NOVEMBER, 1926 TECHNICAL BULLETIN 21

AGRICULTURAL EXPERIMENT STATION

KANSAS STATE AGRICULTURAL COLLEGE
MANHATTAN, KANSAS

BACILLARY WHITE DIARRHEA IN FOWL

PRINTED BY KANSAS STATE PRINTING PLANT
B. P. WALKER, STATE PRINTER
TOPEKA 1926
11-4689

SUMMARY

The disease, bacillary white diarrhea, is very prevalent in all parts of the country where studies have been made for its detection. This does not mean that every flock is infected, but from a general survey it means that about 75 per cent are suffering from this trouble. The disease affects both adult fowl and chicks. 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.

The organisms are carried from generation to generation largely in the bodies of birds which suffered from the disease as chicks, but which recovered and continued to harbor the organism after reaching the period of production. The organism is then passed off in the egg to infect the embryo or newly hatched chicks. This infection of the ovary of the producing hen reduces productivity, fertility, and hatchability. After hatching, the infected chicks spread the disease widely to noninfected chicks through the dust of the incubator, through droppings, and by contaminating the ground, litter, and feeding and water utensils by their infected excreta.

The amount of actual money loss due to these birds is difficult to determine because of the confusion which exists concerning the cause of death in fowl. An accurate diagnosis of chick diseases is impossible outside a research laboratory. Numerous other causes of death produce clinical symptoms and pathological changes almost identical with those caused by *S. pullorum* infection. Thus, it is necessary to develop a method for correct differential diagnosis of various diseases before adequate data can be collected to determine this point.

There has been devised no adequate method of treatment for this disease. The only method of control consists of three steps: (1) The testing of the serum of all birds in the flock by means of the agglutination test; (2) the elimination of all reactors from the flock immediately after they are discovered; and (3) the careful use of strict sanitary measures for the runs, houses, and drinking and feeding utensils.

The data collected in this investigation indicate that a large per cent of infection may take place after birds have matured. The infectious material may be transferred through feeding and drink-

ing utensils and from contaminated litter. It may be transferred both actively from the male to the hen or passively from an infected hen to a healthy hen by the male bird.

The agglutination test has been found to be the only method yet devised for detecting the carriers of the disease. This test is very highly effective in locating the diseased "carriers." The exact details of making the test have not been agreed upon by all laboratory workers, but it is a well-known fact that only skillful technicians should be allowed to use it. The chief difficulty which has arisen to date is the inability of the various workers to determine what dilution of the serum should be used to determine the greatest per cent of dangerous carriers. Some birds which recover and become immune will react to this test. From the limited information available it appears that the lowest dilution of serum which can be used and still allow a reading is the best one to use. This must, however, be used in conjunction with a higher dilution to avoid the prozone which may appear in badly infected birds.

The length of time necessary to rid a flock of this disease will depend upon many factors, among which are regularity and the thoroughness with which the flock is tested. In the judgment of the writers the flock should be tested twice a year until all reactors are eliminated. This applies to the utility as well as the breeding flock. As long as there are infected birds on the farm there will be more or less spread of disease material to the breeding stock. In many cases the organisms are eliminated through the droppings, These may be carried on the shoes of the workmen, by sparrows and other birds, by wind and insects, and in various other ways. Care used in following sanitary precautions after the removal of all reactors is also an important factor in ridding the flock of this disease. Sanitary precautions are useless if all reactors are not first removed from the flock. A valuable bird is equally as dangerous as one of little value. There are also certain factors which predispose to infection. These include improper care, inadequate diet, and insanitary living conditions. If the flock is properly cared for, properly tested twice a year, properly freed of all reactors, and kept in clean sanitary surroundings the disease should be eliminated in two or three years,

Great care should be exercised in the introduction of adult birds, eggs, and chicks from the outside. Practically all the bacillary white diarrhea has been introduced into Kansas and distributed to different communities because untested birds were introduced into

disease-free flocks in the past few years. For example, all the birds shipped directly and all the flocks receiving birds from a certain breeder in an eastern state have been found to be infected with these organisms as shown by the agglutination test. *Birds should not be accepted by any purchaser of breeding stock without being guaranteed free of this disease.* Such a guaranty will not work a hardship on anyone and will eradicate the chief method of the spread of the disease. The purchaser of breeding stock can well afford to pay a premium for all tested stock.

TABLE OF CONTENTS

BACILLARY WHITE DIARRHEA IN FOWL[1]

L. D. BUSHNELL, W. R. HINSHAW, AND L. F. PAYNE

INTRODUCTION

Bacillary white diarrhea in domesticated fowl is rapidly assuming the lead in causing losses of poultry in this country. It is a highly acute infectious disease of young chicks which usually affects the birds a few days after being hatched and a chronic or acute infectious disease of adult fowl. The cause is a specific bacterium, *Salmonella pullorum.* Although the disease is generalized in the body of the chick the most marked external symptom is that of diarrhea. From the fact that the droppings of the chicks are white and the causative factor is a bacterium, the name "bacillary white diarrhea" has been given to this disease.

Study of this disease have been conducted by the Department of Bacteriology of the Kansas Agricultural Experiment Station for several years, and the present bulletin contains the important information which has been secured to date.

HISTORICAL REVIEW OF LITERATURE

Rettger (1900) reported his studies on a disease of young chicks which was very common and destructive in the eastern part of the United States. He succeeded in isolating the organism causing the trouble and named the disease "white diarrhea." Since that date Rettger and his associates have published numerous reports of their investigations and their work has also been substantiated many times in all parts of this country and in a few instances in Europe.

Rettger (1901) again reported an epizoötic of this disease on adjoining farms involving the loss of hundreds of hen-hatched chicks. Losses were as high as 80 per cent and occurred when the chicks were from one to four weeks of age. This disease was the same as reported by him in 1900,

Rettger and Harvey (1908) and Rettger (1909) reported work with other epizoötics of the disease and the recovery of the organism above mentioned. They succeeded in reproducing the disease successfully by artificial inoculation, and Rettger named the organism *Bacterium pollorum.*[2]

---

1. Contribution No. 79 from the Department of Bacteriology, and No. 38 from the Department of Poultry Husbandry.

2. The name was recently changed to *Salmonella pullorum* by the Society of American Bacteriologists.

Milks (1908) described an outbreak of this disease in birds of five to six weeks of age. The symptoms and post-mortem findings were those of white diarrhea and an organism similar to that described by Rettger was isolated.

Pernot (1908) of Oregon made a study of chicks dead in the shell and isolated an organism from the heart blood and unabsorbed yolk. He also showed that this organism was pathogenic for chicks in the shell and for young chicks after hatching. This organism was probably not of the *S. pullorum* type.

Rettger and Stoneburn (1909) concluded that the hen was the original source of infection of the chick; that a certain per cent of chicks on infected farms have the disease when hatched; and that the mortality depends upon the virulence and numbers of the organism, the mode and time of infection, and doubtless upon the vitality of the chicks. While a large number of infected chicks die under four weeks of age, some may survive the infection. These are likely to be weak and stunted and seem particularly susceptible to other disorders.

Rettger and Stoneburn (1911) published further results on the influence of direct infection of chicks with cultures of *S. pullorum.* They also report work to confirm the earlier observations that the hen was the source of infection of the chicks.

Rettger, Kirkpatrick, and Stoneburn (1912) made a study of the comparative susceptibility of chicks of different ages. The chicks were artificially infected. They concluded that the period of grave danger to chicks lies within the first three days after hatching. There is comparatively little danger to chicks of strong vitality after the first 48 hours. When the vitality is low, infection may take place as late as the fourth or even the fifth day.

They also report a study of infection of mature stock. From their experiments they found that of all hens used as checks not one laid an egg which was known to be infected with the white diarrhea bacterium. Nor was there a single indication, when the hens were killed and the ovaries examined, that the ovaries were infected with *B. pullorum.* On the other hand, eight hens kept in litter-infected pens laid eggs which were found to be infected and nine revealed infection of the ovary by direct observation after the hens were killed. Another was in all probability infected.

These writers report on extensive experiments in feeding sour milk. They considered it a good means of preventing, or at least

holding in check, epizoöties of white diarrhea. They state that the sour milk should be fed early and should be kept before the chicks constantly. They found that sour milk had an important stimulating effect on the growth and vitality of chicks, and for that reason alone it is a most valuable food.

These authors also report in this bulletin the extremely important findings on adult infection with this disease. Normal adult hens exposed in the same pen with infected hens became infected after eight to sixteen months. It was also found that birds exposed to litter and feed containing this organism became infected.

Gage (1911) published reports from Maryland confirming the work reported by Rettger and others. He found the disease to be due to infection by *Bact. pullorum,* and that the hen was the original source of infection and transmitted the organism from the ovary to the chicks through the eggs.

Jones (1910) (1911) published results of his investigations and was able to confirm the findings of Rettger and Stoneburn, and others. He considered the disease as being largely local in the ovary of the adult fowl and was able to produce such infections by intravenous inoculation of *Bact. pullorum* cultures.

Jones (1911) reported an epizoötic among adult fowl due to *Bact. pullorum.* This trouble apparently arose from feeding incubated eggs from a flock which had been giving poor hatching results. After 16 days several of the adult birds, which had eaten eggs, were taken sick. In all, about 50 died. The infection in adults is characterized by necrotic spots in the liver, spleen, and pancreas, and by large necrotic nodules in the heart muscle; also by the presence of a fibrinous exudate on the capsule of the liver and spleen and on the pericardium.

Jones (1913) demonstrated that the agglutination test was of great value in detecting fowl harboring *Bact. 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 the per cent of actual infection.

> The blood serum of all infected fowl 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, 1:1,000, and 1:2,000.

Bushnell and Maurer (1913) reported on the value of milk soured by *B. bulgaricus* in control of the disease. It was found that this treatment was beneficial in reducing mortality. This was especially

true when the milk treatment was begun before infection had occurred. At the end of eight weeks the birds receiving milk showed 26 per cent less mortality than those in the check pen.

Gage, Paige, and Hylan (1914) published the results of their investigation on white diarrhea infection in fowl and concluded that the examination of eggs for the presence of *Bact. pullorum* gave results that were too irregular to be of practical value in the diagnosis of this disease. They recommend the macroscopic agglutination test and state that their results with this test substantiate those of Jones. They also found that it was possible to produce an ovarian infection of the hen by means of an intravenous injection of cultures of *Bact. pullorum.* Some of the birds showed the ovarian infection for two years after injection of the cultures.

Rettger (1914) reviewed the results of earlier studies on the transmission of the infection from the hen to the chick

Rettger, Kirkpatrick, and Jones (1914) reported that female chicks which are infected with *Bact. pullorum* when small may develop into permanent bacillus carriers and be a constant danger to young and old stock. This carrier condition was established in fully 25 per cent of the infected flock. They also reported on the use of the macroscopic agglutination test for recognizing white diarrhea bacillus carriers. They also reported further experiments on the use of sour milk and found it to be of equal value to milk soured by the Bulgarian sour milk bacillus of Metchinkoff. An appendix is included in this bulletin in which they discuss the post-mortem findings of the disease and give a description of *Bact. pullorum.* In one case a bird's blood reacted positively to the agglutination test, but on post-mortem examination the ovaries were found to be normal. However, a tumor which contained a pure culture of the organism of bacillary white diarrhea was found in the thoracic cavity.

Rettger, Kirkpatrick, and Jones (1915) issued a fifth report on their work. In this report they summarized their work on the use of the agglutination test. In all, they had examined 14,617 individual fowl from 107 flocks. Of these they found 1,440, or 9.85 per cent, reactors (infected). Of the 13,831 hens 1,417, or 10.24 per cent, were positive and of the 786 males tested, 23, or 2.9 per cent, reacted. The testes of two of the males harbored *Bact. pullorum* in large numbers. In four of the reacting males pericarditis and infection of the heart sac with the same organism were observed.

The retesting of flocks, which on first examination by the agglutination test contained bacillus carriers and from which the reactors

were removed, gave widely different results. In four flocks, out of a total of 13, no reactors were found at the time of the second test. In the other nine, the infection varied from 0.6 to 25.7 per cent, the number in each instance being decidedly less than at the first test.

Rettger, Kirkpatrick, and Jones (1916) in a sixth report included the results of testing 22,354 fowl. Of this number 21,317 were hens and 1,037 were males. Of the hens, 9.3 per cent, and of the males, 2.1 per cent, were found to be infected. The infection in various flocks ranged from 0 to 56.3 per cent. The number of chicks hatched, 166,460, was 56.7 per cent of all eggs set. The mortality during the first three weeks was 10.2 per cent.

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, and consider that this failure is due to the fact that certain birds have been infected for too short a time for the production of sufficient agglutinins to react to the test. Table I shows their data on this point.

TABLE I.—Value of one agglutination test in reducing infection.

BREED.	First test.		Second test.	
	Number of birds.	Per cent reactors.	Number of birds.	Per cent reactors.
Leghorns	782	3.50	240	0.00
Rhode Island Reds	1,343	11.25	499	1.80
Barred Rocks	272	22.80	157	7.60
Wyandottes	984	20.80	440	14.50
Total	3,381	13.16	1,336	6.37

Rettger, Kirkpatrick, and Card (1915) found that milk feeding reduced mortality from all causes, and if fed soon enough and for a sufficiently long period, greatly reduces the death rate caused by bacillary white diarrhea. Sweet and sour milk were apparently of equal value in this respect.

Smith and Ten Broeck (1915) called attention to the similarities between the organism of fowl typhoid, *Bact. sanguinarium*[3] of Moore and *Bact. pullorum* of Rettger. They conclude that it is possible to distinguish between these organisms on the basis of acid and gas production in dextrose and mannite, since the former failed in this respect. The fact that the *Bact. sanguinarium* pro-

3. This organism is the cause of a disease in fowl called "Fowl typhoid," and has been named *Bacillus sanguinarium*.

duced acid in maltose also differentiated it from the *Bact. pullorum.* Toxin production is identical for both. The agglutination tests are definite enough to group the fowl typhoid and pullorum types together, and both show an intimate relation to the human typhoid bacillus.

Gage and Paige (1915) discuss the agglutination test for the detection of carriers in the breeding stock. They used dilutions of 1:100 and 1:200 of the serum on a polyvalent antigen containing six strains of the organism obtained from various sources. Birds showing a positive agglutination in serum dilutions of 1:100 were considered as reactors. The infection in flocks varied from none to as much as 50 per cent. They recommend the use of the agglutination test as a means of eradicating the disease.

Rettger, Hull, and Sturgis (1916) studied the danger of food poisoning in human beings from consuming eggs infected with the organism. They report the results of feeding cultures of the organism to rabbits, guinea pigs, kittens, and white rats. The rabbits usually died from the feeding, while young kittens showed symptoms of severe poisoning. They studied the resistance of the organism to cooking. Infected eggs were fried, boiled, scrambled, and poached in the usual manner. It was found that boiling for four minutes would not kill the organism in all cases. Frying eggs on one side was not effective in rendering them sterile. Frying on both sides, scrambling, or poaching was effective in this respect.

Gage and Martin (1916) made a careful study of the pathological changes in the intestine of chicks artificially infected with cultures of *Bact. pullorum.* The sections revealed a marked injury, especially of the small intestine. The mucosa showed marked hyperemia, hemorrhagic exudation, and leucocytic infiltration. In the individuals in which the disease had run a longer course there were exhibited processes of regeneration. There were many instances of thickening of the intestinal wall and marked injury to the tissue probably due to the toxic substances produced. The findings indicate that the disease may be considered as an acute catarrhal inflammation.

Taylor (1916) compares the characteristics of *Bact. sanguinarium* and *Bact. pullorum.* He concludes that while the lesions produced by the two organisms and their biological characters are very similar, there are enough differences to warrant the conclusion that they are distinct organisms.

Rettger and Koser (1917) reported on a comparative study of

Bact. pullorum and *Bact. sanguinarium.* Their findings agree with those of Smith and Ten Broeck reported earlier. They report a marked reduction of nitrates to nitrites. The Voges-Proskauer reaction was negative and while *Bact. pullorum* produced small amounts of acid in milk, *Bact. sanguinarium* lacked this property. The authors found a distinct difference between these two organisms in their ability to ferment maltose, dextrin, and dulcite. *Bact. sanguinarium* attacked these substances with appreciable acid production, while *Bact. pullorum* failed to affect them and exhibited a tendency to produce alkali. They state that the methyl-red test is of practical value in the differentiation of these organisms, *Bact. sanguinarium* producing acid while *Bact. pullorum* did not. They found no change in the agglutinative ability of immune sera produced against these organisms. By immunization tests it was found that the fowl typhoid bacillus did not protect against fatal doses of *Bact. pullorum,* although the bacillus of bacillary white diarrhea appears to immunize against *Bact. sanguinarium.*

Ward and Gallagher (1917) reported on the value of the intradermal test for the detection of carriers of *Bact. pullorum.* Cultures grown for a month and stored for several weeks, then killed by use of phenol gave the best results. The results reported are quite irregular but give fairly close agreement with autopsy findings. They emphasize the importance of reading the test at a certain time. This is after the initial swelling due to injury has disappeared and before that due to the specific action of the product has disappeared. They indicate that 48 hours is too late and at 24 hours there might be some false results and recommend reading at about 30 hours. Of the birds artificially infected, about 90 per cent gave positive reactions while 6 per cent failed to react to the test and 3 per cent showed no reaction and no lesions. The injection of this material caused the birds to react to the agglutination test later.

Winslow, Kligler, and Rothberg (1917) class *Bact. pullorum* with *B. paratyphoid A* as a Group III of the colon-typhoid bacteria. The characteristics of this group are glucose, rhamnose, and mannite positive and lactose and xylose negative. The authors consider Type III as one in which the reaction falls in one day to between pH 5.0 and 5.5 and drops 0.2 to 0.4 in the next four days. In discussing these fermentations the authors state:

"There is a well marked metabolic gradient, inosit (as might be expected from its composition) being least attacked, dulcite and sucrose coming next,

then glycerin, salacin, and lactose, then rhamnose and arabinose and finally xylose, mannite, and glucose."

Hadley (1917) discusses the relation between the Alpha and Beta group of paratyphoids in reaction on litmus milk. Alpha is usually stated to give a permanent alkaline reaction after an initial acidity. He compares various strains of the *paratyphoids A and B* and *Bact. pullorum, B. enteritidis,* and *B. avisepticus.* He found that all the strains of *paratyphoid A* gave an initial acidity which returned to neutral by the second day; that the strains of *paratyphoid B* gave no initial acidity (though the tubes lightened somewhat) and gave an alkaline reaction beginning about the eighth day. By the time the *paratyphoids A* had returned to neutral, the *paratyphoids B* showed a moderately strong reaction. The pullorum strains showed an initial acidity which passed quickly into a neutral, then to an alkaline reaction about the thirty-eighth day, sometimes earlier. At the end of the ninetieth day the only difference between the A and B types was a stronger alkalinity and a deep wine red translucency for the B types by transmitted light. This was not shown by the A types. The pullorum type showed this characteristic. Both types give a terminal alkaline reaction. There appears to be in this respect no fundamental qualitative difference between the two bacterial types, but one involving quantitative relations only.

Goldberg (1917) reported on a study of the fermenting properties of *Bact. pullorum* (Rettger) and *Bact. sanguinarium* (Moore). His *Bact. pullorum 5* is an atypical strain which did not produce gas in any of the carbohydrates used. It is in this respect similar to the original Rettger strain.

The other strains of *Bact. pullorum* produced gas and marked acid in dextrose, mannite, galactose, levulose, arabinose, and mannose. From these carbohydrates *Bact. sanguinarium* produced acidity but no gas. In isodulcite four strains of *Bact. pullorum* produced gas and marked acidity while *Bact. sanguinarium* produced only slight acidity at first, the amount of the acidity gradually increasing on prolonged incubation. In dulcite the strains of *Bact. sanguinrium* produced marked acidity and no gas while the first four strains of *Bact. pullorum* produced slight acidity and gradually changed to an alkaline reaction on prolonged incubation. In dextrin the results were similar to those in dulcite except that acidity is not so marked. In lactose, saccharose, starch, sugar-free broth, adonite, salicin, inulin, raffinose, and erythrol, all strains of *Bact. sangui-*

narium and four of *Bact. pullorum* produced slight acidity and became alkaline after prolonged incubation. (Xylose showed a marked increase in acidity after sterilization.) One year later the work was repeated with dextrose, mannite, and glactose with identical results. The atypical *Bact. pullorum No. 5* produced acid in milk in 24 hours and coagulation in 12 days. Whey did not separate from the curd. No gas appeared in any carbohydrates used, but more acid was produced than in other strains. The author considers that this may be a doubtful strain.

Krumweide and Kohn (1917) studied the paratyphoid-enteritidis group of bacteria and found that this group uniformly and promptly fermented rhamnose, whereas the typhoid bacillus did not attack this carbohydrate. These authors made a special study of the anaërogenic strain, *Bact. sanguinarium,* and the aërogenic strain, *Bact. pullorum.* Their results agree in general with those of Smith and Ten Broeck. The results of agglutination tests also agree with those of the earlier workers. It was found that the avian strains would not remove the specific agglutinins for *Bact. typhosus* from antityphoid sera. They also found a lower cross agglutination of *Bact. sanguinarium* and *Bact. pullorum.*

Hadley and others (1917) reported on infections caused by *Bact. pullorum* in adult fowl. These infections showed a clinical picture and pathological manifestations indistinguishable from those of fowl typhoid. The authors believed that the latent infection was stimulated into active manifestations of fatal generalized infection as the result of intestinal irritation, or of physiological changes following the feeding of a ration containing a large proportion of roughage in the form of oat husks. They also call attention to two forms of *Bact. pullorum.* An "A" type, which is gas forming and whose immune serum agglutinates *Bact. typhosus* about equally with its homogeneous antigen, and which is pathogenic for young chickens only. The "B" type, resembling the type "A" in its chief characteristics but differing in the following respects: (1) It does not form gas in any carbohydrate; (2) its serum (like the antifowl typhoid serum) does not agglutinate at high dilutions, human typhoid antigens; and (3) it is able to produce natural generalized infections in adult fowl, but only to a slight degree, if at all, in young stock.

Hadley, Elkins, and Caldwell (1918) present the results of a long study of the various poultry pathogens including *Bact. pullorum.* This report fills a bulletin of 216 pages and contains an excellent

bibliography. They agree with earlier workers that *Bact. pullorum* is closely related to *B. paratyphoid A,* especially in its action on milk. The *Bact. pullorum* does not manifest a very close relation to *B. paratyphoid A* in antigenic action but is more closely related to *B. typhosus.* Three epizoöitics in adult fowl are reported in which the apparent causative agent was *Bact. pullorurn.* In one of these typical leukemia was the most obvious and characteristic symptom. They state that the present methods of diagnosis of *Bact. pullorum* infections by agglutination reactions are not satisfactory because they fail to differentiate between (1) ovarian and nonovarian infections; (2) culminated and current infections; (3) *Bact. pullorurn* and infections with the fowl typhoid bacillus, which is widely disseminated among adult stock; and (4) infections caused by *Bact. pullorum A* and *Bact. pullorum B.*

Mulsow (1919) made an exhaustive study of these two groups of organisms. His findings confirm those of earlier workers on this subject. The fact is noted that some of the strains produce acid and even gas in maltose. Agglutination tests have shown that there is an antigenic relation between those organisms and *B. typhosus, B. enteritides,* and *B. abortus-equinum.* Such antigenic relations were not observed between these avian strains and *B. avisepticus, B. dysenteriae, B. paratyphosus A and B, B. suipesticus, B. proteus,* and *B. coli.* Absorption tests will differentiate quite readily between *B. typhosus* and these avian strains.

Feeding experiments indicate that laboratory cultures of these organisms will rarely produce injurious effects on laboratory animals. Both organisms, when grown under proper conditions, produce toxins which are quite poisonous to rabbits. The action of the toxin appears to be the same for the two strains. These strains may be differentiated from *B. typhosus* by their lack of motility, their fermentation reactions in rhamnose and sorbite, and absorption tests with immune sera. *Bact. pullorum* may be distinguished from *Bact. sanguinarium* by the inability of the former to ferment dulcite, while the latter ferments this carbohydrate. The author considers that there is sufficient difference between the two organisms to regard them as separate types.

Scherago and Benson (1919) made a brief report on their investigations in testing for bacillary white diarrhea. These authors report one poultry raiser who lost 1,800 chicks in one year from this disease and the following year the losses in the same flock reached 90 per

cent of the chicks hatched. They also report their results from use of the intradermal test and conclude that the test is so inconsistent that it is practically worthless so far as a diagnostic agent for *Bact. pullorum* infection in adult fowl is concerned. The reasons for these variations are due to the fact that swelling may result from the introduction of even sterile water and the introduction of foreign protein is likely to give the same results. The previous introduction of intradermal fluid had caused at least 85 per cent of the birds tested to react to the agglutination test regardless of their reactions in the original test.

Rettger, Kirkpatrick, and Card (1919) report on an examination of over 21,000 hens by means of the agglutination test. They have found comparatively few flocks of any size which have been tested and found to be free from ovarian infection. 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. They state that 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. The authors attempted to determine the influence of the male upon the spread of the infection throughout the flock. The males showed a rather low per cent of infection, 2.1 per cent, and only two of about, 55 examined showed the infection in the testicular tissue. While the male is of little importance in the direct transmission of the infection he may be a passive carrier of these organisms. To determine this, cultures of *Bact. pullorum* were introduced into the cloaca of pullets. Later it was found that 25 per cent of the birds subjected to oviduct inoculation became permanent reactors, and on examination of the different organs and tissues, these birds at the completion of the experiment were found to possess abnormal ova which had every indication of being infected and from which *Bact. pullorum* was isolated with ease. The suggestion is made that the passive transfer of these organisms by the male bird may be reduced to a minimum by segregating the males during the greater part of the year and allowing them to run with females during the breeding season only.

Winslow, Kligler, and Rothberg (1919) describe *Bact. pullorum* (Rettger) as follows:

"Gram negative, nonspore-forming, nonmotile rods. Colonies on gelatin

somewhat intermediate between the thin translucent irregular colonies of *B. typhosus* and the convex regular colonies of *B. coli.* Produces acid and gas in media containing hexoses, mannitol, rhamnose, arabinose, and sorbitol; but not in maltose, dulcitol, xylose, lactose, sucrose, salicin, dextrin, raffinose, inulin, and adonitol. Milk first turned slightly acid, later, but only slowly, neutral or slightly alkaline. Lead acetate not reduced. Gelatin not liquefied. Indol not produced. Exhibits group agglutination with *B. typhosus.* Causative agent of bacillary white diarrhea in young chicks and found (without definite pathological symptoms) in ovaries of adult fowls."

These authors consider that in view of the lack of correlation between gas production and power to attack various carbohydrates, exhibited by the Morgan bacillus and the two varieties of *Bact. pullorum,* they are less inclined to lay stress on the former than on the latter characteristic. They do not agree with Hadley and others (Krumweide and Kohn, 1917) who believe that *Bact. gallinarum* is more closely related to *B. typhosus* than is *Bact. pullorum.* All these organisms are, however, allied in their agglutinative reactions.

Hadley, Caldwell, and Heath (1919) consider that *Bact. pullorum* (Rettger) is typically a gas-producing organism. Occasionally, however, an anaërogenic strain is observed. Certain strains of this organism isolated from eggs or from chicks dying of bacillary white diarrhea or from adult stock, and which had been maintained for some years in the laboratory, were grown in extract and in infusion glucose broth in Smith fermentation tubes. From the data collected it appears that many strains of *Bact. pullorum* that do not produce gas in extract media may produce gas in infusion media; also that when any amount of gas is produced in extract media a larger amount is produced by the same strain in infusion media. Gas production in sugar media is apparently strongly influenced by environmental factors independent of the presence of specific fermentable sugar.

They also studied *Bact. pullorum* infection in adult stock and made a record of three widely separate epizoötics among adult fowl, each involving a considerable mortality. They isolated in pure culture, as the only microörganism present in the blood and organs, an organism conforming to *Bact. pullorum* in all essential respects except that it is not aërogenic. It should be added that the clinical features of the disease, as well as the pathological findings at autopsy, differed in no important respect from those of fowl typhoid. In the ovaries and the eggs of birds dying in one of the epizoötics mentioned, aërogenic strains were also found but these were not found in the other organs of the body, in the heart blood, or in the

pericardial or plural exudates. The writers postulate two types of *Bact. pullorum:* (1) *Bact. pullorum A,* aërogenic and found only in infections of young stock or as a latent ovarian infection in adult stock; (2) *Bact. pullorum B,* anaërogenic and observed only as an agent of active infection in adult stock. Slight fermentation differences between these subtypes may also exist. An explanation of this diversity of type, together with the difference of selective action in the tissues of the fowl, is not at present possible.

Gage and Flint (1922, 1923, and 1924), of Massachusetts, have issued several reports on the testing work of the Massachusetts Agricultural Experiment Station. They state that the elimination of bacillary white diarrhea depends upon two factors: First, the finding of the infected birds in the breeding flock through the application of the blood serum test; and second, the removal of these birds from the breeding flock and the protection of the growing flock from infection. By the test a damaged mature breeding bird is located, and by being eliminated from the breeding flock, a step to an impaired day-old chick is intercepted. The following plan for testing and control has been devised and is now in effect in that state.

TESTING PLAN No. 1.—Tests on Birds One Year of Age or Older

A flock of hens is to be considered free from infection when reactors do not exist in the breeding flock after the following plan has been carried out:

1. First test at the age of one year or older.
2. Second test on nonreactors, six to twelve months later. By this plan each adult hen in the breeding flock will have been tested twice.

TESTING PLAN No. 2.—Tests on Pullets

A flock of pullets is to be considered free from infection when it is the product of a flock which has already been tested twice, and when on test it contains no reactors. Three tests are thus required, as follows:

1. Original test, when the birds are eight to twelve months old.
2. Nonreactors, tested again six to twelve months later.
3. Third test made on the offspring of the birds already tested twice.

TESTING PLAN No. 3.— Alternate Plan for Tests on Pullets

A flock of pullets is to be considered free from infection when produced as prescribed, and when, on the last of the tests, no reactors are found to exist. The procedure is as follows:

1. Original test when birds are eight to twelve months old.
2. Nonreactors tested again four to six weeks later.
3. Offspring of the above flock tested as pullets.
4. Nonreactors tested again four to six weeks later. In other words, the test is applied to pullets and their offspring. By this plan each bird is tested twice before being bred.

Note.—It is mutually understood that pullets are eligible for testing two months after flock has attained a 20 per cent egg production. Male buds may be used for breeders if tested once and found nonreacting.

CONTROL MEASURES

1. All breeding birds, male or female, to be leg-banded with bands furnished at cost by the Department of Veterinary Science and Animal Pathology of the Massachusetts Agricultural Experiment Station.

2. All birds showing a positive agglutination reaction to be removed from the breeding flock. Reacting birds to be disposed of if possible, but if found necessary to keep them as egg producers, to be kept under strict quarantine.

3. Male birds not to be housed during the breeding season with other than breeding stock.

4. New stock brought on the place, whether adults, day-old chicks, or hatching eggs, to come from stock shown to be free from bacillary white diarrhea as determined by the agglutination test.

Note.—The Department of Veterinary Science and Animal Pathology of the Massachusetts Agricultural Experiment Station does not issue certificates of any kind or enter any agreement to guarantee flocks. It will, however, cooperate with poultry associations or groups of poultrymen who may wish to issue certificates of merit or accredit their own flocks.

Testing Plan No. 2, which is a test on pullets, has been the most generally followed and appears to be the most satisfactory to the poultrymen.

Bransfield (1925) reports a rapid increase in the use of the agglutination test for the control of the disease in Massachusetts. His paper shows a marked decrease in the per cent of disease in the birds tested and an increase in number of disease-free flocks within the state from which it is possible for purchasers to obtain disease-free chicks and eggs. Taking an average of the 99 flocks tested by the Massachusetts Plan No. 2, it was found that the original infection was reduced more than 75 per cent. This plan offers the quickest method for cleaning up the infection, but since very few have followed it the author was unable to draw definite conclusions as to its value.

The value of Massachusetts Plan No. 2 is shown by the following: In 1919 a flock was tested which revealed 27.5 per cent of the birds in the breeding pen to be infected. Less than 15 per cent of the chicks from this pen matured. In 1920 the infection had dropped to 20 per cent, the following year to 6.5 per cent, and for the next year the flock was declared free from the disease. From the 1,110 tested birds 11,600 eggs were incubated, and of the 8,700 chicks hatched, 92.9 per cent were reared.

Tables II and III show the value of consecutive as opposed to nonconsecutive testing.

TABLE II.—Consecutive versus haphazard testing.

NUMBER OF FLOCKS TESTED.	First year.		Second year.		Third year.		Fourth year.		Reduction in infection.
	Number of birds tested.	Per cent infected.	Number of birds tested.	Per cent infected.	Number of birds tested.	Per cent infected.	Number of birds tested.	Per cent infected.	
Consecutive Testing Plan. Results satisfactory. (Gage and Flint.)									
13	4,380	11.8	5,503	10.0	6,131	4.3	2,890	0.4	*Per cent.* 11.8 to 0.4
Nonconsecutive Testing Plan. Results unsatisfactory.									
3	1,915	5.4	2,328	1.0	No testing.	No testing.	2,328	10.6	Increase in infection. 1.0 to 10.6

The results of testing the same flock over a series of years are shown in Table III.

TABLE III.—Average per cent reduction of infection in flocks tested consecutively for two or more years.

NUMBER OF YEARS TESTED.	Number of flocks.	Per cent of infection.				
		1919-'20.	1920-'21.	1921-'22.	1922-'23.	1923-'24.
5	8	22.51	8.11	9.50	3.59	2.06
4	10	.....	16.74	15.42	4.85	1.90
3	10	.....	.....	21.04	10.22	8.61
2	24	.....	.....	.....	13.25	5.51

Note.—This table illustrates two points concerning this test: (1) The value of a continuous testing program, and (2) the great difficulty of completely eradicating this disease once it is established.

Bushnell and Beaudette (1922) prepared a scheme for the rapid diagnosis of the more common poultry diseases. Since it is difficult to make a differential diagnosis of fowl cholera, fowl typhoid, and white diarrhea infections by post-mortem examination, a scheme for bacteriological testing is necessary. The following outline will illustrate a simple method which has been used successfully by these authors.

TABLE IV.—Outline of plan.

DISEASE.	Dextrose.		Lactose.		Maltose.		Saccharose.		Indol.
	a	g	a	g	a	g	a	g	For-ma-tion.
Fowl typhoid	+	—	—	—	+	—	—	—	—
Fowl cholera	+	—	—	—	—	—	+	—	+
Bacillary white diarrhea	+	+	—	—	?	?	—	—	—

Key to Symbols: +, positive; —, negative; ?, variable reaction. **By means of this scheme a definite diagnosis may be made in 48 hours.**

Hitchner (1923) reported on the presence of a fatty substance in the blood sera of fowl which affected the agglutination test. Tests set up with the serum of certain birds are obscured by a yellowish to white emulsion-like suspension that is of less specific gravity than is the test fluid and therefore floats on the surface. 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. He found that birds starved less than 48 hours were apt to show this phenom-

enon. But he also found that starving caused the birds to fall off in production about half the first week and they did not return to normal for over two weeks. He recommended starving the fowls for 36 hours before bleeding.

Gage (1922) reports on a study of 112 different strains of *Bact. pullorum* isolated from various places in Massachusetts. The fermentation tests showed that the organism fermented dextrose, galactose, mannose, mannite, levulose, xylose, and arabinose; that it was negative in glycerine, maltose, adonite, dulcite, lactose, dextrine, saccharose, inulin, erythrol, and raffinose. In salicin there was slight acidity in a large per cent of the strains. The aërogenic property of these strains is persistent. The cultures did not coagulate or peptonize milk; they formed H_2S in lead acetate medium; did not produce indol; and did not reduce nitrates. It was found by this author that in every test made, the *Bact. pullorum* immune serum agglutinated typhoid antigen better than typhoid serum agglutinates *Bact. pullorum* antigen. There has never been demonstrated any indication of an affinity of interagglutinability between *B. aviseptic*us and the pullorum and sanguinarium types.

Beaudette, Bushnell, and Payne (1923*a*) studied an organism from the unabsorbed yolk of chicks dead in the shell and found it very similar to *Bact. pullorum*. The chief difference from the latter organism was its ability to cause slight fermentation in maltose. This organism was very pathogenic for developing embryos, causing a mortality of 100 per cent in the inoculated eggs, compared to 16.6 per cent in the uninoculated eggs. Eggs inoculated with nonpathogenic bacteria and sterile salt solution did not cause an appreciable death rate in embryos. *B. paratyphoid A and B* caused death of all the inoculated embryos.

Beaudette, Bushnell, and Payne (1923*b*) reported on the relation of *Bact. pullorum* infection to the hatchability of eggs. It was found that there was a considerable difference between the fertility and hatchability of eggs from infected and noninfected hens. The losses in fertility and hatchability for 1922 were 19.05 per cent, and for 1923, 31.6 per cent.

Beaudette (1923*a*) reported on the use of a single-tube method in making agglutination tests for carriers of *Bact. pullorum*. The dilution in this case was approximately 1:80 and was obtained by using a single drop (1/20 to 1/25 of a ml.) of the serum in four mls. of a highly diluted antigen. A table is introduced in which the

author compares the results of his test with the serial dilution method commonly used.

Beaudette also (1923*b*) made a study of the agglutinins for *Bact. pullorum* in hens' eggs. It was found that the albumin of eggs from hens infected with *Bact. pullorum* contained agglutinins specific for this antigen in most cases. In these cases there was marked agglutination in albumin dilution of 1:32 and some mere complete in 1:64. The albumin from eggs of noninfected birds rarely showed agglutination in dilutions of 1:8.

Steiner (1924) in a popular circular on the disease, bacillary white diarrhea, recommends the use of bichloride of mercury, 7 grains; calcium sulphocarbolate, 15 grains; and sodium sulphocarbolate, 15 grains. Also bichloride of mercury, 15 grains to a gallon of sour milk, is recommended.

May (1924) conducted some experiments on the infection of eggs. The experiments carried out in his laboratory indicate that there is no increase of infection in the yolks of eggs laid by a hen undergoing artificial immunization or even suffering from infection with poultry pathogens. In no case were the organisms inoculated into hens obtained from the yolks of their eggs. It was found that all hens either fed or inoculated with living cultures of typical *Bact. pullorum* and autopsied showed an infection of the ovary by this organism. In each case it was recovered in pure culture. The blood serum was extremely variable in regard to germicidal power. In about one-half of the flocks there was no indication of any germicidal power while in the other half the serum in a 1:4 dilution was strongly germicidal. This variation seems to show some relationship to the cultures used. The albumin from eggs of normal hens showed only a slight inhibitory effect on the growth of this organism when it was tested in a 1:4 dilution. There was no definite correlation between agglutination titre of blood serum or egg albumin and germicidal power.

Jorgenson (1924) studied the influence of milk cultures of *B. acidpohilus* and immune serum on this disease. Two methods of procedure were used in the experiment, one to feed the exposed and infected chicks with milk cultured with *B. acidophilus,* checking the results with untreated controls. The other method was to feed infected chicks with immune rabbit serum. Twelve chicks clinically ill and six half-day old chicks from another flock free from bacillary white diarrhea were used in the experiment, *S. pullorum* cultures were isolated from the infected chicks' feces. Ten of the infected

chicks and four of the noninfected ones were fed ten mls. of the cultured milk every four hours, the chicks being fed individually with a clean pipette. Control pens of two well birds and two infected chicks were not fed the cultured milk. Oatmeal and water constituted the remainder of the ration and the birds were kept in a warm brooder house under as nearly normal conditions as possible.

Of the ten infected chicks fed *B. acidophilus* milk, 3 died. Of four noninfected chicks fed *B. acidophilus* milk, none died, but one developed a slight attack of the disease. Both infected chicks that were not fed *B. acidophilus* milk died. Both noninfected chicks not fed *B. acidophilus* milk developed the disease and died.

Ten healthy half-day old chicks were fed two five-ml. doses of the cultured milk and two hours later given 0.01 ml. of a virulent culture of *S. pullorum* each and placed in an infected brooder. They were given access to milk cultured with *B. acidophilus* and to oatmeal. Only one of the ten chicks died. Seven chicks ill with the disease and four that were exposed to it were given five-ml. doses of rabbit serum immune to *S. pullorum.* Five of the seven sick birds recovered. None of the exposed chicks died.

Reports from the field since *B. acidophilus* milk was tried in the experiment quoted seem to indicate that there is some therapeutic value to the cultured milk, according to the author.

Rice (1924) studied over 100 strains of the organism, *Bact. pullorum,* isolated from eggs, chicks, and adult birds and it was found that many strains failed to produce gas in dextrose, mannite, and levulose. Some failed even to produce acid and this ability to produce gas was not constant for a strain: "A nongas producer might subsequently become a gas producer and *vice versa.*"

Discussing the question of adult carriers, Rice says:

"Four chicks, all obviously ill, were received, of which two died and were found to have bacillary white diarrhea. The two survivors were reared to adult stage and became reactors. To answer the question more fully, a reacting cockerel (one of the above) and reacting hens, all natural cases, were mated. Bacillary white diarrhea appeared among the resulting chicks but the losses were small. The survivors, 23 cockerels and 24 pullets, were reared to an average of seven months without observation to ascertain if at any time they had passed through an obvious attack. The agglutination test was applied to the 47 birds at this age and only one cockerel and three pullets gave a clear reaction, while two pullets gave a very slight reaction. Thus, at the age of seven months, 88 per cent of the survivors were definitely noncarriers."

All the cockerels were kept together until they were about 10 months old. Fifteen cockerels which were noncarriers at seven

months old were again tested, when two gave a moderate reaction and two gave a very slight reaction. Eleven gave no reaction. These 15 cockerels were killed immediately and examined for *Bact. pullorum* with negative results. It may be concluded from the experiment that between the age of seven and ten months nonreacting birds may become partial reactors by contact with a reactor. The development of a partial reaction is evidence that infective material became available to them, directly or indirectly, from their reacting companions during the seventh to tenth months of life but that *Bact. pullorum* failed to establish itself in their bodies as evidenced by negative bacteriological examination. An alternative, but unlikely explanation, would be that the development of a partial reaction was the delayed result of infection of chicks.

Six strong reacting hens and one nonreacting hen were kept together for 17 months. The single nonreactor was still a nonreactor at the end of the period. Six birds which failed to pass the test in October, 1922, and 22 birds which failed to pass in October and November, 1923, were retested in January and February, 1924. Two birds in the first lot, or 40 per cent, and five birds in the second lot, or 23 per cent, gave not the slightest reaction and were killed and examined for *Bact. pullorum* with negative results. The important conclusion can be drawn from the experiment that a large proportion of positive reactors become negative reactors, even during a period of four months. The reactor is not, then, necessarily a reactor for life. Recovery can take place and the birds may be returned with safety to the breeding flock. This would explain the occurrence of slight mortality in the late as compared with the early hatches from the same breeding flock which contains reactors. Another explanation of the phenomenon would be that the carriers (hens; owing to degeneration of the ovaries would not continue to lay as many eggs as the noncarrier and thus fewer eggs from the carriers are set for hatching. During the investigations, 21 birds which gave completely negative reactions were killed immediately after testing and a bacteriological examination made. In no case did the 21 completely negative reactors yield *Bact. pullorum.* Twenty-six birds which gave an agglutination reaction of varying intensity were killed immediately after testing and examined for *Bact. pullorum.* Seventeen birds yielded *Bact. pullorum,* including two which gave only a partial reaction. Nine birds did not yield *Bact. pullorum,* including one bird which gave a strong reaction and one bird which gave a partial reaction.

The author concludes from the above experiments that a completely negative reaction, except in rare cases of recent infection, means that the bird is not a carrier, but that a reaction does not necessarily mean that the bird is a carrier, and further, that the intensity of the reaction does not indicate whether it is a carrier or a noncarrier. The position, then, is that if it is desired to eliminate all carriers with certainty from the breeding stock, all birds which give even slight reaction must be excluded, but such eliminations may be expensive in that noncarriers will be included in the discards.

Rice recommends that all tests should be incubated 15 hours before reading and states that fat in serum may be reduced by starvation prior to bleeding. He found that birds affected with fowl typhoid would give an agglutination reaction indistinguishable from that given by carriers of *Bact. pullorum.* The intradermal test was not satisfactory,

Post-mortem examinations may or may not reveal any abnormalities. Only one-half of the birds which yielded *Bact. pullorum* in recent investigations showed apparent changes in the ovary, the ova being, in varying degrees, angular and discolored with firm contents. *Bact. pullorum* was recovered indifferently from both apparently normal and from degenerated ova. It was found that the ovary of a carrier commonly showed both healthy and diseased ova. The organisms were recovered from both functioning and nonfunctioning ovaries.

Brunett (1924) reported a number of instances of diagnoses of bacillary white diarrhea made on chicks over two weeks of age. The infection in these chicks appeared to be either of a chronic type or the chicks were infected later in life than commonly occurs. The lesions in these chicks were pin-point abscesses scattered rather abundantly throughout the liver, heart, lungs, and gizzard. *Bact. pullorum* was isolated from the heart blood and abscesses in these cases.

Beaudette (1925*a*) published a review of the literature concerning bacillary white diarrhea of fowl. He concluded that an infected bird is one whose blood serum is capable of agglutinating the organism in dilution of 1:100, and he stated 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 fowl may retain the agglutinins, but not the infection, from having had the disease as chicks. He does not recommend testing young birds but considers that the breeders should be tested annually until the flock has been shown to be free on at least two consecutive tests.

Beaudette (1925 *b)* found several cases of fowl typhoid in chicks from one to five weeks of age. The post-mortem appearance of these cases was much the same as that of bacillary white diarrhea and the agglutination test, using blood serum from one of the flocks, gave results which would not differentiate the two diseases. This writer considered that the disease may be transmitted from the hen to the chick through the egg. The ovarian infection by this organism could not be differentiated from that of the organism of bacillary white diarrhea.

Kaupp and Dearstyne (1925) determined the limits of acid tolerance for the growth of *Bact. pullorum.* This was found to be between 0.6 and 0.7 per cent of lactic acid when tested in milk. These authors also discuss the value of milk and acid from a physiological standpoint.

Under the direction of Dr. M. A. Jull (1925), the United States Department of Agriculture, a plan of accreditation and certification was adopted at the National Conference held at Kansas State Agricultural College, Manhattan, Kan., August 10 and 11, 1925.

The general plan of the accreditation and certification work is divided into four parts: First, accreditation; second, accreditation combined with testing for bacillary white diarrhea; third, certification; and fourth, certification plus testing for bacillary white diarrhea. The organization of the plan is such that one phase of the work leads logically to the next and means that each succeeding step represents a definite effort to raise the standard and to improve the quality of breeding stock. Some such plan as this should and probably will come into general use.

Brunett (1925) made a study of *B. acidophilus* milk culture in the control of this disease. He found that, while such milk was an excellent food and prevented all disease, it could not be looked upon as a means of curing birds already infected. This would be true also of all intestinal antiseptics,

Krumwiede, Cooper, and Provost (1925) reported close absorption relations between *B. typhosus, Bact. pullorum,* and *Bact. sanguinarium.* They studied the relationships of these organisms carefully, especially in regard to the quantitative factor. Of special interest is the absorptive capacity of *B. typhosus* with the *Bact. pullorum* and *Bact. sanguianrium,* antisera, in spite of the fact that *B. typhosus* is only feebly agglutinated by these sera. The differences elicited between *Bact. sanguinarium* and *Bact. pullorum* are

very slight, so slight in fact, that they could be looked upon as merely reflecting the differences in agglutinability obtained with the unabsorbed sera. From this aspect one could say that an actual difference has not been demonstrated between two cultures of similar host origin which differ culturally and in the disease picture produced.

H. R. Baker (1925) reported a study on the reduction of this disease in flocks tested more than one year. Of four flocks tested two consecutive years the reactors were considerably reduced in numbers. Flock No. 1 reduced from 3.5 per cent to 0.3 per cent; flock No. 2, from 29.0 per cent to 7.69 per cent; flock No. 3, from 2.3 per cent to no reactors. The average number of reactors in 10 Delaware flocks was between 8 and 9 per cent.

A. H. Baker (1925) found that various antiseptics, as mercury biniodid and formalin, caused either hemolysis or prevented good separation of blood serum, when used for preservation of blood for agglutination tests. Boric acid in dilutions of 1:100 to 1:500 allowed good separation of serum and restricted growth of contaminating organisms for six to eight days, with no effect on the agglutination titre of the serum. The author recommends the use of 0.1 c. c. of a 5 per cent solution of boric acid in saline placed in a vial of 2.5 c. c. capacity. This when filled with blood gives a boric acid concentration of 1:500. This method has been in use for months and the author states that fowl blood will travel safely and permit satisfactory testing when so preserved.

NATURE OF SALMONELLA PULLORUM

The cause of the bacillary type of white diarrhea in fowls is a bacterium, *Salmonella pullorum.* This organism is capable of affecting both chicks and adult birds and is carried from generation to generation within the body of adult females and males. The organism belongs to the so-called colon-typhoid group of bacteria, and is commonly spoken of as belonging to the group of paratyphoids, since it produces gas from dextrose, but not from lactose. More recent investigators consider the term paracolon as being more appropriate for this group, since it is more closely related to the colon than to the typhoid group of bacteria in many of its characteristics.

An examination of 83 cultures from various sources in Kansas and elsewhere shows the following characteristics: A gram-negative rod with rounded ends, arranged singly, nonmotile, and without

spores or capsules. On an agar slant: moderate, filiform to slightly spreading, slightly raised, glistening, smooth, translucent, nonfluorescent. Growth in broth; moderate clouding, no surface growth, and slight granular sediment. The colonies on agar are small, raised, glistening, with smooth to slightly irregular surface. The edge is smooth to undulate. Litmus milk slowly changed to slightly acid and later to neutral or slightly alkaline in most cases. No coagulation or digestion of the casein takes place. Nitrates are reduced and H_2S and indol tests are negative. The organism grows best at 37 degrees C. and is a facultative anaërobe.

The fermentation of the various carbohydrates is shown in Table V.

TABLE V.—Fermentation of various carbohydrates by *Salmonella pullorum*.

	Acid.	Gas.	Remarks.
Monosaccharides:			
Dextrose	+	+	
Levulose	+	+	
Galactose	+	+	
Disaccharides:			
Maltose	±	±	Irregular.
Saccharose	—	—	
Lactose	—	—	
Trisaccharides:			
Raffinose	—	—	
Pentoses:			
Arabinose	+	+	
Xylose	+	+	
Polysaccharides:			
Dextrin	—	—	
Starch	—	—	
Inulin	—	—	
Triatomic Alcohols:			
Glycerol	?	—	Some cultures produce slight acidity.
Hexatomic Alcohols:			
Mannite	+	+	
Dulcitol	?	—	Irregular acid.
Sorbite	—	—	
Glucosides:			
Salicin	—	—	
Methyl Pentose:			
Rhamnose	+	+	

Note.—Tests closed after three weeks incubation at 37 degrees C.

The action of this organism on the monosaccharides agreed in most cases with the results reported by other writers on the subject. The action on maltose was variable. Fifty-four of the cultures did not act upon this carbohydrate while eight formed acid and gas from it. Of these one was from an adult bird, five were from

chicks, and two were obtained from other laboratories. Many of our cultures produced a distinct acid reaction from glycerine after three weeks incubation, while one otherwise typical culture produced a small amount of gas in three weeks. None of the remaining cultures produced gas even on prolonged incubation. Eleven of the cultures produced a slight amount of acid but no gas from dulcite. The acidity in this case was indicated by the use of bromthymol-blue. The acidity was first noted after about one week of incubation, and in some cultures not until after two weeks.

Fourteen per cent of the cultures of the writers failed to show gas after three weeks in any of the carbohydrates tested. These would correspond to the findings of Hadley and others (1917) Type B of this group of organisms. None of these organisms produced gas, but all produced acid in dextrose, levulose, galactose, mannite, arabinose, xylose, and rhamnose. Two produced acid in dulcite after one week and none produced acid in maltose in three weeks. In the presence of immune serum they could not be distinguished from the aërogenic type.

In speaking of these two types, Hadley et al. (1917) state:

"1. *Bact. pullorurn A:* possessing the chief characteristics presented by Rettger; a gas-forming type whose immune serum agglutinates *B. typhosus* about equally with its homologous antigen; pathogenic for young chickens only. 2. *Bact pullorum B:* resembling the type A in its chief characteristics but differing in that (1) it does not form gas in any carbohydrate; (2) its serum (like antifowl typhoid serum) does not agglutinate in high dilutions, human typhoid antigens; (3) it is able to produce natural generalized infections in adult fowls, but only to a slight degree, if at all, in young stock."

He also states that the A type is strictly dextrin-dulcite-negative with a terminal alkalinity in the cultures while the B type manifests a slightly delayed lessening of the initial acidity, sometimes after a slight increase; also a terminal alkalinity of a lower degree than in case of the A type.

The results of the writers agree with those reported by most other investigators except in nitrate reduction and xylose and glycerine fermentation. The writers found all cultures reduced nitrates to nitrites to some extent, and a few fermented xylose and maltose and glycerine. No attempt was made to classify the organisms or to make a comparative study with other organisms of this group.

A serological examination was made on these organisms using blood serum from two birds which showed natural infection. One bird was a male and the other a female. There was considerable

variation in sensitiveness of the different antigens to the action of these sera. The titre of the sera on those antigens which agglutinated best was 1:160. Twenty-one of the cultures agglutinated satisfactorily in dilutions of serum of 1:80 to 1:160. The remainder could not be considered as suitable for the preparation of antigens. This point is of considerable importance in practical field and laboratory work in the selection of an antigen to be used in routine tests. Many cultures which are culturally typical show poor antigenic qualities.

Antigens were also made from each culture and tested against the sera of several birds which had been immunized against killed cultures of this organism. Table VI shows the action of this serum upon its homologous antigen, and Table VII the per cent of cultures showing complete agglutination in various dilutions of this serum. This immunizing antigen contained five typical strains of these organisms, which were grown separately and pooled before injection into the birds. Three of these cultures were from chicks and two from hens which were found to be carriers. These cultures had been grown on agar for about one year before use.

TABLE VI.—Action of serum on homologous antigens.

DILUTION OF SERUM.	1:320	1:640	1:1280	1:2560	1:5120	Check.
Degree of agglutination (a).....	4	4	4	3	2	0

(a) The figure "4" represents complete agglutination; "0" represents no agglutination.

TABLE VII.—Per cent of cultures showing complete agglutination in various dilutions of immune fowl serum.

DILUTION OF SERUM.	1:320	1:640	1:1280	1:2560	1:5120	Check.
Per cent of cultures showing complete agglutination.....	97.8	95.1	71.2	43.6	7.4	0

Total number of cultures used, 83.

These results indicate great variations in the ability of different antigens to be agglutinated by the sera of the same immune birds. The lack of agreement in results from different laboratories on the same sample of serum is probably due largely to this discrepancy in reports of action on various antigens. The reasons for these variations are not known at present.

INCIDENCE OF THE DISEASE

It is impossible to determine the exact distribution of this disease at the present time. There has been no careful epizoölogical study of it, except in a very few states.

THE DISEASE IN ADULT FOWLS

There is considerable evidence that adult birds may become infected from other adults through contact. This point is difficult to prove, since the infection in some birds remains latent for long periods of time and some writers believe that birds recover from the infection. Such infection is, however, a possibility and should not be overlooked.

General Distribution of the Disease

Rettger, Kirkpatrick, and Stoneburn (1912) found that normal adult hens exposed in the same pen with infected hens likewise become infected after eight to 16 months. They also found that birds exposed to litter and feed contaminated with *S. pullorum* became infected.

Jones (1912) reported an acute outbreak of this disease in adult hens due to feeding infected eggs which failed to hatch,

Gage, Paige, and Hylan (1914) found that it was possible to produce ovarian infection in adult hens by the injection of pure cultures of *S. pullorum.* Some of these birds showed the diseased condition two years after the injection of the cultures.

Hadley *et al.* (1917, 1919) believe that there are two types of *S. pullorum* organism. One type infects adult birds more commonly than chicks. A generalized infection is produced in adult birds by this organism. The other type localizes in the ovary of the hens and affects chicks.

Rettger, Kirkpatrick, and Jones (1914) conducted extensive experiments to find whether or not chicks which survived an attack of bacillary white diarrhea became permanent carriers of the disease, They found that of 138 chicks which grew to maturity and lived until the termination of the experiment, 88 had been infected with cultures while quite young. The remaining 57 were not subjected to this treatment but served throughout the investigation as checks. Of the 88 that were infected as chicks, 21, or 23.9 per cent, gave a positive agglutination test when about a year old. On the other hand, of the 57 pullets which were not naturally infected, but one, or 1.7 per cent, gave a positive agglutination test and showed evidence of ovarian trouble. This bird was kept in a house close to

the infected birds and may have been infected later in the season. They consider that the carrier condition was established in fully 25 per cent of the infected flock. They reported later (1915) on an examination of 14,617 individuals by the agglutination test and found 10.24 per cent of the hens and 2.9 per cent of the males to be reactors. In 13 flocks subjected to a second test the infection varied from 0.6 to 25.7 per cent. In a later report (1916) they state that 0.3 per cent of the hens and 2.1 per cent of the males were found to react to the agglutination test. The per cent of infection in various flocks ranged from 0 to 56.3 per cent.

Gage and Paige (1915) found the infection in Massachusetts flocks to vary from 0 to 50 per cent.

Reports by Gage and Flint (1922, 1923, 1924) give the data on distribution of the disease in Massachusetts. Of 110 flocks examined during 1921 and 1922, consisting of 29,875 birds of several breeds, they found 27.5 per cent to be free from the disease. In 1922 and 1923 of 121 flocks examined, consisting of 33,602 birds of several breeds, they found 23.9 per cent to be free from the disease. The former showed 72.5 per cent and the latter 76.1 per cent of the flocks infected. These figures are very similar to those which were obtained in Kansas.

Bransfield (1925) from this same laboratory reported that in 74 flocks tested for two or more years the per cent of infection had dropped to 2.94 per cent, and of the 156 flocks tested for the first time, 79, or 50.6 per cent, were infected. This leaves 49.4 per cent of the flocks free from the disease. Gage and Flint (1924) reported on the results found by the initial tests on several flocks. Their results are given in Table VIII.

TABLE VIII.—Per cent of infection on initial tests.

Number of flocks tested.	Per cent of infection.
8	22.51
10	16.74
10	21.04
24	13.25

H. R. Baker (1925) in Delaware reports an average of between 8 and 9 per cent infection in 10 flocks tested.

Rettger, Kirkpatrick, and Card (1919) state that the spread of this disease in an adult flock may be slow or stationary or may

spread to involve 20 to 25 per cent of the entire flock in 12 to 15 months. They made the important observation that 25 per cent of the birds subjected to oviduct inoculation with pure cultures of the organism, later became permanent reactors and possessed infected ova. They suggested that the male may act in the passive transfer of the organisms and should be removed from the flock for the greater part of the year.

May (1924) found that all hens, either fed or inoculated with living cultures of typical *S. pullorum* and autopsied later showed an infection of the ovary by this organism. In each case it was recovered in pure culture.

Rice (1924) reared 47 chicks from reacting adults to an average of seven months of age. At this time four of the birds gave a clear reaction to the agglutination test. The remaining birds were negative. He concludes that, at the age of seven months, 88 per cent of the survivors were definitely noncarriers. This would leave 12 per cent as reactors. A few months later some of these birds were again reactors showing that they had become infected either directly or indirectly from their mates. Later work led him to believe that a reactor may not be a reactor for life, and that a positive reaction may or may not mean that the bird is a carrier.

The Disease in Kansas

In a test made on 74 flocks in Kansas in 1924 it was found that 78.3 per cent showed reactors and that 31.5 per cent of the total birds tested were found to be reactors. Table IX shows something of the prevalence of the disease in Kansas. The data included in this table were obtained by direct examination or by agglutination tests made in this laboratory. The increased number of infected flocks found does not represent entirely a spread of the disease to new territory but merely the fact that there is a greater recognition of the value of having a diagnosis made.

TABLE IX.—Incidence of the disease in Kansas.

YEAR.	Number of flocks infected.	Number of counties represented.
1919-'20	6	———
1920-'21	12	———
1921-'22	8	Testing started spring of 1922.
1922-'23	31	———
1923-'24	102	25
1924-'25	153	41

During the past four years the writers have examined and tested blood from 227 flocks in 71 counties in Kansas. Among the 8,220 birds so examined 2,891, or 35.1 per cent, were found to be infected with this disease. The accompanying map (fig. 1) shows the distribution of bacillary white diarrhea in Kansas, as determined from the agglutination test reports and autopsy records of this laboratory from April 1, 1921, to July 1, 1925.

As a rough estimate it is probable that about 75 per cent of the flocks and 25 per cent of the birds in infected areas are carriers of

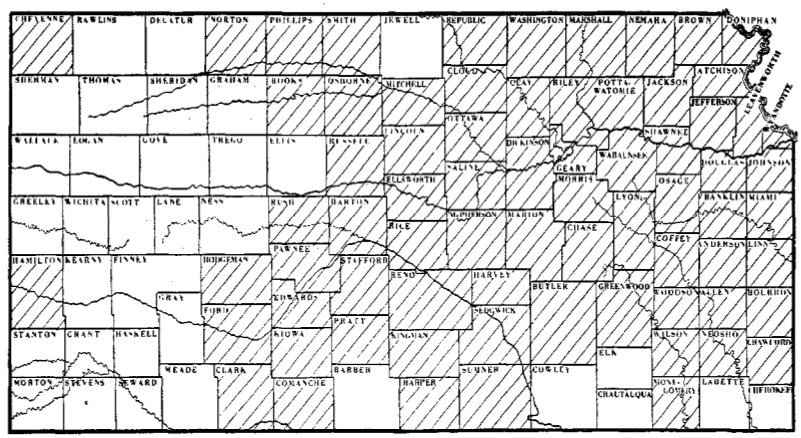

FIG. 1.—Map of Kansas showing the distribution of bacillary white diarrhea.

this disease. Estimating the poultry population of Kansas at 16,000,000 birds and the number of carriers on the basis of the data given above, it may be estimated that there are approximately 3,000,000 carriers in the state. This nucleus of infection is sufficient to keep the disease alive for some time to come unless it is eradicated.

THE DISEASE IN CHICKS

Rettger (1901) reported an epizoötic of bacillary white diarrhea among young chicks in which the losses were 80 per cent of the chicks involved. Scherago and Benson (1919) report losses of 90 per cent of the incubated chicks in one outbreak. Bransfield (1925) discusses the value of the agglutination test in reducing chick losses. In 1919, a flock of birds was tested which revealed 27.5 per cent of the hens to be infected and less than 15 per cent of the chicks from this pen matured. After three years testing this flock was declared

to be free from bacillary white diarrhea. Of the 8,700 chicks hatched, 92.9 per cent were reared.

Termohlen (1925) reported on a flock in which the losses were 652 per 1,000 chicks incubated. Canfield (1925) considers that the livability of chicks from infected hens is 22.4 per cent and from noninfected hens, 94.0 per cent.

The exact loss due to this disease will never be known, due to the fact that there are so many complicating factors. Unfortunately other things will kill chicks. Under this heading may be included: (1) Filthy runs, (2) lice and mites, (3) improper ventilation, (4) dampness, (5) lack of sunlight, (6) overcrowding, (7) chilling, (8) overheating, (9) impure water, (10) spoiled feed, (11) inadequate diet, (12) suffocation, (13) other infections, etc. Many of these cause a white diarrhea. The droppings from chicks a few days of age will always be white since they contain nothing but the urates from the kidneys. In most of the deaths due to the above causes there will also be found an unabsorbed yolk and ochre-colored liver so characteristic of the infectious type of diarrhea.

To make a comparative study of the symptoms and lesions of some of the miscellaneous chick diseases, 60 half-day-old chicks were secured and divided into six groups as follows:

Lot 1—10 chicks—controls.

Lot 2—10 chicks—infected by subcutaneous injections of 0.5 ml. of a pooled 24-hour broth culture of *S. pullorum.*

Lot 3—10 chicks—infected as above using 0.5 ml. of a 24-hour broth culture of *E. sanguinarium.*

Lot 4—10 chicks—infected as above, using 0.5 ml. of a 24-hour broth culture of *P. avicida.*

Lot 5—10 chicks—chilled by placing in a refrigerator having a temperature of about 40 degrees F., for 10 minutes.

Lot 6—10 chicks—overheated by placing in an oven having a temperature of about 115 degrees F., for 15 minutes, two times at intervals of four hours.

A summary of the mortality in these groups is given in Table X. The experiment was terminated after 10 days, the majority of the deaths occurring from the fourth to the seventh day.

TABLE X.—Summary of results of a comparative study of some miscellaneous chick diseases.

Lot No.	1	2	3	4	5	6
HISTORY.	Controls.	Bacillary white diarrhea.	Fowl typhoid.	Fowl cholera.	Chilled.	Overheated.
Total dying	0	7	6	5	10	8
Per cent dying	0	70	60	50	100	80

The autopsy results were checked with a bacteriological examination and the respective organisms isolated from the lots which were inoculated. The symptoms seen in all these lots were identical. Lesions were similar except in case of the chicks infected with *P. avicida.* In these chicks areas of congestion simulating lesions of fowl cholera in adult birds were noted. Also, in these chicks, the ochre-colored liver was not as predominant a feature as in the other lots.

Unabsorbed yolks were observed in all cases, and a yellow or ochre-colored liver was seen in all but a few cases. In the bacillary white diarrhea cases, a general, icteric condition of all the organs was more noticeable than in the other cases. In chicks which did not die before a diarrhea was noticed the droppings were always of a whitish color.

A second and similar experiment yielded approximately the same results.

From this it may be seen that there is great confusion in the diagnosis of chick diseases. It should be emphasized, also, that great care must be used in making a correct diagnosis. Outside the laboratory this is almost impossible.

SYMPTOMS AND PATHOLOGY OF THE DISEASE

While there is so much confusion at the present time concerning losses of chicks, there are certain characteristics which may be used to designate the bacillary type of white diarrhea. These may be described somewhat in detail as follows:

The chicks appear stupid and remain under the hover or hen most of the time. They may remain by themselves and peep constantly, or utter a short cry of pain when attempting to void the excrement. The feathers become rough and the wings droop. They eat little and appear to be unable to pick up food. The characteristic whitish discharge from the vent soon makes its appearance,

although in very acute cases the chicKs die before showing signs of diarrhea. The discharge may be creamy white or mixed with brown. It appears to be more nearly white in very young chicks. In many cases this clings to the down in sufficient quantity to cause occlusion of the vent, This condition is known to poultrymen as "pasting up hehind." Frequently the chicks become "short-backed" and usually "big-bellied." The abdomen may protrude to the rear so that it bunches out behind, out of line with the vent. In some cases the chicks die without warning and show few symptoms. In other cases they will live for a long time and show all the above mentioned symptoms. On post-mortem examination the liver is often found to be of yellow (ochre) color and the yolk of the egg is unabsorbed in most cases. The crop is usually empty of food, and the intestines are pale and empty while the ceca are filled with grayish material which is usually soft but may be of cheesy consistency similar to that seen in coccidiosis.

The unabsorbed yolk will vary from the size of a grain of wheat to a nearly full-sized yolk. It may vary in color from yellowish to green and the contents may be fluid or cheesy. This material does not possess a putrid odor.

The chick as a whole appears very much emaciated and the muscles are icteric. The other organs of the body are normal in appearance and texture but are usually icteric.

In the adult hen the infection is usually localized in the ovaries, but may be found localized in other parts of the body, within or outside of cysts, or it may be generalized. In the ovary of the hen the infection is characterized by the presence of angular, firm, discolored ovules varying greatly in size. The number of ovules infected will vary in different individuals. These may rupture and be followed by generalized peritonitis, or a constriction of the oviduct and cyst formation. In some cases the intestine is so bound together by adhesions that it is nearly impossible to dissect it out.

Occasionally one will see abscesses on the skin of the body and legs from which pure cultures of *S. pullorum* can be isolated. One such bird was recently brought to this laboratory. Several abscesses which were tumor-like in structure were found on the skin of the posterior part of the abdomen, and on the legs. The legs had the appearance of a serve outbreak of scaly leg. The ovary of this bird showed typical lesions of *S. pullorum* infection and pure cultures were isolated from the abscesses on the abdomen and legs, and from the ovary. The blood serum of this bird caused complete agglutination

of *S. pullorum* antigen in 1:20, a partial agglutination in 1:40, and no agglutination in 1:80 dilution of the serum.

Brunett (1924) found this disease in chicks of two weeks of age. The chief lesions were small abscesses in the liver, heart, lungs, and gizzard, while Beaudette (1925) found ovarian lesions typical of bacillary white diarrhea from which he isolated *E. sanguinarium.* At this station one case is on record in which a pure culture of *P. avicida* was recovered from the ovary of a bird which showed no outward symptoms of the disease. The owner of the flock from which this bird came keeps trapnest records and reports that it had been a good layer until about five months before it was sent to the laboratory. It then quit laying and developed male characteristics. On post-mortem examination, the ovary showed typical lesions of those seen in *S. pullorum* infection. Pure cultures of *P. avicida* were isolated from several of the misshapened ova. The bird was a negative reactor to the agglutination test a few months previous to the autopsy and again at the time of autopsy. No acute or chronic outbreak of fowl cholera has been reported from the flock from which this bird came, for at least three years.

SOURCES AND MODES OF INFECTION

The control of an infectious disease rests largely upon the knowledge of the source and modes of transmission of the infectious material. Rettger (1900) determined these facts for bacillary white diarrhea in chicks in 1900. Since that time virtually nothing new has been developed in relation to this disease except that the agglutination test has been applied to the detection of carriers of the infection. Rettger and Stoneburn (1911) found that the disease followed a definite cycle. Certain infected chicks do not die but become adults and still harbor the organisms in the ovaries. The eggs from these hens may be infected and infect the embryo and the chick in the shell. Many of the infected embryos die in the shell. Some chicks die because they are unable to get out of the shell, some die shortly after hatching, and some live to become adult carriers. When the infected chicks are placed in contact with healthy chicks, they may transmit the infection to the normal chicks through the fecal material.

With this information in mind it is an easy matter to control this disease in a flock. The method of control consists of three steps:

1. All birds should be tested by means of the agglutination test.
2. All reactors should be removed immediately.

3. Strict sanitary precautions should be followed. The above points are outlined more in detail below. The accompanying scheme (fig. 2) illustrating the distribution and elimination of the disease is introduced in order to show the complexity of the problem and a simple method for its solution.

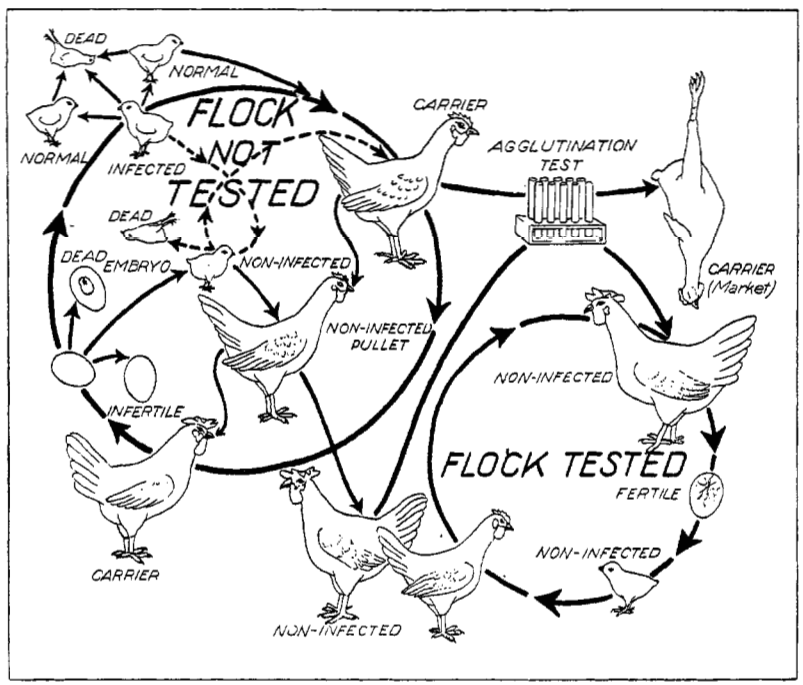

FIG. 2.—Chart showing the possibilities of the agglutination test in controlling bacillary white diarrhea in poultry flocks.

The agglutination test will be discussed in detail later but it should be mentioned in this connection. This test can be successfully made only in a laboratory and by one trained in laboratory manipulation. It is also the only test which has been devised to date which can be used for the detection of the disease in the living bird. While this test is not infallible, it is highly successful in the hands of a competent worker.

The removal of the reactors from the flock appears to be the greatest stumbling-block to the success of the above outlined procedure. When promising birds are found to be carriers, the owners object to removing them from the flock. Of course, this may work a hardship in case of valuable birds, but it is necessary to the success of disease

eradication. A bird valued at $25 is as great a menace to the health of the flock as a bird valued at $1. With the ordinary farm flock, birds are almost as valuable on the market as they are for breeding purposes.

It has been found that the carriers are often poor producers. Their eggs may be infertile, and the embryos may die in the shell as well as after hatching into chicks. When everything is taken into consideration, it is far better to cull all reactors closely. The leaving of only one bird in a flock of one thousand will make a focus of infection and again start the disease.

An illustration of the influence of a few carriers upon the dissemination of the disease is shown by the following case: The owner of a flock of disease-free birds (as shown by the test) introduced some untested birds from the outside. The eggs from these birds were placed in a large incubator with eggs from nonreactors and hatched. For the first three hatches there were heavy losses and the flock was again tested and the two reactors located and removed from the flock. The next hatch showed considerable loss probably due to contaminated incubator and brooders. After this the losses stopped for the next four hatches from this flock.

These two reactors were brought to the laboratory, killed, and autopsied. The titre limit of the serum was determined for both. It is given in Table XI.

TABLE XI.—Titre of serum of birds 29 and 33.

Dilution of serum.	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control.
Bird 29.....	4	3	2	2	0	0	0	0	0
Bird 33.....	4	4	4	4	4	4	3	2	0

The ovary of bird No. 29 appeared atrophied. The entire organ was removed to broth and the *S. pullorum* isolated in pure culture. The other organs of the body appeared normal.

Bird No. 33 showed typical lesions of the disease in the ovary and a pure culture of *S. pullorum* was isolated. Sixty dead chicks from this flock were brought to the laboratory. From 42 of these *S. pullorum* was obtained in pure culture. These included eleven from hen No. 33 and one from hen No. 29. Unfortunately but a small number of the chicks which died during the period of trouble, or later, were secured, so the exact losses due to this infection could not be checked. The per cent of loss for one of these hatches was 15.5 per cent and

for another 28.2 per cent, while subsequent losses for four hatches (after the reactors were removed) averaged 7.6 per cent.

From the above it may be said that 12 of the chicks which were delivered to the laboratory died of direct infection from the hens while the remainder (30 in number) died from contact infection. These two birds constituted but 0.8 per cent of the total hens in the flock, showing the folly of allowing even a very small per cent of reactors to remain. Even low-titre reactors may be a source of danger.

It has been suggested that a flock may contain two per cent reactors without serious results. To follow such a suggestion will merely propagate this disease among the chicken population of this country as long as chickens are raised. The damage which would result from such practice will depend upon several factors, some of which are listed as follows:

1. Sanitary care used in flock control.
2. Resistance of the birds to infection.
3. Virulence of the organism present.
4. Localization of the disease in the body of the carrier.

Most of these points are so obvious as to need no discussion. It may be said that the greater the sanitary care the fewer the number of organisms which will be passed from one bird to another. In the case of chicks the skill used in brooding and feeding will, in many cases, determine the success of the hatch. Some breeders manage infected flocks and have practically no trouble when they care for their own chicks, but chicks from the same hatch sent to other localities and other owners die in large numbers. For this reason the probable losses from an infected flock can only be estimated.

Organisms vary greatly in virulence, or their ability to combat the natural protective forces of the body, The greater the virulence the smaller the number of organisms necessary to set up an infection, and *vice versa.*

The point at which the disease localizes in an adult will determine the per cent of infection of chicks to be expected from this individual. It has been found that a relatively small number of eggs contain the organisms though the hen may be a strong reactor to the agglutination test. Table XII by Gage *et al.* (1914) is a good illustration of this point.

TABLE XII.—Irregularity of egg infection by *S. pullorum* in reacting hens.

HEN NO.	Number of eggs laid before *S. pullora* was detected.	Laying period in days.	Per cent of eggs infected.
1	11	20	9.1
2	8	16	12.5
3	5	6	20.0
4	12	20	8.3
5	7	16	14.3
6	7	10	14.3
7	8	15	12.5
8	5	8	20.0
9	4	12	25.0
10	21	39	4.8
11	13	38	7.7
12	21	33	4.8
13	11	24	9.1
14	19	58	5.3
15	12	61	8.3

In discussing this point Gage *et al.* state that they agree with Rettger and Stoneburn (1911), that the diagnosis of this disease by testing the egg is impractical. In some instances, however, the egg testing has given results with the examination of the first few eggs. Gage found that if a bird is badly infected, persistence in egg testing will usually yield a positive result. Of the 619 eggs tested from hens during July, August, and the first part of September, 32 were found to contain the organism. Of these birds, Nos. 8, 9, and 15 had been detected as carriers by egg examination before the experiment started and the remainder had been inoculated with a virulent culture some time before, so all were known to be infected.

In spite of the fact that the infection of the egg by carriers is so irregular, they usually produce enough infected chicks to infect many others in the brood soon after hatching.

Fortunately for the health of the uninfected chicks, the hatchability of the eggs from infected birds is quite low in many cases. This fact prevents a large number of infected chicks which might later become carriers, from hatching. The age of the chick is of some importance in the dissemination of this disease. This is well shown by an experiment made by Rettger, Kirkpatrick, and Stoneburn

(1912). The chicks in this experiment were of White Leghorn stock free from bacillary white diarrhea infection, and were strong and vigorous. The various lots contained 26 chicks each. Mortality observations were obtained for a period of about four weeks. The results are shown in Table XIII.

TABLE XIII.—Comparative susceptibility to bacillary white diarrhea of chicks infected at different ages.

PEN NO.	Description.	Per cent of mortality.
1	Check (not infected)	11.50
2	Infected when 36 hours old	33.50
3	Infected when 60 hours old	23.33
4	Infected when 84 hours old	19.25
5	Infected when 108 hours old	7.70

In Table XIV these writers give the influence of a similar infection upon a group of chicks which had been hatched from a flock of birds lightly infected with bacillary white diarrhea and showing a low vitality. These chicks were given an artificial infection of the organism of bacillary white diarrhea through the drinking water.

TABLE XIV.—Comparative susceptibility to bacillary white diarrhea of chicks infected at different ages.

PEN NO.	Description.	Per cent of mortality.
1	Check (not infected)	16.66
2	Infected when 24 hours old	72.00
3	Infected when 48 hours old	38.00
4	Infected when 72 hours old	30.00
5	Infected when 96 hours old	20.00
6	Infected when 120 hours old	16.00

From their work these writers conclude that "this infection with serious consequences to the chicks seldom, if indeed at all, takes place after the third or fourth day." The fact is also emphasized that the survivors in the infected pens lagged behind the chicks in uninfected pens. This agrees with the findings of Jones (1910) who found that chicks were most susceptible during the first 24 hours of life.

THE INCUBATOR AS A SOURCE OF INFECTION

A large number of inquiries concerning incubators as a possible means of spreading bacillary white diarrhea, led the writers to study this problem. The complete report of the experiments on such dissemination has been published (Hinshaw, Upp, and Moore, 1926). A few observations and the preliminary results of this study, however, are presented herewith.

In a study of the bacteriology of various types of incubators, it was found that the bacterial content of the air in the forced-air-draft type of incubator increased very markedly at hatching time. This increase is due to two fecal types of bacteria that disappear upon cleaning and disinfection of the incubator and reappear at hatching time. These facts pointed to a possibility of a similar spread of *S. pullorum,* if infected chicks were present.

To prove that infectious material can be spread throughout the incubator, sterile chick down was saturated with a 24-hour culture of *S. pullorum,* and then air dried. This dried infected down was placed in a compartment of a forced-air-draft incubator just as the chicks were starting to hatch. Seventy-five controls were removed before the down was placed in the incubator and the remainder of the chicks, which were just hatching, left in the incubator for 38 hours. A total of 117 chicks hatched and were placed in a cleaned and disinfected room which had never been used for the brooding of chicks. None of the controls died from bacillary white diarrhea while 33, or 28.2 per cent, of the exposed chicks died from this disease.

In three other experiments, chicks were infected by saturating their bodies with *S. pullorum* culture as soon as the eggs were pipped, or immediately after hatching. These infected chicks were used to supply a source of infected down and normal chicks exposed to these in each compartment of the incubator. The summary of the results of these experiments is given in Table XV.

In the three experiments 316 chicks were under observation; 151 of these were either artificially infected or exposed to chicks artificially infected in the same compartment of the incubator; 140 were exposed to the artificially infected chicks but in the opposite end of the incubator. The 25 controls were not exposed, and were hatched in a cleaned and disinfected machine. Higher per cent mortality was observed in chicks exposed in the infected compartment of the machine than in the chicks exposed in the noninfected compartment. This was also true in the experiment where

chicks were exposed to infected down. Therefore, it seems that there is greater chance of incubator dissemination where normal chicks are in close quarters with the infected ones.

TABLE XV.—Summary of results obtained in transmission of bacillary white diarrhea in incubators.

TREATMENT.	Total number of chicks.	Total dying.		Total dying from bacillary white diarrnea.		Total dying from other causes.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Chicks hatching in infected compartment of incubator	151	102	67.55	90	59.6	12	7.9
Chicks hatching in noninfected compartment but exposed to infected compartment	140	43	30.71	38	27.14	5	3.6
Controls	25	7	28.0	00	00	7	28.0

The results given in this article suggests a source of bacillary white diarrhea dissemination not reported previously. However, since small numbers are involved, the experiments should be repeated, using larger numbers of chicks and better controls. Chicks from naturally infected eggs should also be used.

In these preliminary experiments it was impossible to get more than one incubator with which to work, and the only eggs which were available were some which were being hatched for determination of hatchability. All of these were from birds which were negative to the agglutination test for bacillary white diarrhea, and records of hatchability and livability were available on 3,505 eggs incubated from this flock during a period of six months previous to the time these studies were started. The livability of the 2,438 chicks hatched was over 90 per cent. No outbreaks of bacillary white diarrhea had occurred in this flock.

In all the experiments reported above, 529 chicks were used and 111 (20.98 per cent) of these were controls. Only one control chick died with bacillary white diarrhea, and this occurred in the first experiment to determine if chicks could be infected through the nostrils. In this case, the infected chicks were separated only by a loose board partition, and infection could easily be carried by the attendant, by mice, or by infected down carried by the air. In contrast to this one (0.9 of 1 per cent) which died of bacillary white diarrhea in the controls, 170 or 40.68 per cent, of the exposed chicks

died with the disease. In all of these cases the diagnosis was confirmed by bacteriological examination.

The period of incubation of the disease is from 43 to 72 hours. By a study of results summarized in the experiment where the infection depended entirely upon the chicks getting some of the down or other infective material, it was found that it was four days before the first death occurred in the infected compartment of the machine, and on the ninth day the maximum number of deaths was recorded. After 22 days no more deaths occurred and the chicks appeared normal. With the exception of one instance, no losses from bacillary white diarrhea were experienced in any experiment after the fourteenth day from the date of exposure. In this case the chick died on the twenty-second day.

In a study of the autopsy records, it was found that it takes about 24 hours longer to produce infection in chicks exposed to infective material which is in circulation in the incubator, than in chicks contaminated by saturation of their bodies with *S. pullorum.* Also, chicks infected by saturating their bodies before they break from the shells, die sooner than those treated from 12 to 24 hours after they are out of the shell. The question of how the disease is produced after such infection is a problem which still remains to be solved. Probably, when the culture is injected around the chick in the shell, some of it reaches the digestive tract through the mouth or the allantoic circulation. This may account for the earlier apex of mortality in these chicks than in those infected in the same manner after they are hatched. The infected down may reach either the digestive tract or the respiratory tract through the mouth or nostrils.

The question of whether the chick down, pieces of shell, and droppings from naturally infected chicks are a similar source of dissemination yet remains to be settled. There is no doubt but that fecal organisms spread throughout the incubators of the forced-air-draft type; and since it has been proved that the infected droppings are one of the chief sources of infection, it is logical to assume that *S. pullorum* can be transmitted throughout the machine by the air current. This may also be true with other types of machines. It is planned to report on this phase of the problem later.

One hatcheryman, coöperating with the writers, says that he has seen typical examples of just such infection, but no definite data are available to prove it. One field example has been reported

through the kindness of Mr. George Robertson, assistant Dominion poultry husbandman, Canadian Experimental Farms, Ottawa, Canada. He reports as follows:

"In one instance, we had eggs from a breeder whose flock we afterward discovered was badly infected with bacillary white diarrhea. I will not give you the results of the whole machine, but simply the results of a line of exhibition Leghorns which we were hatching . . . at the same time.

"The two hatches immediately preceding the hatch referred to gave a chick mortality of 2 per cent. The hatch immediately following the hatch referred to, gave 100 per cent viability. In the infected hatch the mortality was 85 per cent. In this connection I might point out that the chicks did not come in contact at all. All the chicks were in wire pedigree baskets and were in separate drawers from the eggs from the infected flock. I might say that the flock from which these White Leghorn eggs came was free from pullorum. We got pullorum from the chicks of the infected flock taken from the machine, and also from unhatched eggs from the infected flock which were in the machine."

The writers are not ready to make the assertion that bacillary white diarrhea can be spread in incubators naturally infected, but believe hatcherymen should be careful to prevent such dissemination by using hatching eggs from tested flocks only. The custom hatchery problem is one which needs more attention, and every precaution should be taken to insure the owners of custom-hatched chicks that their chicks are not being exposed to infection in the incubator. This can be done (1) by insisting that clients buy eggs from tested flocks only, and (2) by carefully cleaning and disinfecting incubators after each hatch. Custom eggs should not be hatched with eggs intended for hatching commercial chicks, unless it is known that such eggs come from tested flocks.

INFLUENCE OF THE DISEASE ON FERTILITY AND HATCHABILITY

The influence of the disease on fertility and hatchability is of considerable importance to the commercial hatcheryman. In many cases there is a marked reduction in fertility and hatchability between the eggs of reacting and nonreacting birds. This difference is shown in Table XVI.

TABLE XVI.—Hatchability and fertility of eggs from reacting and nonreacting hens for one year.

	Number of hens.	Eggs set.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent total set hatched.
Infected.....	19	387	166	221	57.1	121	100	45.2	25.8
Noninfected...	164	5,066	483	4,583	90.4	1,673	2,910	63.4	57.3
Total....	183	5,453	649	4,804	88.0	1,794	3,010	62.6	55.2

This table shows that 18.2 per cent more of the fertile eggs hatched from nonreacting hens than from the reacting ones. It will also be noted that the fertility of eggs from nonreacting hens was 33.3 per cent more than those from reacting. The difference in hatchability of fertile eggs from reacting and nonreacting hens was greater in this flock for 1923 than for 1922. Yet the removal of reacting birds would have only increased the hatchability of fertile eggs for the entire flock by 0.8 per cent. Of course, these results will vary in every flock, depending upon the per cent of infected fowl and the per cent of their eggs used for hatching. The individual hatching records of the infected fowl are shown in Table XVII.

From Table XVII it may be noted:

1. That some reacting birds do not lay eggs at all, or lay very few, (See birds 2, 7, 9, 11, 14 and 17.)

2. That some reacting birds continually lay eggs which are infertile. (See birds 3, 8, 12, and 19.)

3. Although some reacting birds lay fertile eggs, many fail to hatch. (See birds 4, 7, 17 and 18.)

4. Although some birds are reactors they lay a normal number of fertile eggs which hatch well. (See bird 15.)

The fact should again be emphasized that the loss from this disease will depend upon several factors, all of which are not easily-recognized. In the first place the per cent of fertility and hatchability of the flock will depend upon the ratio between the number of re-

TABLE XVII.—Individual hatching records of infected birds.

HEN NO.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
1	28	8	20	71.4	9	11	55.0	39.3
2	0	.....	.....	.....	.....	.....	.....	.....
3	25	22	3	12.0	0	3	100.0	12.0
4	28	4	24	85.7	15	9	37.5	32.1
5	22	11	11	50.0	6	5	45.5	22.7
6	35	0	35	100.0	18	17	48.6	48.6
7	3	0	3	100.0	2	1	33.3	33.3
8	37	35	2	5.4	0	2	100.0	5.4
9	6	3	3	50.0	3	0	0.0	0.0
10	33	12	21	63.6	14	7	33.3	21.2
11	0	.....	.....	.....	.....	.....	.....	.....
12	25	18	7	28.0	5	2	28.6	8.0
13	13	0	13	100.0	7	6	46.2	46.2
14	7	7	0	0.0	0	0	0.0	0.0
15	34	1	33	97.1	12	21	63.6	61.8
16	27	6	21	77.8	12	9	42.8	33.3
17	8	2	6	75.0	5	1	16.7	12.5
18	29	15	14	48.3	11	3	21.4	10.3
19	27	22	5	18.5	2	3	60.0	11.1
Total	387	166	221	57.0	121	100	45.2	25.8

actors and nonreactors in the flock. The removal of 5 per cent of the total flock as reactors will have much less effect upon subsequent results than the removal of 50 per cent. The care which the flock receives, the virulence of the organisms, the resistance of the birds to infection, and the location of the disease lesions in the hens will all influence the relation of this disease to the success of handling the flock.

Tables XVIII to XXVII will show a more detailed record of the hatchability of eggs from birds known to be reactors and known not to be reactors. These records are included to show the effect of the disease upon fertility and hatchability over a period of years. The reacting birds in this case were all of good vigor and high productivity. Thus they are not a good group to select for comparison, because they are probably far above the average reactors in most respects.

TABLE XVIII.—Record of birds known to be infected with *S. pullorum*.

HEN NO.	Year.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.	Eggs examined.	*S. pullorum* found.	Titre of serum.
1	1920	61	8	53	86.9	50	3	5.6	4.9	2.0	0	1:160
	1921	44	16	28	63.6	12	16	57.1	36.4	.....	.....	
	1922	34	2	32	94.1	5	27	84.4	79.4	.....	.....	
	1923	25	22	3	12.0	0	3	100.0	12.0	.....	.....	
2	1919	61	10	51	83.6	44	7	13.7	11.4	2	1	1:160
	1920	35	0	35	100.0	28	7	20.0	20.0	.....	.....	
	1921	34	11	23	67.6	18	5	21.7	14.7	.....	.....	
	1922	28	6	22	78.6	15	7	31.8	25.0	.....	.....	
3	1920	61	6	55	90.1	44	11	20.0	18.0	1	0	1:80
	1921	48	40	8	16.6	3	5	62.5	10.4	.....	.....	
	1922	22	3	19	86.4	13	6	31.6	27.3	.....	.....	
4	1920	63	0	63	100.0	47	16	25.4	25.4	4	3	1:80
	1921	45	10	35	77.8	12	23	65.7	51.1	.....	.....	
	1922	33	1	32	97.0	3	29	90.6	87.9	.....	.....	
	1923	28	8	20	71.4	9	11	55.0	39.3	.....	.....	
5	1920	67	18	49	73 0	37	12	24.4	17.9	2	0	1:40
	1921	47	34	13	27.7	10	3	23.1	6.4	.....	.....	
	1922	32	29	3	9.4	1	2	66.7	6.2	.....	.....	
	1923	29	15	14	48.2	11	3	21.4	10.3	.....	.....	
6	1920	65	40	25	38.4	16	9	36.0	13.8	1	1	1:40
	1921	55	42	13	23.6	8	5	38.4	9.1	.....	.....	
	1922	31	31	0	0.0	0	0	0.0	0.0	.....	.....	
7	1919	67	9	58	86.6	43	15	25.8	22.3	0	0	1:40
	1920	56	3	53	94.6	42	11	20.7	19.6	.....	.....	
	1921	21	5	16	76.2	12	4	25.0	19.0	.....	.....	
	1922	1	0	1	100.0	1	0	0.0	0.0	.....	.....	
Total or av.	.....	1,093	369	724	66.2	484	240	33.1	21.9	.....	.....	.....

TABLE XIX.—Records of hens known to be free of *S. pullorum* infection.

HEN No.	Year.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
8	1920	59	7	52	88.1	33	19	36.5	32.2
	1921	45	25	20	44.4	8	12	60.0	26.7
	1922	26	6	20	76.9	14	6	30.0	23.1
9	1920	74	45	29	39.2	23	6	20.7	8.1
	1921	41	17	24	58.5	18	6	25.0	14.6
	1922	54	25	29	53.7	17	12	41.4	22.2
	1923	39	32	7	17.9	5	2	28.6	5.1
10	1921	50	29	21	42.0	3	18	85.7	36.0
	1922	24	0	24	100.0	7	17	70.8	70.8
	1923	32	16	16	50.0	14	2	12.5	6.3
11	1922	35	4	31	88.6	7	24	77.4	68.6
	1923	34	2	32	94.1	10	22	68.7	64.7
	1924	2	0	2	100.0	2	0	0.0	0.0
12	1922	34	0	34	100.0	2	32	94.1	94.1
	1923	30	0	30	100.0	11	19	63.3	63.3
	1924	29	0	29	100.0	3	26	89.6	89.6
13	1922	37	1	36	97.3	2	34	94.4	91.9
	1923	25	2	23	92.0	9	14	60.9	56.0
	1924	29	26	3	10.3	1	2	66.6	6.8
14	1922	40	0	40	100.0	15	25	62.5	62.5
	1923	39	20	19	48.7	7	12	63.2	30.8
	1924	25	1	24	96.0	9	15	62.5	60.0
Total or av....		803	258	545	67.8	220	325	59.7	40.5

In Table XVIII is included records from seven carrier hens. From these birds, 1,093 eggs were incubated during a course of four years. These eggs showed a fertility of 66.2 per cent and a hatchability of fertile eggs of 33.1 per cent and a hatchability of total eggs incubated of 21.9 per cent.

In Table XIX is included a record of seven normal hens from the same flock over a period of the same years. Of the 803 eggs incubated, 67.8 per cent were fertile and the hatchability of fertile eggs was 59.7 per cent and the hatchability of total eggs incubated was 40.5 per cent. The reactors show 1.6 per cent lower fertility, 26.6 per cent lower hatchability of the fertile eggs, and 18.6 per cent lower hatchability of eggs incubated than did the nonreactors.

In a publication from this laboratory in 1923 (Beaudette, Bushnell, and Payne, 1923*b*) a report was made on 34 birds known to be infected with *S. pullorum.* It was found that 1,462 fertile eggs were obtained from the 34 infected birds under observation. Of these, 553, or 37.8 per cent, hatched, and of 25 eggs containing dead embryos, 12, or 48 per cent, yielded pure cultures of *S. pullorum.* These 12 infected eggs were produced by eight hens.

The figures from Table XVIII are somewhat misleading since these were birds retained in the flock because of physical vigor, etc. A similar number of birds in a general flock not under such careful observations would probably show much poorer results. Diseased birds have been held in the experimental flocks of this station for long periods of time with no eggs, or very few eggs, being laid. In case of one bird, two eggs were laid during the breeding season. Both were fertile and both hatched. This gave a fertility of 100 per cent and a hatchability of 100 per cent. However, this bird was practically valueless as a breeder because of its low productivity.

TABLE XX.—Data showing the influence of age upon fertility and hatchability of reactors and nonreactors—summary of Tables XVIII and XIX.

YEAR.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
Reactors								
1	445	91	354	79.6	281	73	20.6	16.4
2	330	145	185	56.1	115	70	37.9	21.3
3	207	82	125	60.4	52	73	58.4	35.2
Nonreactors								
1	329	86	243	73.8	88	155	63.8	47.1
2	238	66	172	72.3	70	102	59.3	42.9
3	197	74	123	62.4	60	63	51.2	31.9

Table XX shows a peculiar phenomenon. Both the reactors and nonreactors show a general decrease in fertility, while in hatchability the reactors show a gradual increase and the nonreactors shows a gradual decrease with the increased age of the birds. It may be that these particular reacting birds had recovered from the diseased condition and were immune. This point is still open for discussion since no examination was made of these birds to show whether they were carriers of the disease at the end of the period.

One hen presents an interesting case. The per cent of hatch for her in 1920 was 25.3, which was below the flock average for that year. In 1921 and 1922 the average for this bird was considerably above the flock average. The agglutination test was first made on this bird June 2,1921, at which time the agglutination was complete in a dilution of 1:640, indicating a strong reaction. The next test

was made on January 25, 1922, and the agglutination was found to have dropped to complete in 1:80 and partial in 1:160. A third test was made on April 13,1922. At this time there was no agglutination above 1:40 dilution. These results are shown in Table XXI.

TABLE XXI.—Data showing decrease in agglutinins in serum of fowl 25.

DATE.	Titre of serum.	Hatchability.	
		Hen 25.	Flock.
June 11, 1921	1:640	25.3	32.0
January 25, 1922	1:160	65.7	53.9
April 13, 1922	1:40	90.6	63.9

This bird was apparently rapidly recovering from this disease as indicated by decreased agglutinin content of the blood serum and increased hatchability of her eggs.

Table XXII shows the influence of age upon both reactors and nonreactors. The reactors are divided into three groups according to the titre of the serum. The group of "high reactors" showed a complete agglutination reaction in a 1:160 or above in dilution of serum. The "medium reactors," a complete agglutination reaction in 1:80, but not in 1:160; while the low reactors showed complete agglutination reactions in 1:40, but not above that dilution of serum. The nonreactors did not show any agglutination in dilution of 1:20.

The records for the reactors all show high fertility and low hatchability of the fertile eggs. The nonreactors show a somewhat lower degree of fertility but more than double the hatchability. It is not possible to show the relative productivity of these groups of birds because records were not obtained over the same period in all cases.

The low hatchability is an important point to emphasize in retaining reacting birds within a flock. Many of these birds lay as many eggs during the season as do nonreactors and possess an equal degree of fertility, but show much lower degree of hatchability of the fertile eggs and livability of the chicks.

TABLE XXII.—Influence of age of bird upon fertility and hatchability by reactors and nonreactors.

Hen No.	Year.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.	Titre of serum.
					High Reactors					
23	1st	72	10	62	86.0	48	14	22.5	19.4	1:160
1	1st	61	8	53	86.9	50	3	5.6	4.9	
2	1st	61	10	51	83.6	44	7	13.7	11.4	
25	1st	58	0	58	100.0	44	14	24.1	24.1	
Total or av.,		252	28	224	88.8	186	38	17.0	15.1	.....
					Medium Reactors					
3	1st	61	6	55	90.1	44	11	20.0	18.0	1:80
4	1st	63	0	63	100.0	47	16	25.4	25.4	
Total or av.,		124	6	118	95.1	91	27	22.0	21.8	.....
					Low Reactors					
5	1st	67	18	49	73.2	37	12	24.4	17.9	1:40
6	1st	65	40	25	38.4	16	9	36.0	13.8	
7	1st	67	9	58	86.6	43	15	25.8	22.4	
Total or av.,		199	67	132	66.3	96	36	27.3	18.1	.....
					Nonreactors					
8	1st	59	7	52	88.1	33	19	36.5	32.2	0
10	1st	74	45	29	39.2	23	6	20.7	8.1	
11	1st	50	29	21	42.0	3	18	85.7	36.0	
12	1st	35	4	31	88.6	7	24	77.4	68.6	
13	1st	34	0	34	100.0	2	32	94.1	94.1	
14	1st	37	1	36	97.3	2	34	94.4	91.9	
15	1st	40	0	40	100.0	15	25	62.5	62.5	
Total or av.,		329	84	243	73.9	85	158	65.0	48.0	.....

TABLE XXII.—*Concluded.*

Hen No.	Year.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.	Titre of serum.
					High Reactors					
23	3d	18	3	15	83.3	9	6	40.0	33.3	
1	3d	34	2	32	94.1	5	27	84.4	79.4	1:160
2	3d	34	33	23	67.6	18	5	21.7	14.7	
25	3d	1	1	0	0.0	0	0	0.0	0.0	
Total or av.,		87	17	70	80.4	32	38	54.3	43.7	.....
					Medium Reactors					
3	3d	22	3	19	86.4	13	6	31.6	27.3	1:80
4	3d	33	1	32	97.0	3	29	90.6	87.9	
Total or av.,		55	4	51	92.7	16	35	68.6	63.6	.....
					Low Reactors					
5	3d	32	29	3	9.4	1	2	66.6	6.2	
6	3d	31	31	0	0.0	0	0	0.0	0.0	1:40
7	3d	21	5	16	76.2	12	4	25.0	19.0	
Total or av.,		84	65	19	22.6	13	6	31.5	7.1	.....
					Nonreactors					
8	3d	26	6	20	76.9	14	6	30.0	24.0	
10	3d	54	25	29	53.7	17	12	41.4	22.2	
11	3d	32	16	16	50.0	14	2	12.5	6.3	
12	3d	2	0	2	100.0	2	0	0.0	0.0	0
13	3d	29	0	29	100.0	3	26	89.6	89.6	
14	3d	29	26	3	10.3	1	2	66.6	6.9	
15	3d	25	1	24	96.0	9	15	62.5	60.0	
Total or av.,		197	74	123	62.4	60	63	52.0	31.9	.....

TABLE XXIII.—Influence of age on reactors and nonreactors.—Summary.

No. of birds.	Year.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
				High Reactors					
4	1st	252	28	234	92.9	186	38	16.2	15.1
	3d	87	17	70	80.4	32	38	54.3	43.7
				Medium Reactors					
2	1st	124	6	118	95.1	91	27	22.8	21.8
	3d	55	4	51	92.7	16	35	68.6	63.6
				Low Reactors					
3	1st	199	67	132	66.3	96	36	27.3	18.1
	3d	84	65	19	22.6	13	6	31.5	7.1
				Summary of Reactors					
9	1st	575	101	484	84.2	373	101	20.9	17.6
	3d	226	86	140	61.9	61	79	56.4	34.9
				Nonreactors					
8	1st	329	86	243	73.9	85	158	65.0	48.0
	3d	197	74	123	62.4	60	63	52.0	31.9
				Average per Bird—Reactors.					
9	1st	64	11	54	84.3	41	11	20.3	17.1
	3d	25	9	16	54.0	7	9	56.2	36.0
				Average per Bird—Nonreactors.					
8	1st	41	11	30	73.2	11	19	63.3	46.4
	3d	25	9	16	64.0	8	8	50.0	32.0

Tables XXIII and XXIV are introduced to show the influence of the disease process upon the same birds over a period of years. These tables were obtained by rearranging Table XXII and were chosen because they were obtained from birds which had been under observation for at least three years. Table XXIII is arranged, as described above, into groups depending upon the reaction of the

Serum in the agglutination test. In the summary comparing the averages per bird from the groups of reactors and nonreactors it may be seen that the per cent of fertility decreased in both cases, but more for the reactors, while the hatchability for the two groups is just reversed from the condition usually expected. In case of the reactors, the hatchability increased 17.3 per cent in three years, while for the nonreactors it decreased 16.1 per cent during this period. The summary for the two groups of birds is shown in Table XXIV. From such a small group of birds it is impossible to draw definite conclusions and the data is introduced only since it raises the interesting question of how to account for the results obtained. Apparently these differences are not due entirely to chance.

TABLE XXIV.—Difference between first and third years.—Summary.

NUMBER OF BIRDS.	Per cent fertile.	Per cent fertile eggs hatched.	Per cent incubated eggs hatched.	Titre of serum.
		High Reactors		
4	12.5 Decrease	38.1 Increase	28.6 Increase	1:160
		Medium Reactors		
2	2.4 Decrease	45.8 Increase	41.8 Increase	1:80
		Low Reactors		
3	43.7 Decrease	4.2 Increase	11.0 Increase	1:40
		Summary of Reactors		
9	22.3 Decrease	35.5 Increase	17.3 Increase	———
		Summary of Nonreactors		
8	11.1 Decrease	13.0 Decrease	16.1 Decrease	No reaction in 1:20

As it happened, the records chosen for Tables XXII, XXIII, and XXIV showed a higher per cent of fertility among the reactors than among the nonreactors.

Table XXV shows the results obtained on a much larger group of birds for the years 1922, 1923, 1924, and 1925.

TABLE XXV.—A comparison of the fertility and hatchability of eggs from reacting and nonreacting hens.

	No. of birds.	Eggs incubated.	Per cent fertile.	Per cent fertile hatched.	Remarks.
1922.					
Reactors	41	861	69.57	53.58	Difference between hatchability (fertile eggs) of nonreactors and reactors, 11.52 per cent.
Nonreactors	218	6,387	77.10	65.10	
Total	259	7,248	76.21	63.86	
1923.					
Reactors	19	387	57.0	45.2	Difference between hatchability of nonreactors and reactors, 18.2 per cent.
Nonreactors	164	5,066	90.4	63.4	
Total	183	5,453	88.0	62.6	
1924.					
Reactors	14	380	92.1	59.43	Difference between hatchability of nonreactors and reactors, 10.33 per cent.
Nonreactors	201	5,966	90.68	69.76	
Total	215	6,346	91.24	68.06	
1925.					
Nonreactors	239	7,567	89.4	70.3	Reactors removed.

The data given in Table XXV is very interesting in regard to the effect of reactors upon fertility and hatchability of eggs from a flock. This does not take into account the loss of chicks after hatching due to the spread of the infectious material to healthy chicks. This table also shows the value of removing all the reactors from a flock and emphasizes again the danger of a few reactors to the entire flock.

Tables XXVI and XXVII are introduced to show the influence of the reacting male upon fertility and hatchability. Table XXVI is of special interest since two groups of birds were selected which had exactly the same productivity for the season. Both the reacting and the nonreacting group laid 98 eggs for the period of observa-

tion. Such a selection should give a good example of what might be expected as a result of infection by the organisms of bacillary white diarrhea.

It will be noted from these figures that the fertility and hatchability of eggs is greater for the nonreacting group of birds. It is unfortunate that there was not a larger group from which to draw

TABLE XXVI.—Data showing influence on hatch of reacting and nonreacting hens and males.

HEN NO.	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
Reacting Hens and Male (a)								
33	35	6	29	82.9	5	24	82.8	68.6
41	37	35	2	5.4	0	2	100.0	5.4
42	6	3	3	50.0	2	0	0.0	0.0
30	20	8	12	60.0	6	6	50.0	30.0
Total or av...	98	52	46	46.9	13	32	69.6	32.7
Nonreacting Hens and Male (b)								
35	35	4	31	88.6	7	24	77.4	68.6
36	37	1	36	97.3	2	34	94.4	91.9
39	6	4	2	33.3	1	1	50.0	16.7
38	20	1	19	95.0	4	15	78.9	75.0
Total or av...	98	10	88	89.8	14	74	84.1	75.5

(a) All birds showed a serum titre above 1:160.
(b) Blood serum of these birds did not show agglutination in dilutions of 1:20.

conclusions. It should be recognized that fertility may depend upon the male bird, but this could not be checked since it is obviously impossible to have a bird both a reactor and a nonreactor at the same time.

It should be noted that there is a difference of 42.9 per cent in fertility and 14.5 per cent in hatchability of fertile eggs, in favor of the nonreacting group of birds.

Table XXVII shows the influence of the infection in the male alone. Two male birds were chosen having records for a period of more than one year. Male 442 was a nonreactor in the 1:20 dilu-

tion, while male 850 reacted with complete agglutination in dilution of 1:160 of the serum. Such a bird would be considered a strong reactor while No. 442 would be considered to be free from the infection. All the hens used in both cases were nonreactors. The pen containing the reacting male bird showed 8.3 per cent lower fertility than the other, but 11.4 per cent higher hatchability of fertile eggs. In this case, while the male bird was a reactor, he may not have had the infection localized in the testes and thus would not have affected the fertility and hatchability in any way (Rettger,

TABLE XXVII.—Data showing influence of the male on fertility and hatchability.

HEN NO. (a).	Eggs incubated.	Infertile.	Fertile.	Per cent fertile.	Not hatched.	Hatched.	Per cent fertile hatched.	Per cent incubated hatched.
Nonreacting Male No. 442								
23	33	7	26	78.8	10	16	61.5	48.5
25	10	1	9	90.0	7	2	22.2	20.0
54	45	12	33	73.3	21	12	36.3	26.7
26	46	8	38	82.6	13	25	65.8	54.3
37	40	0	40	100.0	15	25	62.5	62.5
85	35	1	34	97.1	5	29	85.3	82.9
88	38	1	37	97.4	4	33	89.2	86.8
84	29	1	28	96.6	6	22	78.6	75.9
61	36	1	35	97.2	34	1	2.8	2.8
62	32	20	12	37.5	8	4	33.3	12.5
63	39	20	19	48.7	7	12	63.2	30.8
64	22	11	11	50.0	6	5	45.5	22.7
Total or av.	405	83	322	79.5	136	186	57.7	45.9
Reacting Male No. 850—Titre, 1:160								
73	31	15	16	51.6	2	14	87.5	45.2
42	42	5	37	88.1	12	25	67.6	59.5
43	21	9	12	57.1	0	12	100.0	57.1
44	33	23	10	30.3	9	1	10.0	3.0
41	30	1	29	96.7	9	20	68.9	66.6
47	43	0	43	100.0	17	26	60.4	60.4
27	26	20	6	23.1	2	4	66.7	15.4
28	7	3	4	57.1	3	1	25.0	14.3
29	31	0	31	100.0	4	27	87.1	87.1
Total or av.	264	76	188	71.2	58	130	69.1	49.3

(a) All hens used were nonreactors.

Kirkpatrick, and Jones, 1916, and Beaudette, 1925). However, when the data from a large number of birds are studied results are found to be considerably in favor of the nonreactors. Rettger *et al.* (1919) found that the male might act as a passive carrier of the organisms.

LOSSES FROM THE DISEASE

There are numerous losses due to this disease which are not easily apparent. Such losses as those of low production, low fertility, and low hatchability, as well as low livability. In some cases the low livability may be of less importance as a source of loss than some of the other factors mentioned.

It is difficult to show the influence of low productivity in a well-regulated flock, since most of the nonproducers are quickly culled and sent to market. It is possible to obtain some idea of the losses from a study of fertility, hatchability, and livability from such a flock. Of course, there are many things which cause loss and the best that can be done is to compare losses from reactors and nonreactors in the same flock.

Table XXVIII is introduced to show the influence of the disease upon loss due to lowered fertility and hatchability.

In this flock there was a difference of 7.53 per cent in fertility and 12.9 per cent in hatchability for the first year; 33.4 per cent in fertility and 31.55 per cent in hatchability for the second year; and 1.42 per cent in fertility in favor of the reactors, and 8.55 per cent in hatchability for the nonreactors for the third year. This flock

TABLE XXVIII.—A comparison of the losses in fertility and hatchability of eggs from reactors and nonreactors.

	Number of birds.	Eggs incubated.	Per cent fertile.	Difference in fertility.	Per cent incubated hatched.	Difference in per cent incubated hatched.
1922.				*Per cent.*		
Reactors	41	861	69.57	.....	37.28	.....
Nonreactors	218	6,387	77.10	+7.53	50.19	+12.9
1923.						
Reactors	19	387	57.0	.....	25.76	.....
Nonreactors	164	5,066	90.4	+33.40	57.31	+31.55
1924.						
Reactors	14	380	92.10	.....	54.71	.....
Nonreactors	201	5,966	90.68	—1.42	63.26	+8.55
1925.						
Nonreactors	239	7,567	89.4	.....	62.8	.....

was tested each year, but the reactors were not removed because there was little loss of chicks after hatching. In 1925 all reactors were removed and careful sanitary methods introduced. If the loss to the owner for not introducing these improvements in management earlier be figured, the following results will be noted: In the first year the owner lost 12.9 per cent of 861 eggs incubated from the reactors, or a possible loss of 111 chicks. The second year this loss was 31.55 per cent of 387 eggs incubated, or a possible loss of 122 chicks. For the third year the loss was 8.55 per cent of 380 eggs incubated, or a possible loss of 33 chicks. Considering that chicks are worth 15 cents each, the loss of chicks for the three years would be $39.90. In this case the loss due to decreased fertility and hatchability would have paid for the laboratory test of 10 cents each for 399 birds, or 60.73 per cent of the 657 birds in the flock for the three years. This does not include losses from death of chicks due to this disease after hatching.

The following data have been estimated on the basis of five years' study of this disease in Kansas. The figures may not be applicable to other states and there will be very great variation in individual flocks, but in general the figures are a fair average for Kansas conditions:

Average per cent fertility for nonreactors	90
Average per cent fertility for reactors	70
Average per cent hatchability of fertile eggs for nonreactors	70
Average per cent hatchability of fertile eggs for reactors	58
Average per cent livability of chicks from nonreactors	90
Average per cent livability of chicks from reactors	50
Average per cent infection in Kansas flocks	25
Average per cent of Kansas flocks infected	75

Calculating on the basis of the above figures, Kansas losses have been considered from the standpoint of the hatcheryman who sells day-old chicks and the farmer who purchases these chicks. Losses to the farmer who may hatch chicks on his own farm have not been considered because of lack of data on this point. When the disease is present, however, the losses are probably as great as stated above.

LOSSES TO THE HATCHERYMAN

For each 100 eggs incubated from nonreactors the hatch would be 70 per cent of 90 eggs, or 63 chicks. For each 100 eggs from reactors the hatch would be 58 per cent of 70 eggs, or 40.6 chicks. This gives a difference of 22.4 chicks. If 25 per cent of the birds in the flock are reactors, the difference in loss between reactors and nonreactors would be 5.6 chicks per 100 eggs incubated.

At 15 cents per day-old chick this loss would amount to 84 cents. On this basis the hatcheryman can afford to pay 84 cents per hundred more for eggs from nonreactors than from the average farm flock not tested. This is on the basis of saving in losses due to infertility and improper hatchability alone.

LOSSES TO THE PURCHASER OF DAY-OLD CHICKS

If 100 day-old chicks are purchased at 15 cents each the cost is $15. The normal loss is 10 per cent, leaving 90 chicks. If these chicks are affected with bacillary white diarrhea there is an average loss of 40 per cent, or 40 per cent of 90 chicks, or 36 chicks.

The remaining 54 chicks cost $15, or a total of 28 cents per chick. In reality the 90 chicks cost 17 cents each. This makes a difference in cost per chick of 11 cents. The loss of one chick would be enough to pay for the laboratory charge for the testing of an adult bird.

Considering relative losses between reactors and nonreactors startling results are obtained.

Normal Losses per 100 Eggs Incubated

Losses in fertility, 10 per cent, or 10 eggs, leaves 90 eggs.
Losses in hatchability, 30 per cent, or 27 chicks, leaves 63 chicks.
Losses by death, 10 per cent, or 6.3 chicks, leaves 56.7 chicks.

Normal Losses per 100 Eggs Plus Those Due to Bacillary White Diarrhea

Losses in fertility, 30 per cent, or 30 eggs, leaves 70 eggs.
Losses in hatchability, 42 per cent, or 29.4 chicks, leaves 40.6 chicks.
Losses by death, 50 per cent, or 20.3 chicks, leaves 20.3 chicks.

The difference due to bacillary white diarrhea is 36.4 chicks. At 14.1 cent each[4] this amounts to $5.13 per 100 eggs incubated.

In case of 100 per cent reactors in the flock, approximately 20.3 chicks are obtained from each 100 eggs incubated at a cost of $8, or at a cost of 39.4 cents per chick. From a flock of nonreactors held under similar conditions 56.7 chicks may be obtained at a cost of $8, or at a cost of 14.1 cents per chick. The difference in cost per chick in this particular case is 25.3 cents.

It may be concluded that it is easily worth while to test for this disease and eliminate the reactors. The estimated losses have been very conservative and based upon quite extensive investigations.

Termohlen (1925) cites a flock in which the losses were 652 per 1,000 chicks, and Canfield (1925) considers that the per cent of livable chicks from infected hens is 22.4, while from noninfected

4. The figure 14.1 cents was obtained as follows: Cost of eggs, per 100, $3; cost of incubation, $5; total cost, $8. The 56.7 chicks obtained under normal conditions will cost $8, or 14.1 cents each.

hens it is 94.02. From his experiments he finds that the infected hens lay an average of 136 eggs per year, while the noninfected hens lay an average of 166 eggs. If these eggs are considered to be worth 3 cents each the infection would mean a loss of 90 cents per year per bird. When it is considered that 25 per cent of the flock is usually infected the loss is 22.5 cents per bird. This is easily enough to pay for the entire cost of having birds tested.

TREATMENT OF THE DISEASE BY USE OF SOUR MILK

Since the discoveries of Metchnikoff on the influence of lactic-acid bacteria upon the intestinal flora in health and disease, there has been active interest in this problem. Since bacillary white diarrhea may be considered somewhat of an intestinal disease and the portal of entry is undoubtedly through the intestine the use of lactic-acid-producing organisms was suggested.

Rettger, Kirkpatrick and Stoneburn (1912) found that in every instance the mortality was lowered in birds which received sour milk. In one case the deaths were double that in the pens not receiving it, and in one case the ratio was approximately 3:1.

Bushnell and Maurer (1913) reported on the use of cultures of B. bulgaricus milk in the control of this disease in artificially infected chicks. There was 26 per cent less loss in the pens fed the sour milk culture.

Rettger, Kirkpatrick, and Jones (1914) state that sour milk feeding has a beneficial influence on the growth of chicks, and in lessening mortality from all causes. Milk soured by the Bulgarian bacillus had no distinct advantages over naturally soured milk. It has the disadvantage that it requires time and care in its preparation and is not relished by the chicks to the same extent as naturally soured milk.

Rettger, Kirkpatrick, and Card (1915) found that sweet and sour milk were of equal value as a food for chicks. Its value does not depend upon any acids that may be present, nor upon any particular types of microörganisms; but upon one or more of the natural constituents of the milk. The feeding of sweet or sour milk was not found to be injurious if the milk was clean and not too old,

Jorgensen (1924) studied the influence of *B. acidophilus* milk on this disease. The chicks were fed ten mls. of the culture every four hours. The results of his tests were: Of ten infected chicks fed this milk, three died; of four noninfected chicks fed this milk none died; of two infected chicks not fed milk, two died; of two noninfected

chicks not fed milk, 2 died. In this case the milk soured by *B. acidophilus* appears to reduce the mortality from this disease tbout 70 per cent.

Kaupp and Dearstyne (1925) determined the acid tolerance of *S. pullorum.* This was found to be 0.6 to 0.7 per cent of lactic acid when tested in milk.

Brunett (1925) claims that *B. acidophilus* milk may be effective in preventing chicks from getting the disease, but that it would be of no value in curing chicks already infected. The same claim is made for antiseptics in drinking water.

Beach (1925) found that it is possible to implant *B. acidophilus* in the digestive tract of a fowl by feeding milk cultures of the organism, but saw no particular advantages over other products containing lactose and forming lactic acid. He did not work with *S. pullorum* in these studies.

The concensus of opinion appears to be that the feeding of sour milk is of little value in the control of this disease, except that it is an excellent food and will increase the vigor of the chicks. The disease is not entirely a disease of the intestinal tract, but is generalized throughout the organs of the body of the chick. The feeding of sour milk would thus be of no value in curing the disease. It may be of value as an intestinal antiseptic and prevent chicks from becoming infected through the food, but would have no influence on the course of the disease once it had become established in the body. Sweet milk seems to be equally as beneficial as sour milk from the standpoint of food value.

TESTING FOR BACILLARY WHITE DIARRHEA

The eradication of this disease is one of the most important problems which confronts the poultry breeder of to-day. In the following pages some of the various tests suggested are described and compared to the test accepted as best at this laboratory.

By this method of presentation it is hoped to develop some standard of procedure which will be universally used. By such a standard, birds tested in any part of the country could be shipped to the purchasers as being free from the disease. The test suggested here is the most effective yet devised for this purpose. It is generally recognized that some modification of the agglutination test suggested by Jones (1913) is a reliable method of eliminating adult carriers of this disease. However, since there are so many modifications of the

test the writers felt the need of a comparative study before choosing the most efficient.

TESTS USED BY VARIOUS AGRICULTURAL EXPERIMENT STATION LABORATORIES

In order to have some basis for work in determining the proper kind of a test to use for detecting carriers of *S. pullorum,* a questionnaire was sent to 44 state laboratories making such tests. Twenty-eight replies were received and a summary of the answers follows:

Number of different methods of testing reported 16

Number using one-dilution test 8

1:10 dilution	1
1:50 dilution	2
1:60 dilution	1
1:80 dilution	3
1:100 dilution	1
Total	8

Number using two-dilution test 18

1:10 and 1:20 dilutions	1
1:20 and 1:80 dilutions	1
1:20 and 1:100 dilutions	1
1:25 and 1:50 dilutions	2
1:40 and 1:100 dilutions	3
1:50 and 1:100 dilutions	7
1:100 and 1:200 dilutions	2
1:100 and 1:500 dilutions	1
Total	18

Number using four-dilution test 1

(1:10, 1:50, 1:100, 1:200)

Number using six-dilution test 1

Microörganisms Used for Test Fluids

S. pullorum 27

E. sanguinarium (from baby chicks) 1

Note.—One investigator is planning on using both *S. pullorum* and *E. sanguinarium* in his test fluid to determine fowl typhoid as well as *S. pullorum* carriers.

Number of strains used in test fluids:

One strain used	5
Two strains used	8
Three strains used	8
Four strains used	4
Five strains used	2
Six strains used	3
Eight strains used	3

Origin of strains used:

Baby chicks	10
Baby chicks and ovaries	7
Baby chicks, ovaries, embryos, and acute adult infection	2

Chemicals used for preservation of test fluids:

0.2 per cent phenol	1
0.25 per cent phenol	1
0.3 per cent phenol	1
0.4 per cent phenol	1
0.5 per cent phenol	19
0.5 per cent phenol or 0.5 per cent formalin	1
0.5 per cent formalin	1
Coal-tar disinfectant (no per cent given)	1
No preservative used	2

Concentration of test fluid used for tests:

To compare with 0.5 McFarland's nephelometer	8
To compare with 0.75 McFarland's nephelometer	6
To compare with 1.0 McFarland's nephelometer	7
To compare with 1.25 McFarland's nephelometer	1
To compare with 1.5 McFarland's nephelometer	1
To compare with 3.0 McFarland's nephelometer	8
To compare with 3.5 Gates' nephelometer	2
To compare with 4.0 Gates' nephelometer	1
To compare with 5.0 Gates' nephelometer	1
Very dilute	8
No attention paid to dilution	1

Is it possible to differentiate between a carrier of infection and an immune bird?

Number answering "No"	9
Number answering "Doubtful"	8
Number answering "Do not know"	8
Number answering "Depends on degree and nature of immunity"	1
No answer given	2

What constitutes a reactor?

Number reporting partial as well as complete agglutination as reactors	10
Number reporting complete agglutination as reactors and partials as suspicious cases	18

Have you isolated *S. pullorum* from birds giving reactions from 1:10 to 1:50, but not in higher dilutions of serum?

Number answering "No"	11
Number answering "Not attempted"	9
Number answering "Yes in 1:10 dilutions"	4
Number answering "Yes in 1:10 to 1:50 dilutions"	4

General Remarks about the Test

The following remarks were taken from the answers given in replies to the questionnaire but not included in the summary:

Beach, of California, uses a two-tube test of 1:25 and 1:50 dilutions, but formerly used 1:50 and 1:100 dilutions. He changed because he felt that some infected birds might react in lower dilutions. He reports that he has some autopsy evidence to support this theory.

Edwards, of Kentucky, reports that he has found that 0.3 per cent phenol when used as a preservative for test fluids has an advantage over 0.5 per cent phenol since it does not cause so much precipitation of lipoidal substances from serum.

Mathews, of Indiana, is the only investigator using *E. sanguinarium,* for preparation of test fluids. A strain isolated from a baby chick is used for preparation of test fluid. This is grown on pork infusion agar for 48 to 72 hours at 37 degrees C., and the antigen or test fluid preserved with phenol. The test fluid is adjusted to a concentration comparing to No. 3 tube of McFarland's nephelometer for testing. No attention is paid to the pH value of the antigen and tests are incubated at 37 degrees C., for 14 to 18 hours before they are read. Either a partial or a complete agglutination constitutes a reactor.

Gage, of Massachusetts, has found that it pays to include a low-dilution tube (1:50) as a safety limit. He does ,not believe that many cases will be missed if only a 1:100 dilution test is used. Antigen or test fluid is preserved with 0.25 per cent phenol. He has found that a smaller per cent of confusing turbidities result if such a per cent phenol is used than when a higher per cent is used.

Mallman, of Michigan, recommends a single strain of *S. pullorum* test fluid and pays no attention to the source of the culture. His only reason for using this culture is its agglutinability. If more than one strain is to be used each must be of a good agglutinating type.

Fitch, of Minnesota, uses serum bouillon for growing cultures for the preparation of test fluids. The bacteria are precipitated by centrifugalization and washed with sterile saline. The test fluid is preserved with 0.5 per cent phenol, adjusted to a concentration comparing to No. 1.5 in the McFarland nephelometer and to a pH value of 7.0.

Beaudette, of New Jersey, has recently isolated *E. sanguinarium* from chicks and is planning on adding a culture of this organism to his test fluid. He uses Buchner tubes for growing the organisms because they are easily inoculated and avoid contamination better than the Kolle flask. He reports that he has seen only about four partial agglutinations in 28,000 tests made with a single-tube 1:80 dilution test.

Rebrassier, of Ohio, reports that cross agglutination as high as 1:80 dilution of serum of other intestinal organisms than *S. pullorum,* may occur and for this reason uses a single-tube 1:100 dilution test. He uses phenol for preserving the concentrated antigens, but not when diluting to the concentration used in the test. He reports that he has isolated *S. pullorum* from a few birds reacting in dilutions as low as 1:10 and not in higher dilutions.

Tittsler, of Pennsylvania, uses a two-tube test of 1:100 and 1:500 dilutions. He believes that pro-agglutination may occur in dilutions of 1:100 so some reactors would be missed if the higher dilutions were omitted. He uses 0.5 per cent formalin for preservation of test fluid.

May, of Rhode Island, uses a single-strain antigen prepared from a culture isolated from a serious outbreak of bacillary white diarrhea in Rhode Island. This strain (No. 224 R. I.)has been found to pick more positive birds than any other strain or combination of strains used. He has observed normal agglutinations in dilutions of 1:80 but not in dilutions of 1:100 with the antigens prepared from culture 224. This is an argument for the dilutions used in routine tests (1:100 and 1:200).

THE K. S. A. C. TEST

Much discussion about the correct test to use for detecting carriers of *S. pullorum* infection has been taking place among laboratory technicians. Hardly two are using the same test at the present time and each has his reasons for his particular method as discussed later. In the test used by the Department of Bacteriology of the Agricultural Experiment Station of K. S. A. C., two tubes of 1:20 and 1:80 dilutions of the serum are prepared as shown in figure 3. Test tubes 12 by 75 mm. are used for the tests. The antigen is put into these tubes with a burette such as is shown in figure 4.

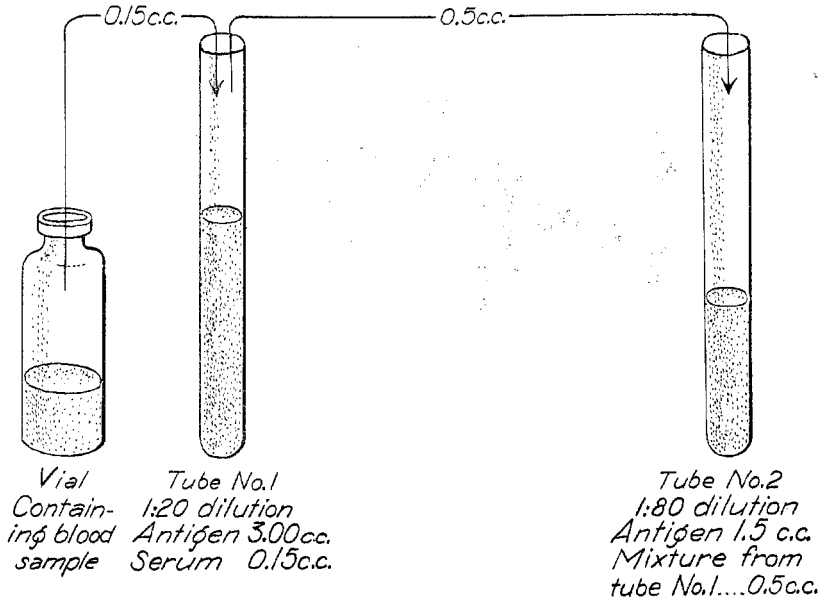

FIG. 3.—Diagram showing method of making dilutions of serum in antigen.

The barrel of the graduate tube is divided into 3 c. c. and 1.5 c. c. spaces. Each 3 c. c. mark is painted red while the 1.5 c. c. marks are painted black. Much speed can be developed with this method and it is as accurate as using a pipet for adding antigen to the tubes. When not in use the burette is kept filled with 0.5 per cent phenol, and is thoroughly rinsed with distilled water and finally with physiological saline before using.

Pipets graduated to deliver 0.15 c. c. and 0.5 c. c. from the tip are used to make dilutions of the serum. The same pipet is used for making both mixtures. The serum-antigen mixture in the first tube

is agitated with the pipet to insure even distribution before making the transfer. A one-eighth-ounce rubber bulb is used on the end of the pipet to remove the serum from the blood clot, make transfers, etc. Wooden blocks which hold 24 tests are used for racks.

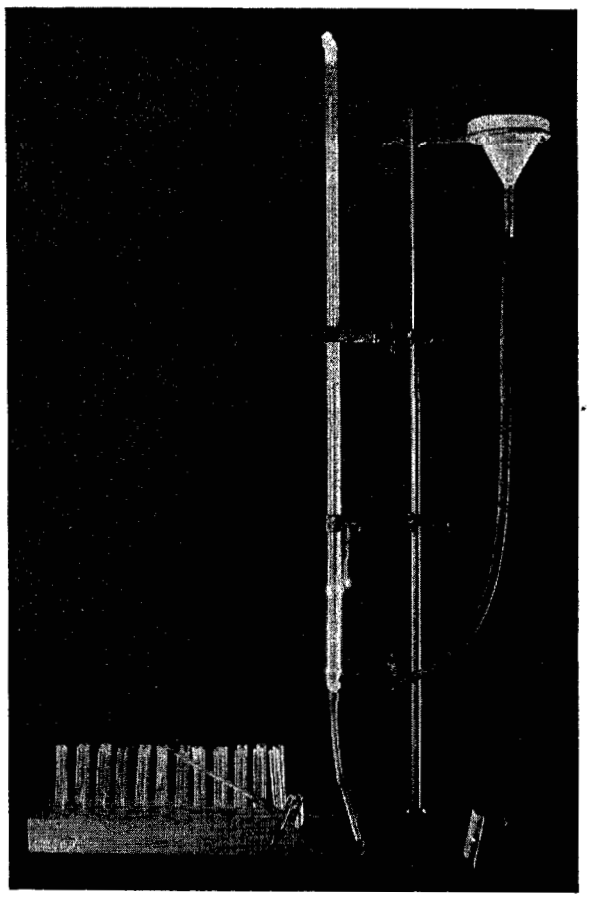

FIG. 4.—Apparatus used in testing for bacillary white diarrhea.

The antigen used is made from six to ten strains of *S. pullorum* obtained from various sources and thoroughly tested. The antigen being used at present consists of eight strains. Three of these were isolated from chicks, three from ova of adult birds, and two from dead embryos (18 days old). Only strains which react strongly to known positive serum are used. Each lot of antigen is tested with known positive serum and against antigens previously proved effi-

cient. All antigens are made from 48 to 72-hour old cultures grown on 2 per cent chicken meat infusion agar having a pH value of 7.2. Physiological saline containing 0.5 per cent phenol is used for washing the culture from the agar and it is stored in very concentrated form in a refrigerator until ready to use. It is diluted to a turbidity which is slightly less than that in tube No. 1 of McFarland's nephelometer when used for tests. When diluted for the test, it is aimed to have an antigen which contains 0.3 per cent phenol as a preservative. Like Gage, of Massachusetts, and Edwards, of Kentucky, the writers have had no trouble with the "fat-like" phenomenon reported by Hitchner (1923) when less than 0.3 per cent phenol is used for a preservative. Formalized antigens do not cause a precipitation of this fat-like substance, but antigens so prepared are not as reliable as when preserved with phenol.

This method necessitates a transfer of serum-antigen mixture from the first to the second tube after it has had a few seconds time for absorption to take place. However, in tests made to determine the possibility of error from this contact of serum and antigen it, was observed that it was possible to allow serum to remain in contact with antigen for one hour without any noticeable effect on the test. At the present time direct antigen dilution of serum is being used in preference to saline dilutions in setting up experimental tests where titre limits are desired. By using such a method much time is saved and the titre is not affected if the dilutions are made within one minute.

Tests are incubated for 20 hours at 37 degrees C., and at room temperature for two to four hours before being read. The agglutinations are more easily read after incubating a short time at room temperature than when read immediately after being taken from the incubator. Partial agglutinations in the 1:20 tube are held for an additional 24 hours at room temperature. All typical agglutination (partial or complete) in either tube are considered as indicating reactors. No "suspects" are recorded. If any test is doubtful, a retest is called for.

COMPARATIVE STUDIES OF VARIOUS TESTS

To compare several one-tube tests that are being used by others, with the K. S. A. C. test, comparative studies were made, using the same antigens. Merely to have some basis for this work, the K. S. A. C. test was considered as having an efficiency of 100, and all comparisons were based on it. The results of the studies made are tabulated in Table XXIX.

TABLE XXIX.—Comparative efficiency of 1:20 and 1:80 single-tube tests, and the K. S. A. C. two-tube test.

TESTS.	One-tube, 1:20	One-tube, 1:80	K. S. A. C., two-tube.
Total number of tests made	5,903	5,903	5,903
Total number negative	3,866	4,403	3,851
Per cent negative	65.5	74.6	65.2
Total number positive	2,037	1,500	2,052
Per cent positive	34.5	25.4	34.7
Per cent reactors missed in comparison with two-tube test	0.24	9.3	0.0
Comparative efficiency of tests	99.76	90.66	100.0

A study of this table shows that 0.24 per cent of the reactors would have been missed if only a one-tube 1:20 test were used. In other words, there were 0.24 per cent of the tests which showed a prozone in 1:20 dilution, but not in the 1:80 dilution. In no flock tested did the prozone extend beyond the 1:40 dilution of serum. If only a 1:80 dilution had been used 9.34 per cent of the reactors would have been missed. Or, the comparative efficiency of the three tests, according to the data given above, is 99.76 per cent, 90.66 per cent, and 100 per cent for the 1:20, one-tube, 1:80, one-tube, and the two-tube tests, respectively.

The efficiency of a single-tube, 1:40 dilution, in comparison to other is shown in Table XXX.

TABLE XXX.—Comparison of single-tube tests of 1:20, 1:40, and 1:80 dilutions, and the K. S. A. C. test.

TESTS.	One-tube, 1:20	One-tube, 1:40	One-tube, 1:80	K. S. A. C., two-tube.
Total number of tests	2,021	2,021	2,021	2,021
Total number negative	1,163	1,208	1,240	1,151
Per cent negative	57.5	59.7	61.3	56.9
Total number positive	858	813	781	870
Per cent positive	42.5	40.2	38.6	43.0
Per cent reactors missed in comparison with two-tube test	0.59	2.81	4.4	0.0
Comparative efficiency of tests	99.41	97.19	95.60	100.0

This table reveals similar results to those in Table XXIX, although a smaller per cent of reactors would have been missed if only the one-tube, 1:80 test had been used. Also more tests showed

prozone reaction, bringing the per cent of missed carriers by the one-tube, 1:20 method higher. It should also be noticed that the single-tube, 1:40 test proved less efficient than the 1:20 but more efficient than the 1:80. In only one of the twelve prozone cases was proagglutination noticed above the 1:20 dilution. In this case the test was negative in each of the 1:20 and 1:40, and partial in 1:80 dilution of serum.

In a third comparative study, the single-tube, 1:10 method was compared with the 1:20 and 1:80 single-tube method and the K. S. A. C. two-tube method. The results are summarized in Table XXXI.

TABLE XXXI.—Comparative results using single-tube tests of 1:10, 1:20, and 1:80 dilutions, and the K. S. A. C. two-tube method.

TESTS.	One-tube, 1:10.	One-tube, 1:20.	One-tube, 1:80.	K. S. A. C., two-tube.
Total number of tests made	150	150	150	150
Total number negative	63	65	98	65
Per cent negative	42	43.3	65.3	43.3
Total number positive	87	85	52	85
Per cent positive	58.0	56.6	34.6	56.6
Per cent reactors missed in comparison with two-tube test	0	0	22	0
Comparative efficiency of tests	(a) 101.33	100.0	78.0	100.0

(a) By the 1:10 test, 1.33 per cent more reactors were found than by the two-tube test.

These tests were made on low-reacting birds to determine if the 1:10 dilution were more efficient than the 1:20 dilution. This accounts for the high per cent of reactors missed by the 1:80 test. Two prozone cases appeared in the 1:10 dilution. Neither of these were negative in the other tests, The four positive birds picked by the 1:10 test, but not by the others, gave only partial reactions and these were rather atypical. This test has other disadvantages which offset its slight increased efficiency. These will be considered in another place.

A fourth study to further compare the efficiency of a single-tube test of 1:40 and 1:80 with a two-tube test of 1:40 and 1:80 dilutions, was made. The results of this study are given in Table XXXII.

In this study the efficiency was based on a two-tube test of 1:40 and 1:80 dilutions instead of on dilutions of 1:20 and 1:80 as in the

other studies. This accounts for the difference in reactors missed by various tests for the 1:40 dilution in Tables XXX and XXXII.

TABLE XXXII.—Comparative study of single-tube tests of 1:40 and 1:80 dilutions with a two-tube test of 1:40 and 1:80 dilutions.

TESTS.	One-tube, 1:40.	One-tube, 1:80.	Two-tube, (standard).
Total number of tests	1,524	1,524	1,524
Total number negative	980	1,086	977
Per cent negative	64.3	71.2	64.1
Total number positive	544	438	547
Per cent positive	35.6	28.7	35.8
Per cent reactors missed in comparison with two-tube test	0.20	7.15	0.0
Comparative efficiency of tests	99.80	92.85	100.0

DISCUSSION OF THE TEST

From these studies it will be seen that there is a marked difference in the efficiency of the various tests. In general, a two-tube test is more efficient in determining reacting birds, but there are arguments for both methods. The advocates of the single-tubemethod have raised various arguments in favor of their test. Beaudette[5] believes that a large per cent of birds which react in dilutions under 1:80 but not in 1:80 are not carriers. These birds may be immune birds, or birds whose serum contains normal agglutinins, or birds suffering from other infections such as fowl typhoid, etc.

Mathews,[5] of Indiana, reports that a 1:10 single-tube test is most efficient. He uses antigen made from cultures of *E. sunguinarium* and dilutes the antigen to a turbidity which will compare with No. 3 tube of a McFarland's nephelometer. He believes that the chance for prozone reactions is lessened where a concentrated test fluid is used. The writers have done no work to approve or disapprove this theory, but have found the less concentrated antigens to give much more clear-cut reactions. More reactors may be missed by failing to detect agglutination in concentrated test fluids than by an increased number of prozone cases.

Stafseth[6] found the single-tube, 1:80 dilution test used by Beaudette to be 89.6 per cent efficient when compared with a two-tube test of 1:40 and 1:100 dilution. Two comparisons showed the Indiana test to be 79.2 per cent and 84.0 per cent as efficient as a

5. Personal communication. 6. Personal communication.

two-tube, 1:40 and 1:100 test. Using *E. typhi* antigen in dilutions of 1:80, which is recommended by some technicians, he found that his tests were 24.0 per cent as efficient as when *S. pullorum* antigen was used in the two dilutions of 1:40 and 1:100. A one-tube test using 1:40 dilution proved 99.42 per cent as efficient as the Michigan two-tube test (1:40 and 1:100), and a single-tube 1:100 dilution test proved 96.52 per cent efficient. In general these figures agree with results obtained in this laboratory.

By studying the results summarized in Table XXX of this bulletin the comparative efficiency of a two-tube, 1:20 and 1:80 dilution and a two-tube, 1:40 and 1:80 dilution test can be determined. Table XXXIII gives a summary of these findings.

TABLE XXXIII.—Comparison of two two-tube methods of testing.

TESTS.	K. S. A. C., 1:20 and 1:80.	Two-tube, 1:40 and 1:80.	Remarks.
Total number of tests	2,021	2,021	———
Total number positive	870	814	12 prozone in 1:20. 1 prozone in 1:40.
Comparative efficiency	100.0	93.6	———

As is shown in the above table the two-tube test used at K. S. A. C. is slightly more efficient than one containing a 1:40 dilution instead of a 1:20. If a similar study is made of Table XXXI it will be seen that in a two-tube test in which a 1:10 dilution tube is substituted for the 1:20 dilution tube, the former will be slightly more efficient. Table XXXIV gives a summary of such a study.

TABLE XXXIV.—Comparison of two two-tube methods of testing.

TESTS.	Two-tube, 1:10 and 1:80.	Two-tube, 1:20 and 1:80.	Remarks.
Total number of tests	150	150	———
Total number positive	89	85	2 prozone cases in 1:10 dilution.
Comparative efficiency	104.7	100.0	———

The above results are those which have been taken only by a comparison of tests and do not take in consideration some of the other factors which may influence the test. It is quite evident that a two-tube test containing one tube of a dilution under 80 or 100 and one tube of either of these dilutions will be more efficient than

a one-tube test of any single dilution under 100 if only such results are studied. Furthermore the advantage lies with the test containing the lowest possible dilution. Also, the one-tube test containing the lowest dilution is apparently more efficient than one containing the higher dilutions.

The following factors should be considered in a study of the test:

1. Correlation of the serum titre and actual infection.
2. Proagglutination and titre of serum.
3. Relation of dilution to fat-like substances which may be in the serum.
4. Correlation of the test with carefully controlled field results.

These factors are only mentioned here to emphasize the fact that in spite of the summaries brought out in this bulletin there may be certain combinations of dilutions which will be more efficient than others though they do not seem so by merely studying tabulated tests. It is by a study of these factors that the test used in this laboratory has been selected. The 1:10 dilutions are more difficult to read and more apt to show atypical results. It is also more difficult to obtain enough of clear amber-colored serum for such a dilution when a two-tube combination is used than when a 1:20 or 1:40 dilution is substituted for the 1:10 dilution. The 1:40 dilution has one advantage over the 1:20 in that the fat-like serum reported by Hitchner (1923) is more apt to interfere in the 1:20 than in the 1:40 dilution. By use of 0.3 per cent phenolized antigen this difficulty has been eliminated. When sera showing this phenomenon are set up using 0.5 per cent phenolized antigen, it is impossible to interpret results. By setting up tests using the same sera but 0.3 per cent phenolized antigen clear-cut tests usually result.

There is no doubt but that there are more prozone cases in lower dilutions and this is one good argument for including a higher-dilution tube in the test. The prozcme phenomena have not been observed in this laboratory in 1:80 dilutions.

CONCLUSIONS FROM STUDIES OF VARIOUS TESTS

A few birds showing agglutination in low dilutions of serum may be immune, or be carriers of infection. However, one will find low-reacting birds which carry actual infection. Two birds were found which gave positive tests in dilutions of 1:20, but which were negative in dilutions of 1:80 and from which pure cultures of *S. pullorum* were isolated. Other investigators in various parts of the country have also isolated the organism from low reactors. There appears

to be little doubt that they may be the source of serious outbreaks of this disease. Also, there is a chance of eliminating birds in the early stages of infection with a low-dilution test. Again, one is just as apt to cull immune birds from a flock with a high-dilution test as with a low dilution and fowl typhoid carriers will sometimes have a serum titre far above the usual dilution test used.

Therefore, until definite experimental work has been done to substantiate the fact that a one-tube test using a 1:80 or 1:100 or a more diluted serum is more efficient than others, it is the opinion of the writers that if a one-tube test is used it should be one using as low a dilution of serum as possible. For general testing a two-tube test of a low and a high dilution will prove most efficient because it avoids prozone cases and determines the largest number of carriers. The small unit value of an average bird will not warrant leaving it in a flock if it is a low reactor, on the supposition that it is an immune bird or a carrier of another disease. It is an advantage to cull out the latter type anyway.

COLLECTION AND TRANSFER OF BLOOD TO A LABORATORY

The proper season of the year for testing blood has not been definitely determined, but it is probably best to test just before the breeding season in order not to throw the birds out of production. Some writers state that it is best to test after the birds have been in production for some time. It is no doubt true that the older the birds and the longer they have been in production the more likely they are to develop agglutinins in the blood stream if they are affected at all. However, with a low-dilution tube in the test these birds will be detected much earlier than with only a high-dilution test. This is an added reason for using such a test as herein recommended.

The instrument for bleeding should be a slender, stiff, slightly curved knife, with a rather broad but very sharp point. The cut should be made lengthwise rather than across the vein. This makes a clean cut which is less likely to result in a hematoma and renders it easier to stop the bleeding after the sample has been collected.

The question of a fatty serum may be corrected to some extent by withholding feed for 18 to 36 hours before bleeding, but this will influence the egg production and it is best to control this trouble by reducing the amount of phenol in the test fluid.

After the blood is drawn it should be carefully cared for. Hemolysis due to contamination, freezing, shaking, or the presence of

water in the collecting vials will render the blood unfit for use. A test which contains hemoglobin will invariably show a precipitate in the tubes and make it impossible to obtain an accurate reading. The blood may be preserved by placing two drops of a 5 per cent solution of boric acid in the vials and drying them in an oven. This amount of preservative will prevent spoilage for several days even in the summer and does not appear to affect the test in any way.

The length of time between drawing and testing blood does not seem to influence the test if the serum is kept sterile and cool. However, the shorter the time blood is held the more clear-cut the test. Blood which has been hemolyzed or contaminated cannot be used. A clear serum free from blood cells and hemoglobin is necessary. For this reason a sample of blood should be used as fresh as possible. The addition of boric acid as described above will aid in preserving the serum in a clear state for several days.

CONCLUSIONS

1. Bacillary white diarrhea is an infectious disease the cause of which is known.

2. This disease causes much greater losses than is usually recognized.

3. The disease may be eradicated from the flock by vigorous methods of testing, culling, and sanitation.

4. The agglutination test is the most reliable method known at the present time for detecting carriers of the disease.

5. It will be impossible to eradicate this disease unless breeders are willing to remove the reactors from their flocks and refuse to purchase anything but tested breeding stock.

LITERATURE CITED

BAKER, A. H.
1925. A USEFUL PRESERVATIVE FOR WHOLE BLOOD. Brit. Jour. Expt. Path. Aug., 1925. Abs. in North Amer. Vet. Vol. 6, No. 12, p. 41.

BAKER, H. R.
1925. STUDIES IN BACILLARY WHITE DIARRHEA IN POULTRY IN DELAWARE. Del. Agr. Expt. Sta. Ann. Rpt. for 1924 (Bul. 139), p. 14.

BEACH, J. R.
1925. THE EFFECT OF FEEDING BACILLUS ACIDOPHILUS, LACTOSE, DRY SKIM MILK, OR WHOLE MILK ON THE HYDROGEN—ION CONCENTRATION OF THE CONTENTS OF THE CECA CHICKENS. Hilgardia Vol. I, No. 8, pp. 145-166.

BEAUDETTE, F. R., BUSHNELL, L. D., and PAYNE, L. F.
1923A. STUDY OF AN ORGANISM RESEMBLING BACT. PULLORUM FROM UNABSORBED YOLK OF CHICKS "DEAD IN THE SHELL." Jour. Infect. Diseases 32:124-132.

1923B. RELATION OF BACTERIUM PULLORUM TO HATCHABILITY OF EGGS. Jour. Infect. Diseases 33:331-337.

BEAUDETTE, F. R.
1923A. SINGLE TUBE METHOD FOR DETERMINING CARRIERS OF BACTERIUM PULLORUM. Jour. Amer. Vet. Med. Assoc. 64 (n.s.17):225-227.

1923B. AGGLUTININS FOR BACTERIUM PULLORUM IN HENS' EGGS. Jour. Immunol. 8:493-499.

1925A. BACILLARY WHITE DIARRHEA. Poultry Sci. 4205-224.

1925B. THE POSSIBLE TRANSMISSION OF FOWL TYPHOID THROUGH THE EGG. Jour. Amer. Vet. Med. Assoc. 67 (n. s. 20):741-745.

BRANSFIELD, P. E.
1925. CONTROL OF BACILLARY WHITE DIARRHEA. Mas. Agr. Expt. Sta. Control Series Bul. 31:1-7.

BRUNETT, E. L.
1924. REPORT OF POULTRY DISEASE INVESTIGATION. Dept. of Path. and Bact. Cornell Univ. July 1, 1923, to June 30, 1924.

1925. BACILLARY WHITE DIARRHEA; FATAL SEPTICEMIA OF CHICKS, Cornell Vet. 15:303-314. Figs. 8.

BUSHNELL, L. D., and MAURER, OTTO
1913. THE USE OF MILK CULTURES OF B. BULGARICUS IN THE PREVENTION AND TREATMENT OF BACILLARY WHITE DIARRHEA OF YOUNG CHICKS, Amer. Vet. Rev, 44:194-207.

6—4689

BUSHNELL, L. D., and BEAUDETTE, F. R.
1922. LABORATORY DIAGNOSIS OF POULTRY DISEASES. Jour. Amer. Vet. Med. Assoc. 60 (n, s. 13) :729-733.

CANFIELD, HAROLD
1925. THE EFFECTS OF BACILLARY WHITE DIARPHEA. Quart. Bul. Mich. Agr. Expt. Sta. Vol. 8, No. 1, pp. 31-32.

GAGE, G. E.
1911. NOTES ON OVARIAN INFECTION WITH BACTERIUM PULLORUM (RETTGER) IN THE DOMESTIC FOWL. Jour. Med. Research 24 (n. s. 19) :491-496.

GAGE, G. E., PAIGE, B. H., and HYLAN, H. W.
1914. ON THE DIAGNOSIS OF INFECTION WITH BACTERIUM PULLORUM IN THE DOMESTIC FOWL. Mass. Agr. Expt. Sta. Bul. 148: 1-20.

GAGE, G. E., and PAIGE, B. H.
1915. BACILLARY WHITE DIARRHEA (BACT. PULLORUM INFECTION) IN YOUNG CHICKS IN MASSACHUSETTS. Mas. Agr. Expt. Sta. Bul. 163. Illus.

GAGE, G. E., and MARTIN, J. F.
1916. NOTES ON THE HISTO-PATHOLOGY OF THE INTESTINE IN YOUNG CHICKS INFECTED WITH BACT. PULLORUN. Jour. Med. Research 34:149-155.

GAGE, G. E.
1922. CONCERNING THE DIAGNOSIS OF BACTERIUM PULLORUM INFECTION IN THE DOMESTIC FOWL. Mass. Agr. Expt. Sta. Tech. Bul. 5:61-88. Fig. 1.

GAGE, G. E., and FLINT, O. S.
1922-1923-1924. CONTROL OF BACILLARY WHITE DIARRHEA. Mass. Agr. Expt. Sta. Control Series Buls. 22, 23, and 27, pp. 8, 10, and 8, respectively,

GOLDBERG, S. A.
1917. A STUDY OF THE FERMENTING PROPERTIES OF BACT. PULLORUM (RETTGER) AND BACT. SANGUINARITUM (MOORE). Jour. Amer. Vet, Med. Assoc. 51 (n.s.4):203-207.

HADLEY, P. B.
1917. NOTE ON THE REACTION OF THE PARATYPHOIDS, ALPHA AND BETA, IN LITMUS MILK. Jour. Bact. 2263-267.

HADLEY, P. B., CALDWELL, D. W., ELKINS, M. W., and LAMBERT, D. J.
1917. INFECTIONS CAUSED BY BACT. PULLORUM IN ADULT FOWLS. R. I. Agr. Expt. Sta. Bul. 172:l-40.

HADLEY, P. B., ELKINS, E. W., and CALDWELL, D. W.
1918. THE COLON-TYPHOID INTERMEDIATES AS CAUSATIVE AGENTS OF DISEASE IN BIRDS: I. PARATYPHOID BACTERIA. R. I. Agr. Expt. Sta. Bul. 174:l-216.

HADLEY, P. B., CALDWELL, D. W., and HEATH, B. M.
1919. BACTERIOLOGICAL NOTES. I. GAS PRODUCTION BY BACTERIUM PULLORUM. II. BACTERIUM PULLORUM INFECTIONS IN ADULT BIRDS. III. RELATION BETWEEN SUCROSE FERMENTATION AND IMMUNIZING POWER BY B. AVISEPTICUS. Jour. Bact. 4:65-69.

HINSHAW, W. R., UPP, C. W., and MOORE J. M.
1926. STUDIES IN TRANSMISSION OF BACILLARY WHITE DIARRHEA IN INCUBATORS. Jour. Amer. Vet. Med. Assoc. 58 (n.s.21):631-641.

HITCHNER, E. R.
1923. THE MACROSCOPIC AGGLUTINATION TEST AS INFLUENCED BY THE FATTY CONTENT OF THE BLOOD SERUM OF FOWLS. Jour. Amer. Vet. Med. Assoc. 63 (n. s.16) :759-763.

JONES, F. S.
1910. FATAL SPETICEMIA OR BACILLARY WHITE DIARRHEA IN YOUNG CHICKENS. N. Y. State Vet. Col. Ann. Rpt., pp. 111-129.

———
1911. FURTHER STUDIES ON BACILLARY WHITE DIARRHEA IN YOUNG CHICKENS. N. Y. State Vet. Col. Ann. Rpt., pp. 69-88.

———
1912 AN OUTBREAK OF AN ACUTE DISEASE IN ADULT FOWLS DUE TO BACTERIUM PULLORUM. N. Y. State Vet. Col. Ann. Rpt., pp. 140-148.

———
1913. THE VALUE OF THE MACROSCOPIC AGGLUTINATION TEST IN DETECTING FOWLS THAT ARE HARBORING BACT. PULLORUM. Jour. Med. Research 27 (n. s.22) :481-495.

JORGENSON, G. E.
1924. B. ACIDOPHILUS AND IMMUNE SERUM IN THE TREATMENT OF BACILLARY WHITE DIARRHEA IN CHICKS. Vet. Med. 5:63-65.

JULL, M. A.
1925. A UNIFORM PLAN OF ACCREDITATION AND CERTIFICATION OF POULTRY AS ADOPTED AT THE STANDARDIZATION CONFERENCE HELD AT MANHATTAN, KAN., AUG. 10 AND 11, 1925. (Unpublished manuscript.)

KAUPF, B. F., and DEARSTYKE, R. S.
1925. THE EFFECT OF LACTIC ACID ON B. PULLORUM. B. AVISPETICUS, B. SANGUINARITUM AND ITS POSSIBLE ROLE IN THE CONTROL OF INTESTINAL DISEASES OF POULTRY. Poultry Sci. 4:242-249.

KRUMWEIDE, C. and KOHN, L. A.
1917. STUDIES OF THE PARATYPHOID-ENTERITIDIS GROUP IV.—THE DIFFERENTIATION OF THE MEMBERS OF THE PARATYPHOID-ENTERITIDIS GROUP FROM B. TYPHOSUS WITH SPECIAL REFERENCE TO ANAEROGENIC STUNS AND OBSERVATIONS ON THE FERMENTATIVE CHARACTERISTICS OF THE AVIAN TYPES. Jour. Med. Research 36:509-518.

KRUMWIEDE, C., COOPER, G., and PROVOST, D. T.
1925. AGGLUTININ ABSORPTION. Jour. Immunol. 10:55-239.

MAY, HENRY G.
1924 THE EXAMINATION OF EGGS FROM INFECTED AND IMMUNIZED HENS, WITH GERMICIDAL TESTS ON ALBUMEN AND BLOOD SERUM. R. I. Agr. Expt. Sta. Bul. 197:147.

MILKS, H. J.
1908. A PRELIMINARY REPORT OF SOME DISEASES OF CHICKENS. La. Agr. Expt. Sta. Bul. 108, pp. 11, figs. 4.

MULSOW, F. W.
1919. THE DIFFERENTIATION AND DISTRIBUTION OF THE PARATYPHOID-ENTERITIDIS GROUP IV. —AVIAN PARATYPHOID BACILLI: A COMPARATIVE STUDY OF B. PULLORUM AND B. SANGUINARIUM. Jour. Infect. Diseases 25 :1-28.

PERNOT, E. F.
1908. AN INVESTIGATION OF THE MORTALITY OFINCUBATOR CHICKS. Oreg. Agr. Expt. Sta. Bul. 103. 16 pp.

RETTGER L. E.
1900. FATAL SEPTICEMIA AMONG YOUNG CHICKS. N. Y. Med. Jour. 71:803-805.

1901. FATAL SEPTICEMIA IN YOUNG CHICKS. N. Y. Med. Jour, 73:267-288.

RETTGER, L. F., and HARVEY, S. C.
1908. FATAL SEPTICEMIA IN YOUNG CHICKENS OR "WHITE DIARRHEA." Jour. Med. Research 18:277-290.

RETTGER L. F.
1909. FURTHER STUDIES ON FATAL SEPTICEMIA IN YOUNG CHICKENS, OR "WHITE DIARRHEA." Jour. Med. Research 21 (n. 8.16):115-123.

RETTGER L. F., and STONEBURN, F. H.
1909. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. Storrs Agr. Expt. Sta. Bul. 60:1-57. Figs. 7.

1911. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. Storrs Agr. Expt. Sta. Bul. 68:279-301. Figs. 7. (Second Report.)

RETTGER, L. F., KIRKPATRICK, W. F., and STONEBURN, F. H.
1912. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. Storrs Agr. Expt. Sta. Bul. 74:153-185. Figs. 8. (Third Report.)

RETTGER, L. F.
1914. OVARIAN INFECTION IN THE DOMESTIC FOWL AND DIRECT TRANSMISSION OF DISEASE TO OFFSPRING. Jour. Expt, Med. 19:552-561.

RETTGER L. F., KIRKPATRICK, W. F., and JONES, R. E.
1914. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. Storrs. Agr. Expt. Sta. Bul. 77:263-309. Figs. 4. (Fourth Report.)

1915. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. ITS ERADICATION BY THE ELIMINATION OF INFECTED BREEDING STOCK. Storrs Agr. Expt. sta. Bul. 85:151-167. Figs. 3. (Fifth Report.)

RETTGER L. F., KIRKPATRJCK, W. F., and CARD, L. E.
1915. MILK FEEDINGAND ITS INFLUENCE ON GROWTH AND MORTALITY. Storrs Agr. Expt. Sta. Bul. **80:1-28.**

RETTGER L. F., KIRKPATRICK, W. F., and JONES, R. E.
1916. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS VI. SECOND PROGRESS REPORT ON THE ELIMINATION OF INFECTED BREEDING STOCK. Storrs Agr. Expt. Sta. Bul. 88 :247-254.

RETTGER L. F., HULL, T. G., and STURGIS, W. S.
1916. FEEDING EXPERIMENTS WITH BACTERIUM PULLORUM. THE TOXICITY OF INFECTED EGGS. Jour. Expt. Med. 23:475-489.

RETTGER, L. F., and KOSER, S. A.
1917. A COMPARATIVE STUDY OF BACTERIUM PULLORUM (RETTGER) AND BACTERIUM SANGUINARIUM (Moore). Jour. Med. Research 35 (n.s.30) : 443-453.

RETTGER, L. F., KIRKPATRICK, W. F., and Card, L. E.
1919. BACILLARY WHITE DIARRHEA OF YOUNG CHICKS. Storrs Agr. Expt. Sta. Bul. 101:75-88. (Seventh Report.)

RICE, J. P.
1924. BACILLARY WHITE DIARRHEA. Natl. Poultry Jour. 5:216-217, and 228-229.

SCHERAGO, M., and BENSON, J. P.
1919. EXPERIMENTS ON THE INTRADERMAL TEST FOR BACTERIUM PULLORUM. Cornell Vet, 9:111-119.

SMITH, TH., and TEN BROECK, C.
1915. A NOTE ON THE RELATION BETWEEN B. PULLORUM (RETTGER) AND THE FOWL TYPHOID BACILLUS (MOORE), Jour. Med. Research 31:547-555.

STEINER, A. J.
1924 BACILLARY WHITE DIARRHEA. Ky. Agr. Expt. Sta. Cir. 33:1-8.

TAYLOR, W. J.
1916. A REPORT UPON AN OUTBREAK OF FOWL TYPHOID. Jour. Amer. Vet. Med. Assoc. 49 (n. s. 2) :35-47.

TBRMOHLEN, WM. D.
1925. BACILLARY WHITE DIARRHEA. Poultry Tribune. April, p. 16.

WARD, A. R., and GALLAGHER, B. A.
1917. AN INTRADERMAL TEST FOR BACTERIUM PULLORUM INFECTION IN FOWLS. Bur. Anim. Indus. Bul. 517:1-15.

WINSLOW, C. E. A., KLIGLER, I. J., and ROTHBERG, W.
1917. FURTHER STUDIES ON THE COLON-TYPHOID GROUP OF BACTERIA. Abs. Bact. 1:27-28.

———
1919. STUDIES ON THE CLASSIFICATION OF THE COLON-TYPHOID GROUP OF BACTERIA WITH SPECIAL REFERENCE TO THEIR FERMENTATION REACTIONS. Jour. Bact. 4:429-503.

11-4689