2 |
| 8 | 114 | 33 | 29.2 | 463 | 110 | 23.5 |
| 20 | 196 | 1 | .5 | 715 | 9 | 1.2 |
| 22 | 50 | 2 | 4.0 | 278 | 125 | 44.9 |
| 24 | 73 | 1 | 1.3 | 318 | 13 | 4.08 |
| 28 | 34 | 0 | 0 | 752 | 1 | .13 |
| 31 | 110 | 5 | 4.5 | 758 | 51 | 6.7 |
| 33 | 105 | 0 | 0 | 78 | 1 | 1.2 |
| 34 | 114 | 0 | 0 | 218 | 0 | 0 |
| Total | 1123 | 47 | 4.1 | 5038 | 328 | 6.5 |

Table 4. Retest on the same individuals in flocks after reactors had been removed.

| Flock | FIRST TEST No.of Birds Tested | No.of Reactors | Per cent Reactors | No.of days between first and second Test | SECOND TEST No. of Birds Tested | No. of Reactors | Per cent Reactors |
|---|---|---|---|---|---|---|---|
| 8 | 463 | 110 | 23.5 | 273 | 305 | 8 | 2.6 |
| 9 | 835 | 299 | 35.8 | 204 | 561 | 15 | 2.6 |
| 15 | 446 | 22 | 4.9 | 60 | 376 | 2 | .54 |
| 16 | 278 | 48 | 17.2 | 231 | 122 | 2 | 1.6 |
| 18 | 510 | 41 | 8.03 | 243 | 427 | 4 | .93 |
| Total | 2532 | 520 | 20.5 | | 1791 | 31 | 1.7 |

in detecting infected birds, since on the retest the reactors found on the first test having been removed, the remaining birds, negative on the first test, showed a very small percentage of infection. These tests were conducted at intervals of two to ten months, on the same individuals, with the exception of Flock 9. While the application of a single agglutination test has been reported to be 100 per cent effective with some flocks, a single test does not, as a rule, eliminate all the reactors from an infected flock (Table 4). It would appear, therefore, that when the agglutination test is being applied to an infected flock with the view of eradicating the disease, rather than controlling it, the agglutination test should be applied at least a second time to the non-reactors of an infected flock, after the reactors have been removed on the first test, before they are bred. If only a single test is applied yearly the eradication of bacillary white diarrhea infection will of necessity, because of the nature of the disease, involve an indefinite period of years. A second test usually detects additional reactors in a flock in which infected birds were found, thus indicating that progressive infection may have taken place prior to or after the first test was made (13). In Flocks 15, 16 and 18, the reactors were removed immediately following the test. In Flock 8, through a mistake on the part of an attendant,

about 25 reactors were retained in the flock, thus exposing a number of birds to further infection after the first test was made. In Flock 9 the owner did not remove all the reactors at once, and furthermore kept some of the reactors for further retests, with the result that the percentage of infected birds was not as effectively reduced as in the case of Flocks 15, 16 and 18. Also, a number of birds not tested previously were introduced on the second test. Table 4 also shows that in five flocks the total average infection was reduced from 20.5 to 1.7 per cent by removal of reactors after the first test. Had all the reactors been promptly removed in each flock, the percentage of infection would, undoubtedly, have been still less. The high value and efficiency of the test, as a means of eradicating the disease, are thus clearly demonstrated.

(b) Retests of Pullets

There is very little information regarding the proper age at which pullets can be tested. Several experiment stations recommend that pullets should not be tested before the flock has reached a production of 20 to 50 per cent or over, but no experimental data have been collected to support this theory.

To throw some light on this subject 396 pullets hatched during the season of 1927 from a reacting flock were

placed in the laying houses. The pullet flock consisted of six different breeds. These birds were hatched, raised and kept under comparable conditions from the day the eggs were placed in the incubator until the end of the first laying year, October 31, 1928. During the first laying year the management and care of all birds was exactly the same for each breed and house.

The birds were bled three times during the year: (1) At the commencement of the laying year, November 17, 1927, (2) In the midst of the laying year, May 17, 1928, and (3) At the end of the laying year, October 19, 1928. The details of the three tests, as far as they apply to all the reactors, together with the age and number of eggs laid when the first agglutination test was made, are shown in Table 5. The pullets that reacted negative to each one of the tests are not included in this table. The age of the negative and positive pullets varied from 186 days to 253 days. The average egg production of the whole flock of 396 birds was 21 per cent and only 15 out of the 70 reactors laid eggs previous to the first test.

The relation between the hatching periods and infection at the commencement of the laying year is shown in Table 6. It will be seen that among the pullets hatched April 5th and May 17th there were no reactors present in any of the five breeds, while those which were hatched on March 9th,

Table 5. Details of Pullet Retests (Reactors)

| Number of Pullets | Age in Days " | No.of Eggs Laidx | Agglutination Test 10-17-27 | 6-17-28 | 10-19-28 |
|---|---|---|---|---|---|
| S.C.W. Leghorns | | | | | |
| 108 | 230 | 0 | x | x | Do |
| 110 | 243 | 0 | x | x | x |
| 122 | 202 | 0 | x | -' | - |
| 152 | 202 | 0 | x | x | x |
| 167 | 202 | 0 | x | - | - |
| 174 | 189 | 0 | x | x | D |
| 190 | 189 | 0 | - | x | x |
| 196 | 243 | 11 | x | D | D |
| 16 | 230 | 0 | x | x | x |
| 21 | 189 | 0 | x | - | - |
| R.I.Reds | | | | | |
| 7104 | 253 | 16 | x | x | D |
| 7119 | 240 | 18 | x | D | D |
| 7137 | 212 | 13 | - | x | x |
| 7143 | 212 | 0 | x | D | D |
| 7144 | 212 | 0 | - | x | x |
| 7147 | 212 | 0 | x | x | D |
| 7148 | 212 | 2 | - | x | D |
| 7156 | 212 | 0 | x | x | x |
| 7157 | 212 | 0 | x | x | x |
| 7159 | 212 | 0 | - | - | x |
| 7167 | 212 | 12 | - | - | x |
| 7169 | 212 | 0 | x | - | - |
| 7179 | 212 | 0 | x | x | x |
| 7187 | 212 | 3 | x | D | D |
| 7188 | 225 | 0 | x | x | x |
| 7191 | 225 | 0 | x | x | x |
| 124 | 239 | 0 | - | - | x |
| 126 | 198 | 13 | - | x | x |
| 133 | 253 | 5 | x | x | D |
| 134 | 212 | 0 | x | x | D |
| B.P.Rocks | | | | | |
| 27245 | 225 | 0 | x | x | x |
| 27153 | 225 | 0 | x | x | x |
| 27171 | 225 | 0 | x | x | x |
| 27177 | 225 | 0 | x | x | x |
| 27187 | 225 | 0 | x | x | x |

Table 5. Continued

| Number of Pullets | Age.in Days " | No.of Eggs Laid x | Agglutination Test 10-17-27 | 6-17-28 | 10-19-28 |
|---|---|---|---|---|---|
| W.Wyandottes | | | | | |
| 27253 | 199 | 0 | x | x | x |
| 27254 | 227 | 0 | x | x | x |
| 27255 | 199 | 0 | x | x | x |
| 27258 | 199 | 0 | x | D | D |
| 27259 | 199 | 1 | x | x | D |
| 27260 | 172 | 0 | - | - | P |
| 27261 | 192 | 2 | x | x | x |
| 27264 | 186 | 0 | x | - | - |
| 27267 | 199 | 0 | x | x | D |
| 27268 | 199 | 0 | x | x | x |
| 27269 | 186 | 0 | - | x | D |
| 27274 | 186 | 0 | x | x | x |
| 27276 | 186 | 0 | x | x | x |
| 27277 | 186 | 0 | - | - | x |
| 109 | 186 | 0 | x | x | x |
| 111 | 186 | 0 | x | D | D |
| 113 | 200 | 0 | - | - | x |
| 116 | 186 | 0 | - | - | x |
| 117 | 200 | 0 | - | - | x |
| 120 | 200 | 0 | - | - | x |
| Black Orpington | | | | | |
| 27281 | 214 | 3 | x | x | x |
| 27282 | 227 | 1 | x | x | x |
| 27286 | 199 | 0 | x | x | x |
| 27290 | 186 | 0 | - | x | x |
| 27292 | 199 | 0 | x | x | x |
| 27293 | 194 | 0 | x | x | D |
| 27294 | 194 | 0 | x | x | x |
| 27295 | 186 | 0 | - | - | x |
| 121 | 186 | 0 | x | x | x |
| 122 | 186 | 0 | x | x | D |
| 123 | 214 | 16 | x | x | x |
| 200 | 227 | 30 | - | - | x |
| Light Sussex | | | | | |
| 77 | 214 | 0 | - | - | x |
| 86 | 199 | 1 | x | x | D |
| 90 | 199 | 0 | x | x | x |

" - age in days when the first test was made
X - number of eggs laid when the first test was made
o - died

Table 6. Distribution of Infection in Pullets by Breeds and Hatches

| Date hatched | March 9 | | March 22 | | April 5 | | April 19 | | May 3 | | May 17 | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Breeds | No. of Birds | No. of Reactors | No. of Birds | No. of Reactors | No. of Birds | No. of Reactors | No.of Birds | No. of Reactors | No. of Birds | No. of Reactors | No. of Birds | No. of Reactors | No. of Birds | No. of Reactors | Per cent Reactors |
| S.C.W.Leghorns | 7 | 2 | 19 | 2 | 27 | 0 | 15 | 3 | 27 | 2 | 19 | 0 | 114 | 9 | 7.8 |
| R. I. Reds | 4 | 2 | 18 | 1 | 31 | 0 | 36 | 8 | 10 | 2 | 6 | 0 | 105 | 13 | 12.3 |
| B. P. Rocks | 1 | 0 | 7 | 0 | 15 | 0 | 0 | 0 | 39 | 5 | 29 | 0 | 91 | 5 | 5.4 |
| W. Wyandottes | | | 6 | 1 | 7 | 0 | 13 | 7 | 17 | 5 | 8 | 0 | 51 | 13 | 25.4 |
| B. Orpingtons | 3 | 2 | 3 | 1 | 0 | 0 | 6 | 3 | 9 | 2 | 0 | 0 | 21 | 8 | 38.0 |
| L. Sussex | 3 | 0 | 3 | 0 | 2 | 0 | 4 | 2 | 0 | 0 | 2 | 0 | 14 | 2 | 14.2 |
| Total | 18 | 6 | 57 | 5 | 82 | 0 | 74 | 23 | 102 | 16 | 64 | 0 | 396 | 50 | 12.6 |

March 22nd, April 19th and May 3rd, showed various degrees of infection in the following order: Black Orpington, 38.0 per cent; White Wyandottes, 25.4 per cent; Light Sussex, 14.2 per cent; R. I. Reds, 12.3 per cent; S. C. W. Leghorn, 7.8 per cent; and B. P. Rock, 5.40 per cent. A summary of the first, second and third tests is given in Table 7. It will be seen that out of 396 birds tested the first time, 50 birds reacted positive to the test. Between the first and second tests, 27 birds died; of these seven were reactors and 20 non-reactors. Of the 369 birds submitted to the second test, 54 birds reacted to the test; eight birds that reacted negatively to the first test reacted positive to the second test; five birds that reacted positive to the first test reacted negatively to the second test. Between the second and third tests 34 birds died; of these 13 were reactors and 21 were non-reactors. Three hundred and thirty-five birds were submitted to a third test; of these 45 showed a positive reaction; 12 birds that reacted negative to the first and second test reacted to the third test only. None of the birds that reacted positive to the second test showed a negative reaction to the third test. A brief summary of the three tests follows:

| | |
|---|---|
| Number of birds reacting positive to one of the three tests | 70 |
| Number of positive birds that died | 20 |
| Number of birds that reacted positive to the first test | 50 |

Table 7. Summary of Three Agglutination Tests on 396 Pullets

| | No.birds tested 10-17-27 | No. reactors | No. birds tested 6-17-28 | No. reactors | Increase in reactors | Decrease in reactors | No.birds tested 10-19-28 | No. reactors | Increase in reactors | Decrease in reactors | Total No. reactors, 12 mos. | Negative birds died | Positive birds died | Total birds died |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S.C.W.Leghorns | 114 | 9 | 107 | 8 | 1 | 3 | 96 | 4 | 0 | 0 | 9 | 12 | 3 | 15 |
| R. I. Reds | 105 | 13 | 96 | 13 | 3 | 1 | 86 | 9 | 4 | 0 | 20 | 11 | 8 | 19 |
| B. P. Rocks | 91 | 5 | 87 | 5 | 0 | 0 | 84 | 5 | 0 | 0 | 5 | 7 | 0 | 7 |
| W. Wyandottes | 51 | 13 | 44 | 11 | 1 | 1 | 38 | 14 | 6 | 0 | 20 | 8 | 5 | 13 |
| B. Orpingtons | 21 | 8 | 21 | 10 | 2 | 0 | 18 | 9 | 2 | 0 | 12 | 0 | 3 | 3 |
| Light Sussex | 14 | 2 | 14 | 2 | 0 | 0 | 13 | 2 | 1 | 0 | 4 | 0 | 1 | 1 |
| Total | 396 | 50 | 369 | 49 | 7 | 5 | 335 | 43 | 13 | 0 | 70 | 38 | 20 | 58 |

| | |
|---|---|
| Number of birds that reacted positive to the second test and not to the first | 8 |
| Number of birds that reacted positive to the third test and not to the first and second | 12 |
| Number of birds that reacted positive to the first test but not to the second and third | 5 |

A detailed study of the birds that showed a positive reaction to the second and third tests reveals some very interesting information. Pullets raised from chicks hatched during the third and fifth hatch apparently did not contract the disease by contact with infected stock on the range. Since none of the pullets from these two hatches reacted to the first or second test, it is concluded that they must have contracted the disease as adult birds by contact either with infected adult birds, droppings, etc. in the absence of a male bird.

Concerning the remaining 15 birds that were exposed to infection during the first, second, third and fifth hatch, and did not show a positive reaction to the first test, but showed a positive reaction either to the second or third test, some exception may be taken to the statement that they contracted the disease as adult birds. An examination of the data shows that seven out of the 15 birds reacted positive to the third test, but did not react to the first or second test, leads to the conclusion that the seven birds contracted the disease as adult birds between the first and second tests.

The eight birds that showed a reaction to the second test, but did not show a reaction on the first test, may have contracted the disease before the first test was made, and may not have had time to develop sufficient agglutinins to show a reaction at the time of the first test, or may have contracted the disease after the first test was made. It is to be noted that 60 per cent of the newly infected birds apparently became infected just prior to and subsequent to the second test, i. e. at least six to seven months after the first test was conducted. Also that a large percentage of the newly infected birds came from those breeds and houses in which heavy infection was found. The number of infected birds among the B. P. Rocks remained stationary.

That recovery from infection takes place very rarely is indicated by the present data: only five birds recovered from the infection, as compared with 20 birds that acquired the infection. Furthermore, the data also indicate that recovery rarely takes place after the birds are fully mature.

The most important conclusions to be drawn from a study of these retests are the facts that: (1) Few birds recover from bacillary white diarrhea infection, (2) infection takes place between adult birds in the absence of a male, (3) The rate at which infection takes place depends upon the extent

of original infection in the flock, (4) With the exception of the few birds that have completely recovered from infection, the reactors have consistently reacted to the agglutination test.

(c) Retests of Experimental Birds

Considerable fault has been found with the agglutination test because of the fact, that, in the hands of some technicians, (1) Positive birds do not always react to the test, and (2) That negative birds are not always free from ovarian infection. In the opinion of some investigators a bird may react positive to the first test, but may not react positive to subsequent tests and then again give a positive reaction and so on throughout a period of repeated tests.

There are two reasons that may account for this condition: (1) The technique of the agglutination test is not sufficiently standardized to assure accurate results from test to test; or (2) The agglutinin content of the blood sera of positive and negative reactors shows marked variations from test to test. Assuming that the test is standard, and that the only variable factor is the agglutinin content of the blood sera of fowls we should be able to answer the following questions: (1) Do birds positive to the test consistently react in the same manner? (2) Do reactors show

marked fluctuations in the significant range of the agglutinin content of their respective sera? (3) Do non-reactors remain negative to the agglutination test, after they have been separated from reactors? (4) Do non-reactors contract the disease from reactors?

There is very little information on this subject in the literature. Most of the data bearing on the problem have been published since Beach of Wisconsin and Beach of California have published their results on the inaccuracy of the test. Further reference to this subject is made in the review of the literature.

Of the many repeated tests carried out in our laboratory on a large number of birds we propose to deal with but one lot of birds. From March 1927 to April 1928 we completed 12 consecutive tests on 79 White Wyandotte, one-year old, positive and negative birds, purchased from an infected flock. Each sample of blood serum was run in a dilution of 1:25, 1:50, 1:100. A reaction in the 1:50 or 1:100 dilution was considered positive. At the beginning of the experiment there were 42 birds that reacted negative to the test. Of these birds 22 were left in contact with reactors in the presence of male birds; nine of these reacted positive to the agglutination test, i.e., during a period of one year 40.91 per cent of the negative birds picked up the infection. The remaining 20 negative birds

were kept in two separate pens of which one bird reacted positive to the agglutination test at the end of the experimental year. There are many ways in which we may account for the appearance of infection in this particular bird; she may have, through the negligence of the attendant, visited the pen in which the positive birds were kept, or she may have become infected through material blown in or brought in from the infected pen.

Of the 37 positive birds at the beginning of the experiment 34 birds showed consistent reactions through all the 12 tests. There were only three birds that failed to react twice, and one bird once to each of the 12 tests.

From the data presented in this paper we may conclude that: (1) Positive birds consistently react to the agglutination tests because; (a) Of the 37 birds that were tested 12 times only two birds failed to react twice and one bird once to each of the 12 tests, (b) Of 50 positive pullets that were tested at the beginning of the year only five failed to react to the second and third tests. (2) While marked fluctuations have been reported in high dilutions we have failed to record a decrease in the agglutinin content of positive birds that would fall below the diagnostic dilution. (3) That non-reactors from an infected flock, usually react negative to the test, after the removal of the reactors. However, in spite of accurate testing and removal

of all reactors there will, as a rule, be found positive birds on a second test. (4) Negative birds may contract the disease from infected birds either in the presence or absence of males.

## VIII - THE EFFECT OF BACILLARY WHITE DIARRHEA INFECTION ON THE FERTILITY AND HATCHABILITY OF EGGS

To determine the effects of bacillary white diarrhea infection in males and females on the fertility and hatchability of eggs a series of hatches have been carried out on a breeding flock.

This flock consisted of 85 birds kept in a 30' x 14' house divided into four pens. Previous to and during the experiment the birds were confined to the pens and received ordinary care. Grain was fed twice a day, 8 a.m. and 4 p.m. Mash, grit, charcoal and water were before the birds all the time. At noon a wet mash containing about 1 per cent cod liver oil was fed in troughs. Green feed consisting of lawn clippings and kale was given whenever available. Mangels were fed occasionally. The birds were trapnested throughout the period of hatching. Eggs were set at intervals of approximately three to four weeks. None of the eggs at the time of setting were over 14 days old. They were not selected for size, quality of shells, etc. All eggs were candled at the end of the seventh and fifteenth day, and pedigreed at the eighteenth day. Eggs from

reactors and non-reactors were hatched in separate machines. With the esception of the natural seasonal variations this series of experiments was conducted under practically similar conditions.

The females were all White Wyandotte mature hens, that have just gone through the first winter molt and were apparently in excellent condition. The males used in these experiments were full grown vigorous one-year old birds, with the exception of one male, that was two years old.

The pens were mated in accordance with the following plan:

| | | |
|---|---|---|
| Pens | I | Positive females x positive males |
| | IIa | Positive females x negative males |
| | IIb | Negative females x negative males |
| | III | Negative females x positive male |
| | IV | Negative females x negative male |

Pen I, II, III and IV were mated to 3, 3, 1 and 1 males respectively. The data from this series of experiments (seven hatches) is summarized by pens in Table 8. Examination of Table 8 shows that the highest percentage of fertility was secured in pen IIb. The second highest pen in fertility was pen IV. The male in this pen was two years old and did not compare in vigor to the rest of the males, which may account for the slightly lower fertility obtained in this pen, as compared with pen IIb.

The difference in fertility between pen III and pens IIb and IV is 5.45 per cent and 1.02 per cent respectively;

Table 8. Summary of Seven Hatches by Pens

| Pen No. | Eggs Set | Infertile Eggs | Dead Germ 1st Test | Dead Germ 2nd Test | Dead in Shell | Chicks Hatched | Hatched % Eggs Set | Hatched % Eggs Fert. | Hatched % Fertility |
|---|---|---|---|---|---|---|---|---|---|
| I | 689 | 99 | 35 | 47 | 239 | 269 | 39.04 | 45.59 | 85.64 |
| IIa | 383 | 56 | 26 | 22 | 115 | 164 | 43.08 | 50.1 | 85.38 |
| IIb | 225 | 14 | 10 | 7 | 68 | 126 | 56.0 | 59.7 | 93.78 |
| III | 290 | 33 | 19 | 11 | 58 | 169 | 58.2 | 65.7 | 88.33 |
| IV | 169 | 18 | 5 | 11 | 41 | 94 | 55.6 | 62.2 | 89.35 |

I - Positive females x positive males.

IIa - Positive females x negative males.

IIb - Negative females x negative males.

III - Negative females x positive male.

IV - Negative females x negative male.

a difference which is not statistically significant. This male was exceptionally vigorous.

In pens I and II in which positive females were mated to positive and negative males the percentage of fertility was 85.65 and 85.38 respectively, i. e. there was no difference in fertility whatsoever.

We may conclude from this data that Salmonella pullorum infection in males apparently does not affect the fertility of eggs when mated to positive or negative females.

There was considerable difference in hatchability of eggs secured from the different matings. The eggs set from the positive females showed consistently lower hatchability than the eggs set from the negative females. The difference in hatchability between the highest and lowest pen was 19.11 per cent. A difference of about 10 per cent was secured between positive and negative females in pen II (a and b). These birds were mated to the same males and as previously stated were kept in the same pen. Under these circumstances a difference of 10 per cent in hatchability of eggs may be considered significant and not due to chance variation.

If we disregard the difference in reaction of the males, and combine all the positive and negative hens together we obtain a difference of 16.5 per cent in hatchability of all eggs set and 15.6 per cent of all fertile eggs.

We may conclude from this data that Salmonella pullorum infection in females definitely lowers the hatchability of eggs.

## IX - THE EFFECT OF BACILLARY WHITE DIARRHEA INFECTION ON THE LIVABILITY OF CHICKS

The practical benefits to be derived from the agglutination test should be evident in the improved livability of chicks from flocks where reactors were found and subsequently removed. There is very little exact information available on this subject, the data necessarily depending upon the information supplied by the poultrymen. Through the office of R.O.P. Poultry Breeders' Association of British Columbia, which is handling the sales of a large number of chicks, were we enabled to check up on the livability of chicks from different breeders by the number of complaints received from their customers, and by requests for adjustments and post-mortem examinations.

Data obtained on the livability of chicks prior and subsequent to the removal of reactors do not readily lend themselves to presentation in tabular form. A brief summary of each case is, therefore, deemed to be the most suitable way of presenting the data. Cases 1 to 5 deal with flocks in which all of the breeders were tested and reactors removed. Without exception, considerable improvement in the livability of chicks, as compared with previous

years, was reported. Since no complaints were received this year from any of the owners or customers, as was the case last year, no opportunity presented itself to hold post-mortem examination on chicks that died and which were apparently considered as normal losses. It is, therefore, impossible to state whether Salmonella pullorum was or was not present in the chicks that died. Cases 5 to 12 deal with flocks in which only a part of the flock was tested (usually one-third to one-half). In some flocks the reactors from the tested birds were removed, while in others all of the reactors were retained in the flock. In each case, however, reactors and non-reactors were deliberately bred from. In practically every instance, disastrous results followed, as judged by reports from owners and customers, post-mortem examination of chicks and recovery of Salmonella pullorum. It is particularly significant to note that nearly all owners of infected flocks have expressed the desire to have some or all of the breeders tested for the disease.

It will be further seen from an examination of the individual cases, that the improvement in livability of chicks was in some instances attributed solely to the removal of the reactors. In certain other cases, some credit should perhaps be given to the improved conditions of hatching, rearing and feeding of chicks this year as

compared with last year. The degree of success in raising chicks that should be attributed to the elimination of bacillary white diarrhea and improved conditions is not known and remains to be investigated. This point deserves much more attention than it has hitherto received. Breeders and hatcherymen are only too prone to attribute losses in chicks known to be caused by bacillary white diarrhea to such factors as chilling, overheating, faulty feeding, etc. In this connection it may be of particular interest to note that data collected on the livability of chicks from flocks in which no reactors were found show that the chicks are raised with negligible losses and are known to do well both for the breeder and for his customers, under all kinds of conditions.

A comparison of the chick mortality in the spring of 1927 and 1928 in tested flocks is briefly given in the following cases:

Reports from Owners of Flocks where all Breeders were Tested

Case 1. In 1927 previous to the removal of the reactors (14.7 per cent) losses of chicks averaged about 30 per cent. In 1928 after the removal of all reactors, losses averaged less than 5 per cent. In this case chicks were fed buttermilk ad lib., and some credit for the good livability of chicks should perhaps be attributed to this factor.

Case 2. In 1927 previous to testing (35 per cent reactors) losses varied from 35 per cent to 50 per cent. In 1928 after the removal of the reactors owner reported normal mortality. Improved livability of chicks attributed by owner solely to the removal of the reactors.

Case 3. 4.9 per cent reactors. In 1927 losses were above those of previous years when no reactors were present in the flock. In 1928 after the removal of the reactors, which were apparently introduced in the flock in 1926, losses of chicks were reported as being negligible.

Case 4. Previous to testing in 1927 (23.5 per cent reactors), bacillary white diarrhea had been suspected in this flock for a period of four years. Complaints were received from many customers each year. In 1928 owner reported great improvement in hatchability of eggs and 3 per cent mortality in chicks up to three weeks of age. No complaints were received this year from customers.

Case 5. 17 per cent reactors. In 1927 losses varied from 25 per cent to over 50 per cent. In 1928 mortality reported normal and chicks thriving uniformly, in spite of crowded conditions.

Reports from Owners of Partially Tested Flocks

Case 6. 15.2 per cent reactors. Part of flock tested. Owner reported considerable difference in favor of chicks hatched from non-reactors.

Case 7. 11.5 per cent reactors. 1-3 of flock tested. Owner experienced extreme difficulty in raising chicks in 1927. Chicks wilted and died up to 75 per cent to 80 per cent of the number hatched. In 1928 numerous complaints were received from customers. Salmonella pullorum isolated from chicks sent in on three occasions. Owner reported that "chicks hatched from non-reactors have given splendid results, both in our hands and with our customers. Will have every breeder (2000) tested this year."

Case 8. 4.08 per cent reactors. 1-3 of flock tested. Salmonella pullorum isolated from chicks sent in by customers, mortality over 50 per cent. Losses on owner's farm not reported.

Case 9. 44.9 reactors. 1-3 of flock tested. Reactors were not removed. Several customers made complaints. Salmonella pullorum isolated from chicks sent in by a customer. Owner reported varying results. Will have every breeder (1500) tested this year.

Case 10. 80.4 per cent reactors. Hatchability and livability poor. Practically all customers complaining. Owner went out of business in 1928.

Case 11. 6.2 per cent reactors. Livability of chicks fair. Many customers dissatisfied. Owner skeptical about the value of the test.

Case 12. 24 birds tested. 75 per cent reactors including an infected male. One customer reported 92 per cent mortality; Salmonella pullorum isolated from four chicks submitted for examination. Another customer reported 60 per cent mortality; Salmonella pullorum isolated from 26 chicks submitted for examination. Many other customers complained, but did not submit chicks for examination. Chicks on owner's farm reported to do well.

## X - THE EFFECT OF BACILLARY WHITE DIARRHEA INFECTION ON EGG PRODUCTION

Rettger and Stoneburn (54) noted that infected hens seem to be poor layers especially in the second year. Doyle, basing his observations on 14 fowls trapnested for a period of 110 days during the months of March, April, May and June, concluded that "the laying powers of the majority of carriers are very seriously impaired as a direct result of the disease." Waite (55) in what he describes as a "simple, continuous method of attack and control" of bacillary white diarrhea in domestic fowls, emphasizes the desirability of picking "the best layers for the breeding pens." He thus suggests that non-reactors are better layers than reactors.

The data presented in this paper are based on the first year egg records of 358 pullets of six different

breeds. These birds were kept under comparable conditions, and were not culled during the first year. They were tested by the agglutination test at the end of the first laying year, some late in October, others early in November. The data on the mean first year egg production are presented in Table 1. The constants were calculated by the usual formula (see "Genetics in Relation to Agriculture" by Babcock and Clausen, 1927) from grouped data, using a class range of 15 eggs (0-14 etc.).

Examination of Table 9 shows that fifty-eight out of the 358 birds, or 16.2 per cent of all the birds tested reacted. The reactors gave a consistently lower egg production than the non-reactors, the differences actually varying in the case of the different breeds, from 21 to 87 eggs. On account of the small numbers of reactors in some breeds, these differences can scarcely be considered significant, although in only two cases are the differences less than three times the probable error. When, however, means are calculated for the combined data of all the breeds, the difference amounts to fifty-three eggs which is ten times the probable error. This difference can certainly be considered significant, since the odds against such a result being due to chance alone, are very high. The fact that in every case the average production of the non-reactors is higher than that of the reactors, although

Table 9. First Year Egg Production of Reactors and Non-Reactors for the Twelve Months Preceding the Test (Nov. 1, 1926 to Oct. 31, 1927)

| | Non-Reactors | | Reactors | | Difference in Egg Prod. | $\frac{\text{Diff.}}{E_{diff}}$ |
|---|---|---|---|---|---|---|
| | No. Birds | Egg Prod. Mean | No Birds | Egg Prod. Mean | | |
| S.C.W.Leghorns | 87 | 244.41 ± 2.63 | 7 | 195.57 ± 19.59 | 48.84 ± 19.77 | 2.5 |
| S.C.R.I. Reds | 63 | 214.38 ± 4.00 | 3 | 127.00 ± 21.86 | 87.38 ± 22.22 | 3.9 |
| B. P. Rocks | 73 | 220.49 ± 2.94 | 22 | 167.23 ± 8.64 | 52.86 ± 9.13 | 5.8 |
| W. Wyandottes | 42 | 195.93 ± 5.43 | 16 | 174.81 ± 6.46 | 21.12 ± 8.44 | 2.5 |
| Black Orpingtons | 17 | 213.47 ± 7.41 | 4 | 153.25 ± 14.47 | 60.22 ± 15.58 | 3.9 |
| Light Sussex | 18 | 182.00 ± 6.84 | 6 | 137.00 ± 10.58 | 45.00 ± 12.60 | 3.6 |
| All Breeds | 300 | 220.00 ± 1.80 | 58 | 166.57 ± 5.03 | 53.43 ± 5.34 | 10.0 |

not necessarily conclusive in itself, further strengthens the conclusion that the difference is due to infection. If the results were due to chance alone, it would be expected that in some cases at least, the average for the non-reactors would be higher than for the reactors.

The individual records for the reactors varied from nine eggs to 283 eggs, whereas the records made by non-reactors varied from four eggs to 305, in the 12 months period. It will be seen, therefore, that the variation in individual records was practically as great for the reactors as for the non-reactors. This is important since it is sometimes assumed that infection can be controlled by culling out the low producers. This has been suggested as one means of eliminating "carriers" from the flock by Waite, as mentioned above. The fact that one reactor made a record of 283 eggs in the 12 months period shows that at least some of the reactors were good layers. A detailed study of the data shows that nine reactors laid over 225 eggs and a considerable number of other birds laid 200 or more eggs. The elimination of the low producers in a flock would therefore not eliminate all reactors to the test for bacillary white diarrhea, although the evidence shows that a "large proportion of them would be culled were all low producers eliminated.

The effects of bacillary white diarrhea infection on

second year egg production were studied in 71 White Wyandotte two-year old hens which were trapnested for six months, from February 15 to August 15. These birds were of the same breeding and kept under comparable conditions. They were bled previous to the beginning of the experiment and once each month after that. Forty-four out of the 71 birds were reactors (one of these birds was negative at the beginning of the experiment, but reacted to the test two months later). The average production for this group of birds was 56.4 eggs. The individual egg records varied from zero eggs to 122 eggs. There were four birds or 9.09 per cent that laid over 100 eggs.

Twenty-seven of the non-reactors laid an average of 99 eggs. The individual egg records varied from 25 eggs to 112 eggs. There were in this group nine birds, or 33.3 per cent that laid over 100 eggs.

The average difference of egg production between non-reactors and reactors was 32.6 eggs over a period of six months. This difference would have undoubtedly increased considerably during the second part of the year, when on the whole, conditions for egg production are not as favorable as during the spring and summer months. It is interesting, however, to note that the highest producer among the 71 two-year old birds was a reactor, number 172, that laid 122 eggs, or 10 more eggs than the highest

non-reactor.

The data on second year egg production of 71 hens, while not as extensive and complete as those of the 358 pullets, substantiate our findings that: (1) There is considerable difference in the egg production of reactors and non-reactors, (2) There is considerable variation in the individual egg records of reactors and non-reactors. Our finding on this point supports the conclusions of Bushnell and Hinshaw (56) that the effects of the disease on the individual are not constant, although the average egg production of reactors is consistently lower than that of non-reactors, (3) Culling out the low producers will not eliminate bacillary white diarrhea from an infected flock (nine out of 58 reacting pullets laid over 225 eggs in one year, four out of 44 reacting hens laid over 100 eggs in six months).

## XI - BACTERIOLOGICAL AND POST-MORTEM EXAMINATION OF ADULT BIRDS

In order to determine the accuracy of a positive or negative agglutination test post-mortem and bacteriological examinations of birds were made accordingly. As soon as the birds were secured, they were bled and retested again. In each case the second test agreed with the original test. The birds were killed in the laboratory and a bacteriological examination was made under "aseptic" conditions. At

least six cultures were taken from each ovary on slanted agar tubes and incubated for 24 hours. As a rule, the organism appeared in pure culture. Each culture was examined by Gram's stain, and if Gram negative it was then plated in several dilutions. Colonies were picked and subsequently examined on dextrose, mannite,maltose, lactose, sucrose, and tested against a positive and negative serum. In the case of dormant ovaries, where it was not possible to examine the individual ova, the ovary was cut into several small pieces, and either sowed in broth or on plain nutrient agar. In only a few cases did we succeed to isolate the organism by this method. We consider the examination of a dormant ovary as very unsuitable material for bacteriological examinations. We have always endeavored to secure for post-mortem examination birds in which the ovary was fully functioning. In such birds we could also make better observations on the presence or absence of typical bacillary white diarrhea ova.

In Table 10 are shown the results of bacteriological and post-mortem examinations of 41 birds secured from one flock. They were all S. C. W. Leghorns and were in excellent condition. One bird was not examined bacteriologically because of dropsy. Of the remaining 40 birds Salmonella pullorum was recovered from 36 birds. From four birds Salmonella pullorum was not recovered and in each

Table 10. Results of Bacteriological and Post-Mortem Examinations of 41 S.C.W.Leghorn Reactors

| No.of Bird | Culture | Condition of Ovary |
|---|---|---|
| 118 | x | Large number of typical BWD ova |
| 119 | - | Ovary completely dormant |
| 120 | x | Internal laying,typical BWD and "bloody" ova |
| 138 | x | Ovary dormant |
| 142 | x | Large number of typical BWD and "bloody" ova |
| 156 | x | Internal laying |
| 181 | x | Ovary active,a few typical BWD ova |
| 182 | x | Typical BWD ovary |
| 183 | x | Typical BWD ovary.Small watery cyst |
| 185 | x | All ova degenerated |
| 187 | x | Dormant, one large "bloody" yolk |
| 209 | x | Typical BWD ovary |
| 224 | x | Dormant, one small "bloody" and one small "hard" yolk |
| 233 | - | Ovary completely dormant |
| 235 | x | Internal laying |
| 244 | x | Internal laying |
| 270 | x | Typical BWD ovary |
| 272 | x | Internal laying (Heart:Pericarditis) |
| 283 | x | Typical BWD ovary |
| 287 | x | Internal laying |
| 291 | x | Ovary completely dormant, two large"hard' yolks |
| 294 | x | Typical BWD ovary |
| 295 | x | Typical BWD ovary |
| 310 | x | Typical BWD ovary |
| 322 | - | Ovary completely dormant |
| 342 | x | Dormant |
| 352 | x | Typical BWD ovary |
| 355 | x | Typical BWD ovary |
| 361 | x | Typical BWD ovary |
| 369 | x | Typical BWD ovary |
| 374 | x | Typical BWD ovary,large watery cyst |
| 386 | - | Internal laying |

Table 10. Continued

| No.of Bird | Culture | Condition of Ovary |
|---|---|---|
| 399 | x | Typical BWD ovary |
| 416 | x | Dormant,typical BWD ovary |
| 422 | x | Typical BWD ovary |
| 425 | No cultures taken | Dropsy |
| 432 | x | Typical BWD ova (Heart:Peri-carditis) |
| 438 | x | Typical BWD ova |
| 447 | x | Active, a few typical BWD ovary |
| 463 | x | Active, two typical BWD ova |
| 340 | x | Internal laying, typical BWD ovary |

x - S. pullorum isolated

- - S. pullorum not isolated

case the ovary was completely dormant. No signs of bacillary white diarrhea could be seen in the very tiny ova and yet it is quite possible that even these were infected with Salmonella pullora, but that the organism was not isolated because of faulty technique.

It is interesting to note that in two birds pericarditis occurred and that eight birds showed signs of internal laying. No other lesions could be observed.

Similar results were obtained from a bacteriological examination of 61 positive birds of miscellaneous breeds obtained from various sources. Salmonella pullorum was isolated from 55 birds. The organism was not isolated from six birds of which three were completely dormant and three were fully functioning. The last three birds were examined very carefully but no lesions could be found in any of the organs.

From 13 negative birds obtained from various sources, we could not isolate Salmonella pullorum.

From 12 birds showing a reaction in low dilutions a faint reaction in 1 to 50 and +++ or ++ reactions in 1 to 25, or 1:10, we failed to isolate the organism of bacillary white diarrhea.

In both the negative birds and in those showing an incomplete reaction we have observed watery cysts attached

either to the ovary or cloaca, as well as other abnormalities, but which could not be considered as indicative of a bacillary white diarrhea condition.

## XII - SUMMARY

Data secured from an investigation of bacillary white diarrhea, a disease which is acute in young chicks and chronic in adult birds are presented. The information secured may be briefly summarized as follows:

(1) Fifteen of the forty-one flocks included in this investigation showed indications of bacillary white diarrhea infection. Salmonella pullorum was isolated from chicks in 13 out of the 15 flocks.

(2) Bacillary white diarrhea infection of adult birds was found in 25 out of 41 flocks to which the agglutination test was applied.

(3) Out of 15,327 tested birds, 1744, or 11.3 per cent, reacted to the agglutination test.

(4) The percentage of infection in flocks tested varied from less than 1 per cent to 80.4 per cent.

(5) All breeds were found to be susceptible to bacillary white diarrhea infection. The degree of infection varied considerably within each breed.

(6) Males were found to be subject to bacillary white diarrhea infection, but there appeared to be a smaller

percentage of infection among them than among the females.

(7) Infection was shown to be introduced into the flocks or further spread within the flocks by:

(a) Introduction of infected breeding stock.

(b) Purchase of day-old chicks from infected flocks.

(c) Feeding of raw eggs from infected birds.

(8) Bacillary white diarrhea infection adversely affected the hatchability of eggs. The difference in hatchability of eggs set from non-reactors and reactors was 16.5 per cent in favor of the non-reactors.

(9) Bacillary white diarrhea infection adversely affected the livability of young chicks, the average mortality ranging in diseased flocks around 50 per cent as compared with 10 per cent in normal flocks.

(10) The difference in egg production between reactors and non-reactors was found to be in:

(a) A flock of 356 pullets, 53 eggs.

(b) A flock of hens trapnested six months, 32.6 eggs.

(11) Bacillary white diarrhea "carriers" can not be culled out from an infected flock on the basis of egg production.

(12) The agglutination test was found to be highly effective in detecting birds infected with bacillary white diarrhea as shown by the fact that the percentage of

infection was reduced from 20.5 per cent to 1.7 per cent after one test and the removal of reactors.

(13) The agglutination test was found to be of value in testing pullets that have just come into production. Only five out of 50 positive pullets that reacted to the first test did not react to subsequent tests.

(14) Negative adult birds, hens and pullets, contracted the disease when left in contact with reactors, either in the presence or absence of males.

(15) Out of 79 birds tested 12 consecutive times:

(a) 37 positive birds reacted consistently to the test.

(b) Nine out of 22 negative birds, kept in contact with reactors, became infected and consistently reacted positive to the test. The remaining 13 birds were negative to all the tests.

(c) One out of 20 birds kept separate from reactors, reacted to the test at the end of the experimental year. The remaining 19 birds were negative to all tests.

(16) In one lot of S. C. W. Leghorns, Salmonella pullorum was isolated from 36 out of 41 positive birds; in another lot of miscellaneous breeds from 55 out of 61 positive birds.

(17) Salmonella pullorum was not isolated from 12 birds showing a reaction in low dilutions and from 13 completely negative birds.

(18) It is concluded on the basis of the data presented in this investigation that bacillary white diarrhea infection may be effectively controlled by means of the agglutination test; that the test is highly effective in detecting "carriers" and that the accuracy of the test can not be doubted, if properly conducted.

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