1943

A study of natural bactericidins in the plasma of the domestic fowl

Estill Everett Schnetzler

Iowa State College

Follow this and additional works at: http://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons, Animal Sciences Commons, and the Microbiology Commons

Recommended Citation

Schnetzler, Estill Everett, "A study of natural bactericidins in the plasma of the domestic fowl " (1943). Retrospective Theses and Dissertations. 13154.

http://lib.dr.iastate.edu/rtd/13154

This Dissertation is brought to you for free and open access by Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
A STUDY OF NATURAL BACTERICIDING
IN THE PLASMA OF THE DOMESTIC FOWL

by

Estill Everett Schnetzler

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Poultry Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

1943
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>11</td>
</tr>
<tr>
<td>Strains of <em>Salmonella pullorum</em></td>
<td>11</td>
</tr>
<tr>
<td>Experimental Stock</td>
<td>12</td>
</tr>
<tr>
<td>Drawing of Blood</td>
<td>14</td>
</tr>
<tr>
<td>Determining the Bactericidal Action</td>
<td>15</td>
</tr>
<tr>
<td>Number of Organisms</td>
<td>17</td>
</tr>
<tr>
<td>Inoculations</td>
<td>17</td>
</tr>
<tr>
<td>Absorption Tests</td>
<td>18</td>
</tr>
<tr>
<td>ANALYSIS OF DATA</td>
<td>19</td>
</tr>
<tr>
<td>EXPERIMENTAL RESULTS</td>
<td>20</td>
</tr>
<tr>
<td>Susceptibility of the Strains of organisms</td>
<td>20</td>
</tr>
<tr>
<td>Breed Comparisons</td>
<td>23</td>
</tr>
<tr>
<td>Relationship with Physiological Functions</td>
<td>25</td>
</tr>
<tr>
<td>The Bactericidal System</td>
<td>31</td>
</tr>
<tr>
<td>Heritable Influences</td>
<td>45</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>66</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>72</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>74</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>78</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A comparison of the strains of Salmonella pullorum in susceptibility to fowl bactericidins</td>
<td>21</td>
</tr>
<tr>
<td>2.</td>
<td>Differences between strains of Salmonella pullorum in susceptibility to fowl bactericidins</td>
<td>22</td>
</tr>
<tr>
<td>3.</td>
<td>Differences in bactericidal activity of the plasma of White Leghorns and Rhode Island Reds</td>
<td>24</td>
</tr>
<tr>
<td>4.</td>
<td>A comparison of the bactericidal activity of the plasma of White Leghorns, White Rocks and New Hampshire with Salmonella pullorum, Strain No. 2</td>
<td>27</td>
</tr>
<tr>
<td>5.</td>
<td>Bactericidal tests taken before, during, and after a complete body molt</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>The effects of heating plasma for 30 minutes at 54°C</td>
<td>33</td>
</tr>
<tr>
<td>7.</td>
<td>Addition of fresh guinea pig plasma to heated fowl plasma</td>
<td>34</td>
</tr>
<tr>
<td>8.</td>
<td>Addition of heated guinea pig plasma to fresh fowl plasma</td>
<td>36</td>
</tr>
<tr>
<td>9.</td>
<td>Reactivation of heated plasma by the addition of fresh plasma</td>
<td>37</td>
</tr>
<tr>
<td>10.</td>
<td>Addition of fresh plasma of high bactericidal activity to heated plasma</td>
<td>39</td>
</tr>
<tr>
<td>11.</td>
<td>Absorption of plasma with cells of Salmonella pullorum, Strain No. 2</td>
<td>40</td>
</tr>
<tr>
<td>12.</td>
<td>The bactericidal activity of the plasma of fowls which reacted positively to the agglutination test for pullorum</td>
<td>41</td>
</tr>
<tr>
<td>13.</td>
<td>The effects of injecting fowls reacting positively to the pullorum agglutination test with dead cells of Salmonella pullorum, Strain No. 2</td>
<td>43</td>
</tr>
</tbody>
</table>
(Tables cont.)

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>The effects of injecting fowls reacting negatively to the pullorum agglutination test with dead cells of <em>Salmonella pullorum</em>, Strain No. 2</td>
<td>44</td>
</tr>
<tr>
<td>15</td>
<td>Summary of results for first generation - 1940</td>
<td>47</td>
</tr>
<tr>
<td>16</td>
<td>Summary of results for second generation - 1941</td>
<td>51</td>
</tr>
<tr>
<td>17</td>
<td>Summary of results for the high and low lines of the third generation - 1942 stock</td>
<td>59</td>
</tr>
<tr>
<td>18</td>
<td>Percentage of organisms killed for the progeny of a high and a low dam mated to a high male</td>
<td>61</td>
</tr>
<tr>
<td>19</td>
<td>Percentage of organisms killed for the progeny of a high and a low dam mated to a low male</td>
<td>62</td>
</tr>
<tr>
<td>20</td>
<td>A comparison of the mean percentage of organisms killed for Mating 250 with that for the high and the low lines</td>
<td>63</td>
</tr>
<tr>
<td>21</td>
<td>A comparison of the mean percentage of organisms killed for Mating 245 with that for the high and the low lines</td>
<td>65</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure No.                                     Page

1. Variation in bactericidal activity of fowl plasma with the eight strains of *Salmonella pullorum*  26

2. Regression of angles corresponding to the percentage of organisms killed on body weight - Rhode Island Red females  28

3. Regression of angles corresponding to the percentage of organisms killed on body condition - Rhode Island Red females  29

4. Regression of angles corresponding to the percentage of organisms killed on sexual maturity - Rhode Island Reds  30

5. Pedigrees and the percentage of organisms killed for the progeny of Sire "LM" - 1940 stock  48

6. Pedigrees and the percentage of organisms killed for the progeny of Sire "10" - 1940 stock  49

7. Pedigrees and the percentage of organisms killed for the progeny of the second generation - 1941 stock  52

8. Pedigrees and the percentage of organisms killed for the progeny of the low line - 1942 stock  57

9. Pedigrees and the percentage of organisms killed for the progeny of the high line - 1942 stock  58
INTRODUCTION

Natural antibodies frequently found in the blood of animals are agglutinins, opsonins, hemolysins, and bactericidins. They exhibit considerable specificity with certain infectious agents and normal constituents of the body. Animals vary in this respect. Differences exist between species and between individuals within a species. A classical illustration of the latter are the ischemagglutinins of human blood. These are definitely known to be gene-determined.

Studies of natural bactericidins reveal wide variations in results. The variation could be due partially to genetic differences in the animals, and to the susceptibility, or genetic differences, in the organisms used. Many other influences could also be contributory causes of variation.

This investigation was designed to study the bactericidal activity of fowl plasma with Salmonella pullorum organisms. Among the questions studied are: Is fowl plasma active in killing Salmonella pullorum organisms? Do strains of Salmonella pullorum differ in resistance to the bactericidal activity of fowl plasma? Are there differences between strains and breeds of chickens in bactericidal activity? What are the effects of certain physiological functions, characteristic of the normal life of the fowl, upon the
bactericidal activity of the plasma? What detectable differences exist in the plasma of fowls showing differences in bactericidal action? Can any of the variation in bactericidal activity be attributed to heritable influences?
REVIEW OF LITERATURE

Nuttal (1886) was the first to make a systematic study of the bactericidal property of blood. He observed that blood possessed the ability to kill certain pathogenic organisms; and that the blood of different animals varied in bactericidal activity. The bactericidal power was lost when the blood was heated to 56°C. for 30 minutes, or after holding for long periods.

The cell-free serum was found by Buchner (1890) to possess the same bactericidal power as the blood. The action of the serum was most marked at body temperature and was thermolabile at 55°C. It was capable of destroying bacteria in the subcutaneous tissue, and the serous cavities of the body without the aid of cells.

Pfeiffer (1894) found that cholera spirilla were rapidly destroyed when injected into the peritoneum of a guinea pig which had recovered from the infection. Complete dissolution of bacteria was observed in portions of the exudate removed from the peritoneum. He found the action to occur only on cholera organisms, and that little action occurred in normal pigs. By injecting small amounts of fresh or heated serum from an infected pig with the bacteria, he found that the animal was protected. Bordet (1899) found that the lytic
phenomenon involved two substances of the blood, alexin (complement) and "substance sensibilisatrice" (amboceptor).

The bactericidal action of the plasma may be influenced by several factors. The most important of these are presented in the review of the following experiments.

Colebrook and Storer (1924) reported that a concentration of 0.25 per cent sodium citrate greatly reduced the killing power of human blood against staphylococcus and streptococcus. Similar results were found when ammonium oxalate and sodium phosphate were used. However, Walsh and Harmsworth (1926) found that sodium citrate, when used in various concentrations, inhibited the growth of staphylococcus in human blood, and added that coagulation of blood enormously increases its bactericidal properties. They later reported (1927) that the blood develops bactericidal properties during the early stages of clotting. Hirudinized blood was more bactericidal than citrated blood.

Citrated chicken blood was found to be highly bactericidal against pneumococci, Bull and Tao (1927). They found citrated rabbit blood very low in bactericidal activity. The action was not retarded by citrate, however, since it was greatly increased after adding immune serum.

Schnetzler and Hartsell (1939) found no change in the bactericidal potency of fowl plasma to *Salmonella pullorum* when 0.4 per cent concentration of sodium citrate was used. Hodes (1941) found that this concentration had no effect upon the
growth of *Salmonella pullorum* either in the presence or absence of serum.

Pettersson (1902, 1926, 1927) confirmed Nutil's (1888) finding of a thermostable agent in the plasma. He referred to the labile fraction as an alpha lysin, which involves the intervention of complement, and to the heat stable fraction as a beta lysin. Gram-negative organisms were reported as being susceptible to alpha lysins while the gram-positive bacteria were susceptible to beta lysins, the activity being of much lower order in the latter instance. Mackie and Finklestein (1932) reported similar results, and found the thermostable substance labile at 60°C. They found no evidence of any relationship between leukins and lysozymes and the thermostable agent. Gram-negative and gram-positive organisms varied widely in resistance to bactericidins of animals of various species. Sheep serum contained more active thermodabile bactericidins than serum of the ox, man, white rat, pig, horse, rabbit, guinea pig or pigeon. The thermostable bactericidins of rabbit serum were more active than those of the rat, horse, human, pig, sheep, ox, or guinea pig. Pigeon serum contained but few thermostable bactericidins.

Mackie and Finklestein (1932) found that the most susceptible gram-negative organisms were the vibrios, and the bacteria of the typhoid-paratyphoid-dysentery group. Only a few gram-negative organisms were not susceptible. Among the
minutes were satisfactory, but a slightly greater bactericidal

Hodges (1941) reported that an incubation period of 20
activity with the stock was 3 to 56 weeks of age.
He also observed that there was an increase in bactericidal
from an average of 50 per cent of the serum of the host at 10°C.
Berger (1945) found the greatest bactericidal activity of

adding fresh serum.

The bactericidal action was restored by
the addition of 25 per cent of the bacterial
component was lost by refrigeration for 24 hours, and approximately 65 per cent after 96 hours.

From plasma to Salmonella pullorum when it was stored at 20°C.

but much more rapidly at the latter temperature. Berger (1945) reported a loss of 50 per cent of the bactericidal strength of
bacteriologic potency when the serum was aged at 10°C. and 24°C.

were used. He reported that there was a progressive loss of
cultures when fresh serum of the host was used, and Pfeifer,
activity occurred when the serum-culture mixtures were incubated for one hour.

Milone (1936) changed the pH of the sera of several different animals by the use of carbon dioxide and nitrogen. He observed a decrease in bactericidal action, regardless of the way the change was made.

Mackie, Finklestein and Van Rooyen (1932) found that normal whole blood and serum-leucocyte mixtures may exert a bactericidal effect when the plasma is inactive, and may possess quantitatively greater bactericidal properties than the plasma, when the plasma is active. Leucocyte suspensions were found to have an initial bactericidal effect on certain organisms but this was followed by a pronounced growth promoting influence. Dead bacteria inhibited the bactericidal action of the plasma.

Mackie and Finklestein (1932) and Gordon and Hoyle (1936) found that by absorption of normal serum with dead bacteria, the bactericidal power was reduced. If the organism was tested against a series of absorbed sera, it was killed most slowly by the serum which had been absorbed by the same organism. Gordon and Johnstone (1940) found that there were not only many species-specific antibodies, but also a vast number of strain-specific antibodies. Or, there was a general bactericidal antibody which was modified by contact with an excess of any particular organism as to render it specifically inactive.
The variation observed between and within species of animals in bactericidal activity is important. Malone, et al (1925) found a difference in bactericidal potency of the sera of Bombay and Madras rats which were known to show outstanding differences in susceptibility to *Pasteurella pestis*. Irwin and Hughes (1933) reported that whole blood from two inbred strains of rats highly susceptible to *Salmonella enteritidis* showed little or no bactericidal action.

Normal sera of the ox, rabbit, and sheep were found to be highly bactericidal for *Brucella suis*, while normal guinea pig serum showed no such activity, Shrigley and Irwin (1936). The bactericidal action could be destroyed by heating to 56.5°C. for 30 minutes. Guinea pig serum would not activate normal ox, rabbit, or sheep sera against this organism. Rabbit complement activated only heated rabbit serum, but ox complement activated almost completely the killing power of heated sera of the rabbit, ox, and partly that of the sheep.

Irwin and Ferguson (1938) found that the bactericidal power of the sera of cows gradually became stronger after the first few weeks following artificial infection with *Brucella abortus*. Guinea pigs were found by Silverthorne (1937) to be protected against meningococcal infection when the bactericidal power had been stimulated by previous vaccination with living organisms. Children vaccinated with fresh virulent strains of meningococci developed bactericidins in their blood. Wulf
(1934) found a thermostable bactericidal substance in human sera during fever; its effect was particularly potent against meningococcus.

Torrey (1908) found bactericidins for gonococcus present in normal and immune rabbit and guinea pig sera. There was considerable variability in the susceptibility of the gonococcus to these antibodies. Natural bactericidins active against a strain of gonococcus were not found in human serum, Abdosh (1936). There was a steady rise, however, in the incidence of bactericidal action in the sera of patients following the process of gonococcal infection. Normal sera from eleven other mammals showed a powerful bactericidal action against this organism.

Roberts, Seversn, and Card (1939) found no difference in bactericidal power of blood from pullorum resistant and susceptible strains of chickens.

Miller (1940) observed bactericidal action in chick embryos. He obtained evidence for a natural bactericidin in chicks, and showed that this action was also a property of the blood of young animals. The bactericidal action was lost when the plasma was heated.

The review of the literature reveals that bactericidal action is a property of the blood of many animals. In some instances, the action was detected only after inoculation. However, blood of animals that apparently had not been exposed to
the disease organism in question, also showed bactericidal activity. Wide variations in bactericidal activity were reported by some of the early workers, and this observation was frequently made in later work. Species of animals differed in bactericidal activity with a given organism and with different species and genera of organisms. Animals of the same species also varied in bactericidal activity.
EXPERIMENTAL PROCEDURE

Strains of Salmonella pullorum

Eight different strains\(^1\) of *Salmonella pullorum* were used in this study. They are known to be different only from the standpoint of isolation, as follows:

- **No. 1** - isolated July 27, 1939, from ovary of White Wyandotte
- **No. 2** - isolated November 16, 1937, from heart of chick six days of age
- **No. 3** - isolated August 6, 1937, from liver of seven months old Rhode Island Red pullet
- **No. 4** - isolated August 2, 1939, from egg yolk of Barred Rock chick seven days of age
- **No. 5** - isolated April 6, 1937, from liver of poult six days of age
- **No. 6** - isolated June 20, 1939, from abdominal exudate of yearling New Hampshire cockerel
- **No. 7** - isolated June 25, 1939, from liver of poult six days of age

\(^1\) The writer is indebted to Dr. Erwin Jungherr, Storrs (Conn.) Agricultural Experiment Station, for furnishing the stock cultures.
No. 8 - "recently isolated" from a chick

These strains were examined at the National Salmonella Center, University of Kentucky, Lexington, Kentucky, and all were serologically typical of the species. Fermentation tests by Bahler (1941) showed also that all strains were typical of the species. Each strain produces smooth types of colonies.

All eight strains of Salmonella pullorum were used in making comparable tests of the bactericidal activity of the plasma of White Leghorn and Rhode Island Red chickens. The one most susceptible to bactericidal activity, No. 2, was used in all other studies.

All stock cultures of Salmonella pullorum were grown on Bacto-Peptone agar medium. After 24 hours of initial incubation at 37.5°C, they were stored in a refrigerator at approximately 5°C. Transfers were made from these as they were needed in running the tests. New stock cultures were made every three weeks. Before using the organism for testing purposes, two 20-hour beef extract broth cultures were made.

Experimental Stock

White Leghorn and Rhode Island Red yearling hens from the Purdue University flocks were used in making studies of breed differences. The White Leghorn stock had not been outcrossed for three years, nor had there been any close inbreeding practiced. The Rhode Island Reds had been secured from the
seven, production for two weeks and eight, production for
eight grades, grade six intermediate production for one week.
In a similar manner. Results of the remaining factors were studied
development as determined by the various factors, were studied
to four. Depending upon the conditions of the root. Sexual
even a grade of live. Intermediate grades ranged from one
even a grade of zero, those well-reared and in good health were
even that were thin, emaciated, or in poor health were even.
the tests concerning their body condition and sexual maturity
were made, a grade was even the tests at the time of running.
All cows were at least 26 weeks of age before any tests
were made.

It is stock

... an early and late than those made on the Purdue University-
make the cooperative studies. The tests were made approx.
look. Only State of Delaware, Penn. State No. 2, was used in
New Hampshire, and white hogs of the Iowa State College
... bacterial tests also were made on the white hogs.

breed were tested simultaneously.

hogs that were tested were need, the same number of each

... disease as determined by the three per cent. All or the cows tested were free of pathy-
percentage of reactors to the Agglutination test never exceeded
whether the breeds were free from pathy disease but the

New breeding stock had been frequently added to the flock.

Piney-Purdue Experiment Station or approximately 50 cows.
three weeks or more. Males that appeared fully mature and females that were ready to lay were given a grade of five. Body weights also were taken at the time of making the tests.

Rhode Island Reds were used in an attempt to produce two lines of stock differing in bactericidal activity. Breeding females were selected on the basis of their mean percentage of organisms killed for the eight strains. Males were selected on the bactericidal activity of their plasma with Strain No. 2. A description of the matings is presented with the experimental results.

Bactericidal tests were made of fowls which reacted positively to the tube agglutination test for pullorum disease. Tests were made of negative fowls from the same flocks when making the tests of the positive reactors.

Drawing of Blood

Chickens

Blood was drawn aseptically from the humoral vein, using a 5 cc. syringe. A concentration of 0.4 per cent sodium citrate was used as an anticoagulant. The blood and citrate were thoroughly mixed by rotating the syringe. The citrated blood was then transferred to a Wassermann tube and centrifuged for 20 minutes. The plasma was mixed immediately with the cell suspension of *Salmonella pullorum*. The time between drawing
the blood and mixing the plasma with the cells was never more than two hours.

Guinea pigs

Blood was drawn from the heart of unanesthetized guinea pigs. Sodium citrate was used as an anticoagulant as described for chickens. Only healthy pigs that had been fed an abundant quantity of green feed were employed.

Determining the Bactericidal Action

Three different procedures were followed. In reporting the results these are differentiated by the last digit of the number of the fowl or the test.

Plan No.1

Bacteria were transferred with a loop from the stock culture to a tube containing 20 cc. of beef extract broth. After 20 hours of incubation at 37.5°C, a second transfer was made into another tube containing the same quantity of beef extract broth. In making the second transfer, a straight needle was used and immersed a definite depth. This procedure assured to some degree a constant number of organisms. After 20 hours of incubation, the beef extract broth culture was filtered through sterile cotton and diluted with physiological saline, making a dilution of 1/16000 of the culture in physiological saline.
Then 0.1 cc. of the cell suspension and 0.25 cc. of plasma were mixed in a sterile tube and incubated in water bath at 37.5°C. for 30 minutes. Controls were prepared by mixing the cell suspension with physiological saline in the same ratio as the cell-suspension-plasma mixture.

After incubation, approximately 10 cc. of melted Bacto-Peptone agar medium cooled to 51°C. were poured in each tube containing the cell-plasma mixtures. The tubes were rolled between the hands, the contents poured into sterile petri dishes, and incubated for 48 hours at 37.5°C. The bactericidal activity of the plasma was determined by comparing the number of colonies on each plate containing the cell-plasma mixture with the number on the control plate.

Plan No. 2

This plan differs from Plan No. 1 in two respects:

1. Instead of using saline for making the cell suspension, a buffer was used. It consisted of a solution of 27 cc., M/10 KH₂PO₄ and 73 cc., M/10 Na₂HPO₄.  
2. The beef extract broth culture was diluted 1/8000 in the buffer. Four times the quantity of cell-suspension and plasma as designated for Plan No. 1 was used. After incubation in water bath, each tube was vigorously shaken and 0.05 cc. of the cell-plasma mixture was transferred with a pipette, graduated in 0.01 cc., to a sterile petri dish. Melted Bacto-Peptone agar medium cooled
to 51°C. was then poured into each plate. All tests were run in duplicate. This plan has the advantage that more fowls can be tested at one time with apparently less variation.

Plan No. 3

Controls were made for each hen by removing 0.05 cc. of the cell-plasma mixture directly after the plasma and cells had been mixed thoroughly. This quantity was placed in a petri dish and melted Bacto-Peptone agar medium added. The remaining portion of the cell-plasma mixture was then incubated in a water bath, the plan from this point being the same as outlined under Plan No. 2. Plan No. 3 reduces the variation due to pipetting.

Number of Organisms

The number of colonies on the control plates usually ranged from 350 to 700. When the number was within this range, the plasma of some fowls would kill practically all of the organisms, while for others, only a few were killed. Since it was impossible to keep the number of organisms constant, the bactericidal activity was expressed in percentage of organisms killed.

Inoculations

The fowls were injected with dead cells of Salmonella
pullorum, Strain No. 2. The cells were grown in beef extract broth for 24 hours and then killed by heating for one hour at 60°C. The inoculum was stored in the refrigerator at approximately 50°C. Injections were made intramuscularly every other day. The first two injections contained 1 cc., the next 2, 1.5 cc., and from there on, 2 cc. for varying lengths of time as stated in experimental results. The inoculum contained approximately 400 million organisms per cubic centimeter as determined by plate count.

Absorption Tests

*Salmonella pullorum*, Strain No. 2, was grown on Bacto-Peptone agar medium. After 48 hours the cells were removed from the medium with a small quantity of sterile physiological saline. The organisms were then killed by heating for 45 minutes at 60°C. After the cells were washed four times with large quantities of saline, 4 cc. of saline were added to 1 cc. of the packed cells and the suspension prepared for the absorption tests by cooling to 0°C. The plasma also was cooled before starting the tests.

The plasma and the washed cell suspension were then mixed in a ratio of 1:1.25 and kept at 0°C. for two hours. After centrifuging, the supernatant fluid was tested for bactericidal activity. Fresh plasma was diluted with an equal quantity of saline and carried along with the absorption tubes as a control.
ANALYSIS OF DATA

Since the number of bacteria in the cell suspensions varied between tests, it was necessary to express the results in percentage of organisms killed. For statistical analysis, the percentages were transformed to angles, giving a distribution more like that of normals. The test of significance would, therefore, be more valid since some of the means were widely separated and approached the lower and upper limits. The means for the strains of organisms ranged from 3.2 per cent to 98.1 per cent. Families showed similar differences between their means. For such data, transformations seemed advisable.
EXPERIMENTAL RESULTS

Susceptibility of the Strains of Organisms

A comparison of the susceptibility of the eight strains of *Salmonella pullorum* to fowl bactericidins is presented in Table 1. All eight strains were susceptible to fowl bactericidins. However, there was considerable variation between them. One cause for variation seemed to be the length of time since isolation. If those strains isolated in 1937 are compared with those isolated in 1939, wide differences can be noted. Strain Nos. 2, 3, and 5 were the first isolated and these were more susceptible to fowl bactericidins. However, Strain No. 7 had been isolated only a short time and it was not significantly less susceptible than Strain No. 5, Table 2.

Strain No. 6, isolated from an abdominal exudate of a New Hampshire cockerel was most resistant to bactericidal action of fowl plasma. It was significantly more resistant than all of the other organisms. Only 36 of the 69 fowls showed any bactericidal action against this strain. The average kill was only 8.1 per cent as compared to the next lowest of 35.0 per cent, Strain No. 4, isolated from an egg yolk of a Barred Rock chick.

The two strains isolated from poults, Strain Nos. 5 and
Table 1. A comparison of the strains of Salmonella pullorum in susceptibility to fowl bactericidins

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Date Isolated</th>
<th>White Leghorns</th>
<th>Rhode Island Reds</th>
<th>Per cent Kill</th>
<th>Per cent Kill</th>
<th>Per cent Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6/20/39</td>
<td>15.1</td>
<td>3.2</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6/2/39</td>
<td>45.7</td>
<td>25.3</td>
<td>35.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7/27/39</td>
<td>56.6</td>
<td>28.9</td>
<td>42.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&quot;Recent&quot;</td>
<td>65.2</td>
<td>34.3</td>
<td>48.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6/26/39</td>
<td>71.6</td>
<td>51.8</td>
<td>61.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4/6/37</td>
<td>76.2</td>
<td>56.6</td>
<td>66.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8/8/37</td>
<td>88.7</td>
<td>66.2</td>
<td>79.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11/15/37</td>
<td>98.2</td>
<td>89.1</td>
<td>94.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>66.9</td>
<td>43.8</td>
<td>55.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Tests were made during months of November and December, 1959.

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>549</td>
<td>415,932.2</td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>1</td>
<td>25,166.5</td>
<td>25,166.5**</td>
</tr>
<tr>
<td>Strain</td>
<td>7</td>
<td>157,750.1</td>
<td>22,532.9**</td>
</tr>
<tr>
<td>Breed x Strain</td>
<td>7</td>
<td>128.8</td>
<td>18.4</td>
</tr>
<tr>
<td>Within subclasses</td>
<td>534</td>
<td>232,956.8</td>
<td>436.2</td>
</tr>
</tbody>
</table>

F values, Breed 57.7, Strain 51.7

** Significant at 1 per cent level.
Table 2. Differences between strains of *Salmonella pullorum* in susceptibility to fowl bactericidins

<table>
<thead>
<tr>
<th>Mean differences - degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain No.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

* Significant at 5 per cent level.

** Significant at 1 per cent level.
7, were relatively susceptible to bactericidins of the fowl. Strain Nos. 2 and 3, isolated from chickens, were more susceptible, but the other four strains were less susceptible than those isolated from poult.s. The species from which these organisms were isolated had no significant relationship to resistance to fowl bactericidins.

Breed Comparisons

Since fowls of two different breeds were used in making the studies of the eight strains of organisms, the data were suitable for studies of breed differences. The results of the bactericidal tests made of the plasma of White Leghorns and Rhode Island Reds are given in Table 3. The plasma of the White Leghorns showed greater bactericidal action with each strain of Salmonella pullorum. The differences between the two breeds ranged from 9.0 to 28.9 per cent for the eight strains. Plasma of the White Leghorns was 23.1 per cent more effective in killing the organisms of all eight strains than that of the Rhode Island Reds. Both the White Leghorns and the Rhode Island Reds showed considerable variation in bactericidal activity.

For three of the strains of Salmonella pullorum the range in bactericidal activity among these fowls was from 0 to 100 per cent of the organisms killed. Strain No. 6, which was most resistant, showed the least range, 0 to 40 per cent. The plasma
Table 3. Differences in bactericidal activity of the plasma of White Leghorns and Rhode Island Reds

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>White Leghorns</th>
<th>Rhode Island Reds</th>
<th>Difference</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
<td>Degrees</td>
</tr>
<tr>
<td>1</td>
<td>56.6</td>
<td>28.9</td>
<td>27.7</td>
<td>16.3</td>
</tr>
<tr>
<td>2</td>
<td>96.2</td>
<td>89.1</td>
<td>9.1</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>69.7</td>
<td>69.2</td>
<td>19.5</td>
<td>14.1</td>
</tr>
<tr>
<td>4</td>
<td>45.7</td>
<td>29.5</td>
<td>20.4</td>
<td>12.3</td>
</tr>
<tr>
<td>5</td>
<td>76.2</td>
<td>56.6</td>
<td>19.6</td>
<td>12.0</td>
</tr>
<tr>
<td>6</td>
<td>15.1</td>
<td>3.2</td>
<td>11.9</td>
<td>12.6</td>
</tr>
<tr>
<td>7</td>
<td>71.6</td>
<td>51.8</td>
<td>19.8</td>
<td>11.8</td>
</tr>
<tr>
<td>8</td>
<td>63.2</td>
<td>34.3</td>
<td>28.9</td>
<td>16.8</td>
</tr>
<tr>
<td>All Strains</td>
<td>66.9</td>
<td>43.8</td>
<td>23.1</td>
<td>15.5</td>
</tr>
</tbody>
</table>

1 - Least significant difference at 1 per cent level, 4.60.

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>549</td>
<td>415,982.2</td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>1</td>
<td>25,166.5</td>
<td>25,166.5**</td>
</tr>
<tr>
<td>Strain</td>
<td>7</td>
<td>157,730.1</td>
<td>22,532.9**</td>
</tr>
<tr>
<td>Breed x Strain</td>
<td>7</td>
<td>123.8</td>
<td>18.4</td>
</tr>
<tr>
<td>Within subclasses</td>
<td>534</td>
<td>232,956.8</td>
<td>436.2</td>
</tr>
</tbody>
</table>

F values, Breed 57.7, Strain 51.7

** Significant at 1 per cent level.
of a fowl may be highly bactericidal against one strain, but it may be very ineffective in killing the organisms of another strain. The fowls retained somewhat the same relative standing in bactericidal activity for each of the eight strains of organisms. Typical cases are illustrated in Figure 1.

Approximately two years later, using Salmonella pullorum, Strain No. 2, comparable bactericidal tests were made of the plasma of White Leghorns, White Rocks, and New Hampshires of the Iowa State College flock. There was a significant difference in bactericidal activity of the plasma of the White Leghorns and the White Rocks, Table 4. The differences between the other breeds were nonsignificant.

Relationship with Physiological Functions

Figures 2, 3 and 4 show the relationship of body weight, body condition, and sexual maturity with the bactericidal action of the plasma. There was no significant regression of bactericidal activity on any of these factors, as seen from the following analysis:

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Degrees of Freedom</th>
<th>Regression Equation</th>
<th>t</th>
<th>Values of t at 5 per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>0.001</td>
<td>137</td>
<td>1237x + 57.47</td>
<td>.06</td>
</tr>
<tr>
<td>Body condition</td>
<td>-0.01</td>
<td>137</td>
<td>56.70 - 149.7x</td>
<td>.14</td>
</tr>
<tr>
<td>Sex. maturity - males</td>
<td>-0.14</td>
<td>82</td>
<td>57.33 - 2.5725x</td>
<td>1.24</td>
</tr>
<tr>
<td>Sex. maturity - females</td>
<td>0.18</td>
<td>139</td>
<td>1.5249x + 53.12</td>
<td>.68</td>
</tr>
</tbody>
</table>

* For regression
Figure 1. Variation in bactericidal activity of fowl plasma with the eight strains of *Salmonella pullorum*
Table 4. A comparison of the bactericidal activity of the plasma of White Leghorns, White Rocks and New Hampshires with *Salmonella pullorum*, Strain No. 2

<table>
<thead>
<tr>
<th>Breed</th>
<th>Per cent kill</th>
<th>Mean of angles - degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Leghorns</td>
<td>96.2</td>
<td>80.2</td>
</tr>
<tr>
<td>White Rocks</td>
<td>86.2</td>
<td>71.4</td>
</tr>
<tr>
<td>New Hampshires</td>
<td>92.1</td>
<td>75.4</td>
</tr>
</tbody>
</table>

Least significant difference, 8.01°.

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>52</td>
<td>5642.8</td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>2</td>
<td>688.4</td>
<td>344.2*</td>
</tr>
<tr>
<td>Error</td>
<td>50</td>
<td>4954.4</td>
<td>99.1</td>
</tr>
</tbody>
</table>

F value, 3.47

* Significant at 5 per cent level.
Figure 2. Regression of angles corresponding to the percentage of organisms killed on body weight—Rhode Island Red females.
Figure 3. Regression of angles corresponding to the percentage of organisms killed on body condition - Rhode Island Red females
Figure 4. Regression of angles corresponding to the percentage of organisms killed on sexual maturity - Rhode Island Reds
The effects of molting upon the bactericidal activity of the plasma are shown in Table 5. This group of fowls was first tested when laying and showing no molt. Approximately ten months later they were tested again when none were laying and they were in a full body molt. The fourth test was taken at the end of the molting period after all of the fowls had started to lay. There was no consistent change in bactericidal activity during the molting period.

Body weight, body condition, sexual maturity and molting, as determined in this study, apparently have no significant influence upon the bactericidal activity of the plasma.

The Bactericidal System

The bactericidal action of the sera of animals is apparently in part dependent upon a thermostable agent, amboceptor, and complement which is thermolabile. Table 6 presents the effects of heating chicken plasma for 30 minutes at 54°C. The bactericidal action was almost completely destroyed. The results were the same for hens of low or high bactericidal activity.

Attempts to reactivate the complement with pooled guinea pig plasma failed, Table 7. After adding an equal quantity of fresh guinea pig plasma, bactericidal action was observed. The bactericidal activity, however, was just slightly greater than that obtained with fresh guinea pig plasma. When heated
Table 5. Bactericidal tests taken before, during, and after a complete body molt

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Dec. 26, 1941</th>
<th>Oct. 21, 1942</th>
<th>Nov. 7, 1942</th>
<th>Feb. 9, 1943</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying - not molting Per cent</td>
<td>Not laying - full molt Per cent</td>
<td>Not laying - full molt Per cent</td>
<td>Laying - not molting Per cent</td>
<td></td>
</tr>
<tr>
<td>2A27</td>
<td>53.6</td>
<td>95.4</td>
<td>98.7</td>
<td>96.3</td>
</tr>
<tr>
<td>2A9</td>
<td>91.9</td>
<td>86.6</td>
<td>97.8</td>
<td>-</td>
</tr>
<tr>
<td>2A22</td>
<td>52.8</td>
<td>78.5</td>
<td>92.6</td>
<td>59.4</td>
</tr>
<tr>
<td>2A10</td>
<td>68.2</td>
<td>78.3</td>
<td>82.6</td>
<td>78.3</td>
</tr>
<tr>
<td>2A4</td>
<td>76.4</td>
<td>76.3</td>
<td>86.3</td>
<td>88.5</td>
</tr>
<tr>
<td>2A9</td>
<td>75.8</td>
<td>69.8</td>
<td>75.9</td>
<td>-</td>
</tr>
<tr>
<td>2A19</td>
<td>58.2</td>
<td>64.5</td>
<td>93.9</td>
<td>31.0</td>
</tr>
<tr>
<td>2A20</td>
<td>45.6</td>
<td>43.9</td>
<td>51.9</td>
<td>56.3</td>
</tr>
<tr>
<td>2A7</td>
<td>76.4</td>
<td>39.7</td>
<td>75.5</td>
<td>32.0</td>
</tr>
<tr>
<td>2A27</td>
<td>68.8</td>
<td>39.7</td>
<td>58.6</td>
<td>49.0</td>
</tr>
<tr>
<td>2A15</td>
<td>54.6</td>
<td>34.0</td>
<td>58.2</td>
<td>40.0</td>
</tr>
<tr>
<td>2A14</td>
<td>44.0</td>
<td>0.0</td>
<td>50.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Mean</td>
<td>63.9</td>
<td>58.9</td>
<td>77.2</td>
<td>57.1</td>
</tr>
</tbody>
</table>
Table 6. The effects of heating plasma for 30 minutes at 54°C

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Organisms killed</th>
<th>Fresh plasma</th>
<th>Heated plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td></td>
</tr>
<tr>
<td>3B10</td>
<td>98.2</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>3B23</td>
<td>96.3</td>
<td>.8</td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>93.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B7</td>
<td>84.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B11</td>
<td>82.5</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B2</td>
<td>68.3</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>3B31</td>
<td>52.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B13</td>
<td>34.3</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B15</td>
<td>33.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B4</td>
<td>33.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B8</td>
<td>32.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B7</td>
<td>29.6</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B3</td>
<td>26.6</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>3B1</td>
<td>25.6</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B32</td>
<td>20.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>
### Table 7: Addition of fresh guinea pig plasma to heated fowl plasma

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Fresh plasma</th>
<th>Heated plasma</th>
<th>Heated plasma + fresh GPP²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>3B4</td>
<td>93.1</td>
<td>0.0</td>
<td>10.0</td>
</tr>
<tr>
<td>3B6</td>
<td>32.1</td>
<td>0.0</td>
<td>10.8</td>
</tr>
<tr>
<td>3B15</td>
<td>33.1</td>
<td>0.0</td>
<td>23.6</td>
</tr>
<tr>
<td>3B3</td>
<td>26.6</td>
<td>2.8</td>
<td>14.7</td>
</tr>
<tr>
<td>3B7</td>
<td>29.6</td>
<td>0.0</td>
<td>14.7</td>
</tr>
<tr>
<td>3B13</td>
<td>34.3</td>
<td>0.0</td>
<td>19.0</td>
</tr>
<tr>
<td>3B11</td>
<td>82.5</td>
<td>0.0</td>
<td>16.2</td>
</tr>
<tr>
<td>3B7</td>
<td>84.1</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3B31</td>
<td>52.0</td>
<td>0.0</td>
<td>11.2</td>
</tr>
<tr>
<td>3B2</td>
<td>68.3</td>
<td>1.1</td>
<td>14.3</td>
</tr>
<tr>
<td>3B32</td>
<td>20.0</td>
<td>0.0</td>
<td>17.5</td>
</tr>
<tr>
<td>3B111</td>
<td>81.1</td>
<td>0.0</td>
<td>7.0</td>
</tr>
<tr>
<td>GPP¹</td>
<td>12.3</td>
<td>0.0</td>
<td>----</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>13.2</td>
</tr>
</tbody>
</table>

¹ - Guinea pig plasma - pooled plasma of six individuals

² - An equal quantity of fresh pooled guinea pig plasma added to heated fowl plasma
guinea pig plasma was added to fresh plasma of fowls of low kill, there was no consistent change in bactericidal action, Table 8.

The tests with guinea pig plasma revealed that neither the heat stable nor the heat labile portions would improve the bactericidal action. The failure of guinea pig plasma to activate the bactericidal activity of heated fowl plasma may have been due to the destruction of the antibodies while heating the plasma, or to the anti-complementary effects of the fowl and guinea pig plasma. Pollard, Hall, and Eichhorn (1943) reported that the complement-fixing bodies of fowl plasma were completely destroyed when heated for 20 minutes at 56°C. It is known, also, that the hemolytic complement of the fowl is anti-complementary to that of the guinea pig, Noguchi and Bronfenbrenner (1911), Hyde (1921), and others. However, the relationship between hemolytic and bactericolytic complement is not fully understood.

Table 9 gives the results of adding the same fowl’s fresh plasma to her heated plasma. In every instance bactericidal action was observed. Among the high kill group of hens, the increase in bactericidal action was proportional to the amount of plasma added. If the antibody had not been destroyed by heating and the complement had been reactivated, the bactericidal action should have been equal to or nearly the same as that obtained with fresh plasma.

The bactericidal action for the fowls of the low kill
Table 8. Addition of heated guinea pig plasma to fresh fowl plasma

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Fresh plasma</th>
<th>Fresh plasma + heated GPP&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>3B17</td>
<td>79.2</td>
<td>38.7</td>
</tr>
<tr>
<td>3B11</td>
<td>33.0</td>
<td>33.3</td>
</tr>
<tr>
<td>3B2</td>
<td>37.8</td>
<td>12.6</td>
</tr>
<tr>
<td>3B4</td>
<td>30.0</td>
<td>47.9</td>
</tr>
<tr>
<td>3B7</td>
<td>41.4</td>
<td>34.4</td>
</tr>
<tr>
<td>3B32</td>
<td>38.7</td>
<td>32.4</td>
</tr>
<tr>
<td>3B20</td>
<td>46.5</td>
<td>32.7</td>
</tr>
<tr>
<td>3B111</td>
<td>51.5</td>
<td>28.2</td>
</tr>
<tr>
<td>3B171</td>
<td>76.2</td>
<td>41.4</td>
</tr>
<tr>
<td>GPP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10.9</td>
<td>---</td>
</tr>
</tbody>
</table>

1 - Fresh pooled plasma of six guinea pigs

2 - Plasma of six guinea pigs, heated for 30 minutes at 54°C. An equal quantity of the heated plasma was added to the fresh plasma.
Table 9. Reactivation of heated plasma by the addition of fresh plasma

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Fresh plasma</th>
<th>Heated plasma</th>
<th>Heated plasma + fresh plasma&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>3B18</td>
<td>99.8</td>
<td>0.0</td>
<td>48.7</td>
</tr>
<tr>
<td>3B8</td>
<td>97.6</td>
<td>0.0</td>
<td>46.2</td>
</tr>
<tr>
<td>3B22</td>
<td>58.9</td>
<td>0.0</td>
<td>24.3</td>
</tr>
<tr>
<td>3B28</td>
<td>77.9</td>
<td>0.0</td>
<td>24.1</td>
</tr>
<tr>
<td>3B4</td>
<td>90.5</td>
<td>16.0</td>
<td>42.0</td>
</tr>
<tr>
<td>3A4</td>
<td>91.1</td>
<td>0.0</td>
<td>49.7</td>
</tr>
<tr>
<td>3B11</td>
<td>80.2</td>
<td>0.0</td>
<td>50.6</td>
</tr>
<tr>
<td>3B41</td>
<td>93.8</td>
<td>0.0</td>
<td>50.5</td>
</tr>
<tr>
<td>3B221</td>
<td>0.0</td>
<td>0.0</td>
<td>33.9</td>
</tr>
<tr>
<td>3B2</td>
<td>19.2</td>
<td>0.0</td>
<td>32.7</td>
</tr>
<tr>
<td>3B23</td>
<td>14.9</td>
<td>0.0</td>
<td>35.9</td>
</tr>
<tr>
<td>3B81</td>
<td>0.0</td>
<td>0.0</td>
<td>28.2</td>
</tr>
<tr>
<td>3B3</td>
<td>0.0</td>
<td>0.0</td>
<td>8.5</td>
</tr>
<tr>
<td>3B12</td>
<td>31.8</td>
<td>0.0</td>
<td>28.4</td>
</tr>
</tbody>
</table>

<sup>1</sup> - Addition of half a quantity of fowl's fresh plasma to her heated plasma.
group was increased by the addition of the same fowl's fresh plasma to her heated plasma. The action was greater than that obtained from the fresh plasma, Table 9. This indicates that there was a deficiency of a heat stable agent in the plasma of the low kill group of fowls. Also, if the killing action were due to complement, alone, the addition of fresh plasma from high kill fowls to heated plasma of low kill fowls should show greater bactericidal action than if the same fowl's fresh plasma were added. This was not found to be true, Table 10. The bactericidal action was about the same. Apparently the fowls of the low kill group were not deficient in complement.

Table 11 presents the results of the absorption tests with dead *Salmonella pullorum* cells of Strain No. 2. A reduction in bactericidal activity occurred in every instance. After adding the supernatant fluid of the absorbed plasma to heated plasma, the bactericidal action was raised slightly above that obtained by the absorbed plasma. Heating the plasma may have partially destroyed the antibodies. If so, it would be impossible to get complete reactivation. The reduction in bactericidal activity obtained by absorption indicates that an antibody is involved in the bactericidal system.

Fowls which reacted positively to the agglutination test for pullorum disease showed significantly less bactericidal activity than negative fowls from the same flocks tested simultaneously, Table 12. It seems that the fowls
Table 10. Addition of fresh plasma of high bactericidal activity to heated plasma

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Fresh plasma</th>
<th>Heated plasma</th>
<th>Heated plasma + fresh plasma</th>
<th>Heated plasma + fresh high plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>3B2</td>
<td>19.2</td>
<td>0.0</td>
<td>32.7</td>
<td>37.7</td>
</tr>
<tr>
<td>3B21</td>
<td>14.9</td>
<td>0.0</td>
<td>35.9</td>
<td>50.0</td>
</tr>
<tr>
<td>3B4</td>
<td>93.8</td>
<td>0.0</td>
<td>50.5</td>
<td>55.2</td>
</tr>
<tr>
<td>3B22</td>
<td>0.0</td>
<td>0.0</td>
<td>33.9</td>
<td>24.0</td>
</tr>
<tr>
<td>3B3</td>
<td>0.0</td>
<td>0.0</td>
<td>8.5</td>
<td>7.6</td>
</tr>
<tr>
<td>3B12</td>
<td>31.8</td>
<td>0.0</td>
<td>28.4</td>
<td>36.6</td>
</tr>
<tr>
<td>3B11</td>
<td>80.2</td>
<td>0.0</td>
<td>50.6</td>
<td>49.2</td>
</tr>
<tr>
<td>Pooled High</td>
<td>77.3</td>
<td>0.0</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

1 - Addition of a half quantity of fowl's fresh plasma
2 - Addition of half quantity of fresh pooled plasma of 3 hens giving high bactericidal activity
### Table 11. Absorption of plasma with cells of *Salmonella pullorum*, Strain No. 2

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Fresh Plasma</th>
<th>Absorbed Plasma</th>
<th>Heated Plasma</th>
<th>Heated Plasma + Absorbed Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
</tr>
<tr>
<td>3B11</td>
<td>90.7</td>
<td>8.6</td>
<td>0.0</td>
<td>17.5</td>
</tr>
<tr>
<td>3B111</td>
<td>73.9</td>
<td>19.3</td>
<td>0.0</td>
<td>51.1</td>
</tr>
<tr>
<td>3B21</td>
<td>77.3</td>
<td>51.4</td>
<td>0.0</td>
<td>58.6</td>
</tr>
<tr>
<td>3B10</td>
<td>65.4</td>
<td>25.2</td>
<td>0.0</td>
<td>35.9</td>
</tr>
<tr>
<td>3B14</td>
<td>53.0</td>
<td>17.3</td>
<td>0.0</td>
<td>23.2</td>
</tr>
</tbody>
</table>

1 - Added half quantity of absorbed plasma to heated plasma.
Table 12. The bactericidal activity of the plasma of fowls which reacted positively to the agglutination test for pullorum

<table>
<thead>
<tr>
<th>Breed</th>
<th>Organisms killed</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Per cent</td>
<td>Positive</td>
<td>Per cent</td>
</tr>
<tr>
<td>White Leghorns</td>
<td>88.8</td>
<td>48.3</td>
<td>40.5</td>
<td></td>
</tr>
<tr>
<td>R. I. Reds and New Hampshires</td>
<td>70.0</td>
<td>22.5</td>
<td>47.5</td>
<td></td>
</tr>
<tr>
<td>Both breeds</td>
<td>80.2</td>
<td>36.9</td>
<td>43.3</td>
<td></td>
</tr>
</tbody>
</table>

1 - Fifty-six fowls, 30 negatives and 26 positives
2 - Averages of three tests - only one test obtained of White Leghorns.

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>55</td>
<td>22627.6</td>
<td></td>
</tr>
<tr>
<td>Positive vs. negative</td>
<td>1</td>
<td>9605.5</td>
<td>9605.5**</td>
</tr>
<tr>
<td>Error</td>
<td>54</td>
<td>13022.1</td>
<td>241.2</td>
</tr>
</tbody>
</table>

F value, 39.8

** Significant at 1 per cent level.
showing positive agglutination tests should have acquired immune bactericidins, and show higher bactericidal action. An attempt was made to increase the bactericidal action of these fowls by artificial immunization with dead cells of *Salmonella pullorum* Strain No. 2. It was found after a series of 15 injections, that except for one fowl, there was no increase in bactericidal action, Table 13. This fowl showed the greatest bactericidal action before the injections were started. All but one of the control fowls gave nearly maximum bactericidal action after immunization.

Table 14 presents the results of immunizing a group of fowls found to be negative by the agglutination test, with dead cells of *Salmonella pullorum* Strain No. 2. The bactericidal activity was increased in all but two of the fowls after 15 injections. These two fowls also failed to show any response after 30 injections. Both fowls gave positive agglutination tests. The increase found in bactericidal action was probably due to the production of immune bactericidins. Since fowls of the low kill group also showed increased bactericidal action, further evidence is obtained that they were not deficient in complement.

The failure of most of the positive agglutination reactors and some of the negative reactors to show an increase in bactericidal action may be due to their inability to produce immune bactericidins. This may be a characteristic
Table 13. The effects of injecting fowls reacting positively to the pullorum agglutination test with dead cells of *Salmonella pullorum*, Strain No. 2

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Organisms killed</th>
<th>Before injections</th>
<th>After 15 injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive agglutination reactors</td>
<td>Per cent</td>
<td>Per cent</td>
<td></td>
</tr>
<tr>
<td>3246</td>
<td>6.7</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>3253</td>
<td>13.9</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>3246</td>
<td>22.0</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>3242</td>
<td>4.8</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>3244</td>
<td>52.9</td>
<td>91.1</td>
<td></td>
</tr>
<tr>
<td>Controls - negative fowls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3B32</td>
<td>15.7</td>
<td>88.2</td>
<td></td>
</tr>
<tr>
<td>3B11</td>
<td>11.8</td>
<td>99.0</td>
<td></td>
</tr>
<tr>
<td>3B111</td>
<td>17.5</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3B17</td>
<td>22.9</td>
<td>99.2</td>
<td></td>
</tr>
<tr>
<td>3B7</td>
<td>14.6</td>
<td>99.9</td>
<td></td>
</tr>
<tr>
<td>3B4</td>
<td>16.0</td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>3B20</td>
<td>22.2</td>
<td>99.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 14. The effects of injecting fowls reacting negatively to the pullorum agglutination test with dead cells of *Salmonella pullorum*, Strain No. 2

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Organisms killed</th>
<th>Before injections</th>
<th>After 10 injections</th>
<th>After 30 injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>Per cent</td>
<td></td>
</tr>
<tr>
<td>3A7</td>
<td>32.0</td>
<td>100.0</td>
<td>91.6</td>
<td></td>
</tr>
<tr>
<td>3A13</td>
<td>46.9</td>
<td>98.8</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>3A27</td>
<td>49.0</td>
<td>21.3</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>3A15</td>
<td>40.0</td>
<td>74.2</td>
<td>63.8</td>
<td></td>
</tr>
<tr>
<td>3A9</td>
<td>24.1</td>
<td>100.0</td>
<td>99.3</td>
<td></td>
</tr>
<tr>
<td>3A10</td>
<td>82.6</td>
<td>99.0</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>3A19</td>
<td>31.0</td>
<td>99.1</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>3A151</td>
<td>92.7</td>
<td>99.2</td>
<td>98.2</td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>88.5</td>
<td>98.9</td>
<td>91.1</td>
<td></td>
</tr>
<tr>
<td>3A271</td>
<td>96.3</td>
<td>75.6</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>3A152</td>
<td>40.0</td>
<td>22.5</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>3A20</td>
<td>56.3</td>
<td>83.2</td>
<td>----</td>
<td></td>
</tr>
</tbody>
</table>
of some fowls and be related to their resistance to specific
diseases.

Heritable Influences

The wide differences observed among fowls raised in the
same flock suggests that heritable influences may be contribut-
ing to the variation. In an attempt to produce lines of stock
differing in bactericidal activity, Rhode Island Red breeding
females, which were tested with all eight strains of Salmonella
pullorum, were selected on the basis of their mean percentage
of organisms killed. The mean for all fowls tested was the
dividing point for the high and low groups. Males were selected
on the basis of three tests made using Salmonella pullorum
Strain No. 2. The average percentage of organisms killed for
Sire "1C" was 26.9 per cent and that for Sire"1W", 58.0 per-
cent. The limited selection did not permit the use of males
differing widely in bactericidal activity. These males were
placed in pens containing high and low kill females.

The first and second generations of stock showed no
significant differences in bactericidal activity between
sexes. Sex was disregarded in analysing the data for these
two generations, because the progeny of some families were
all of the same sex. In the third generation there was a
significant difference between the sexes and an adjustment
was made which will be explained with the results.
The average percentage of organisms killed for the progeny of the first generation was 44.1 per cent, Table 15. The mean of the group from which their parents were selected was 89.1 per cent, Table 3. This reduction may have been caused by changes in the stock or the organism. However, tests of White Leghorns run simultaneously with those of the first generation of stock showed that the organism was still highly susceptible to bactericidins of the fowl.

Duplicate tubes were run simultaneously when making the tests. The small mean square for tubes shows that the variation due to technique is negligible, Table 15.

The mean percentages of organisms killed for the progeny of the first generation are given in Table 15. Only families that had at least three progeny were tested. Some relationship can be observed between the percentage kill of the dam and that of her progeny, among that group of dams mated to Sire "1W". This is not true for Sire "1C". The significant difference between the groups of progeny from the low and high dams of Sire "1W" indicates that selection for bactericidal activity was slightly effective in separating the groups. The difference between the sires was small and non-significant. Figures 5 and 6 give the pedigrees and the percentage of organisms killed for each progeny. The variation between members of a family is wide.
Table 15. Summary of results for first generation - 1940 stock

<table>
<thead>
<tr>
<th>Sire &quot;IW&quot; (58.0 per cent kill)</th>
<th>Sire &quot;IC&quot; (26.9 per cent kill)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam's</td>
<td>Progeny</td>
</tr>
<tr>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>Hights:</td>
<td></td>
</tr>
<tr>
<td>1507</td>
<td>79.8</td>
</tr>
<tr>
<td>1528</td>
<td>45.8</td>
</tr>
<tr>
<td>1536</td>
<td>41.8</td>
</tr>
<tr>
<td>Lows:</td>
<td></td>
</tr>
<tr>
<td>1505</td>
<td>31.3</td>
</tr>
<tr>
<td>1511</td>
<td>22.5</td>
</tr>
<tr>
<td>1517</td>
<td>27.6</td>
</tr>
<tr>
<td>1046</td>
<td>2.8</td>
</tr>
<tr>
<td>1047</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Mean 44.5 Mean for all progeny 44.1

1 - Mean of eight strains
2 - Mean for Strain No.2

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>119</td>
<td>82369.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Sires</td>
<td>1</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Dams for given sire</td>
<td>10</td>
<td>17752.2</td>
<td>1775.2</td>
</tr>
<tr>
<td>High Vs. low-Sire &quot;IW&quot;</td>
<td>1</td>
<td>6827.1</td>
<td>6827.1*</td>
</tr>
<tr>
<td>High Vs. low-Sire &quot;IC&quot;</td>
<td>1</td>
<td>175.0</td>
<td>175.0</td>
</tr>
<tr>
<td>Remainder</td>
<td>8</td>
<td>10750.1</td>
<td>1343.8</td>
</tr>
<tr>
<td>Progeny for given sire and dam</td>
<td>48</td>
<td>61681.9</td>
<td>1285.0*</td>
</tr>
<tr>
<td>Pairs (duplicate tubes)</td>
<td>60</td>
<td>2929.5</td>
<td>48.8</td>
</tr>
</tbody>
</table>

1 - Error term
F value, 5.32
* Significant at 5 per cent level.
Sire "IW"  
(58.0)  

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1507 (79.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1914 (89.9)</td>
<td></td>
</tr>
<tr>
<td>116 (97.3)</td>
<td></td>
</tr>
<tr>
<td>113 (92.4)</td>
<td></td>
</tr>
<tr>
<td>152 (84.7)</td>
<td></td>
</tr>
<tr>
<td>151 (67.9)</td>
<td></td>
</tr>
<tr>
<td>114 (26.5)</td>
<td></td>
</tr>
<tr>
<td>15 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1511 (22.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>164 (91.0)</td>
<td></td>
</tr>
<tr>
<td>119 (91.0)</td>
<td></td>
</tr>
<tr>
<td>160 (87.7)</td>
<td></td>
</tr>
<tr>
<td>162 (47.9)</td>
<td></td>
</tr>
<tr>
<td>1920 (25.8)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1528 (45.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>163 (95.3)</td>
<td></td>
</tr>
<tr>
<td>137 (92.9)</td>
<td></td>
</tr>
<tr>
<td>162 (61.5)</td>
<td></td>
</tr>
<tr>
<td>177 (54.3)</td>
<td></td>
</tr>
<tr>
<td>132 (59.6)</td>
<td></td>
</tr>
<tr>
<td>164 (14.6)</td>
<td></td>
</tr>
<tr>
<td>134 (9.5)</td>
<td></td>
</tr>
<tr>
<td>135 (1.3)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1517 (27.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>128 (62.2)</td>
<td></td>
</tr>
<tr>
<td>174 (38.8)</td>
<td></td>
</tr>
<tr>
<td>130 (24.3)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1536 (41.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>145 (91.4)</td>
<td></td>
</tr>
<tr>
<td>160 (88.3)</td>
<td></td>
</tr>
<tr>
<td>142 (60.8)</td>
<td></td>
</tr>
<tr>
<td>194 (67.9)</td>
<td></td>
</tr>
<tr>
<td>143 (60.4)</td>
<td></td>
</tr>
<tr>
<td>193 (2.0)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1046 (2.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>139 (64.8)</td>
<td></td>
</tr>
<tr>
<td>142 (49.2)</td>
<td></td>
</tr>
<tr>
<td>175 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1047 (34.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>195 (60.9)</td>
<td></td>
</tr>
<tr>
<td>155 (37.8)</td>
<td></td>
</tr>
<tr>
<td>178 (19.2)</td>
<td></td>
</tr>
<tr>
<td>151 (19.2)</td>
<td></td>
</tr>
<tr>
<td>196 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Dam 1505 (31.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 (19.9)</td>
<td></td>
</tr>
<tr>
<td>16 (16.6)</td>
<td></td>
</tr>
<tr>
<td>11 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

1 - Mean percentage kill for all 8 strains of Salmonella pullorum, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in number of the fowl specifies plan used in determining the bacterial activity.

Figure 5. Pedigrees and the percentage of organisms killed for the progeny of Sire "IW" - 1940 stock
Sire "IC" (26.9)

Progeny:

Dam 1609 (88.2)

- 167 (98.9)
- 163 (97.9)
- 145 (34.6)

Dam 167 (78.7)

- 167 (99.9)
- 150 (19.9)
- 120 (17.8)
- 166 (0.0)

Dam 1624 (25.1)

- 166 (99.9)
- 199 (0.0)
- 188 (0.0)

Dam 1630 (7.0)

- 186 (62.9)
- 191 (82.1)
- 19 (65.4)

**Figure 6. Pedigrees and the percentage of organisms killed for the progeny of Sire "IC" - 1940 stock.**

1 - Mean percentage kill for all eight strains of *Salmonella pullorum*, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in the number of the fowl specifies plan used in determining the bactericidal activity.
The small number of progeny from Sire "10" made it impossible to continue the low and high lines as originally planned. Matings were made of fowls that could possibly have been highly heterozygous, since their parents were unlike in respect to the bactericidal activity of their plasma.

The average percentage of organisms killed for the progeny of the second generation was 74.7 as compared to 44.1 for the first generation. Because of necessary changes in technique, comparisons of the two generations should not be made. To produce the second generation, breeding males showing greater bactericidal activity were used, and all but one of the breeding females were from the original high line. The organism was still highly susceptible to bactericidal action of fowl plasma. The mean percentage kill for a group of White Leghorns was 96.2, for New Hampshires 92.1, and for White Rocks 86.2, Table 4.

The results of the second generation of stock are summarized in Table 16. The only significant difference is between the sires, a mean difference in percentage of organisms killed of 9.8. The variation between dams of a given sire was not significant. The important point is the wide variability among the progeny from each dam, Figure 7. The pedigrees and percentage of organisms killed for each progeny are also given in this Figure.
Table 16. Summary of results for second generation - 1941 stock

<table>
<thead>
<tr>
<th>Sires</th>
<th>(61.5 per cent kill)</th>
<th>Sire 119 (42.8 per cent kill)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam's</td>
<td>Per cent</td>
<td>Progeny</td>
</tr>
<tr>
<td>No.</td>
<td>kill</td>
<td>No.</td>
</tr>
<tr>
<td><strong>Higns:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>88.3</td>
<td>12</td>
</tr>
<tr>
<td>162</td>
<td>81.5</td>
<td>11</td>
</tr>
<tr>
<td>163</td>
<td>95.3</td>
<td>14</td>
</tr>
<tr>
<td><strong>Lows:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>14.6</td>
<td>9</td>
</tr>
<tr>
<td>173</td>
<td>20.6</td>
<td>6</td>
</tr>
<tr>
<td>174</td>
<td>36.8</td>
<td>13</td>
</tr>
<tr>
<td>175</td>
<td>0.0</td>
<td>11</td>
</tr>
</tbody>
</table>

Mean 70.5 Mean 81.3

Mean for all progeny 74.7

Analysis of variance of angles corresponding to the percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>119</td>
<td>34289.7</td>
<td></td>
</tr>
<tr>
<td>Sires</td>
<td>1</td>
<td>1481.2</td>
<td>1481.2*</td>
</tr>
<tr>
<td>Dams for given sire</td>
<td>10</td>
<td>2020.4</td>
<td>202.0</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>30788.1</td>
<td>285.8</td>
</tr>
</tbody>
</table>

F value, Sires 5.21
* Significant at 5 per cent level.
1 - Mean percentage kill for all eight strains of *Salmonella pullorum*, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in the number of the fowl specifies plan used in determining the bactericidal activity.  2 - Mean of three tests

Figure 7. Pedigrees and the percentage of organisms killed for the progeny of the second generation - 1941 stock
1 - Mean percentage kill for all eight strains of *Salmonella pullorum*, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in the number of the fowl specifies the plan used in determining the bactericidal activity.

2 - Mean of three tests

*Figure 7.* (cont.) Pedigrees and the percentage of organisms killed for the progeny of the second generation - 1941 stock
- Mean percentage kill for all eight strains of *Salmonella pullorum*, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in the number of the fowl specifies line used in determining the bactericidal activity.

2 - Mean of three tests

Figure 7. (cont.) Pedigrees and the percentage of organisms killed for the progeny of the second generation - 1941 stock
- Mean percentage kill for all eight strains of Salmonella pullorum, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. Last numeral on the left in the number of the fowl specifies plan used in determining the bactericidal activity.

2 - Mean of three tests

Figure 7. (cont.) Pedigrees and the percentage of organisms killed for the progeny of the second generation - 1941 stock
The wide variation obtained between progeny was desirable for selection. The high individuals from the high families and low individuals from the low families were selected from the widely varying groups of progeny. Matings were made involving brothers and sisters, half-brothers and half-sisters, sons and dams, and reciprocal matings between the "highs" and the "lows." The pedigrees and the percentage of organisms killed for each progeny are given in Figures 8 and 9.

In the third generation the mean angle for the females was found to be 67.6 per cent greater than that of the males. It was desirable to adjust for sex differences since comparisons of individual matings were to be made. Each angle for the females was adjusted by multiplying by 67.6 per cent. There was no apparent reason for the difference between the sexes in this generation. The males of each generation were tested simultaneously with the females.

All of the fowls of the third generation were tested twice during a period of eight weeks. The small mean square for tests shows the variation between tests to be small, Table 17. Changes in the fowls and the organisms between tests during this period, as reflected in the bactericidal action of the plasma, were nonsignificant.

A comparison of the bactericidal activity of the progeny of the high and the low lines of the third generation of stock is given in Table 17. The mean percentage of
Figure 8. Pedigrees and the percentage of organisms killed for the progeny of the low line - 1942 stock.
Progeny

Sire "IC" (26.9)  
Dam 1617 (78.7)

Sire 120 (61.3)  
Dam 249 (91.9)

Sire 231 (97.6)  
Dam 276 (83.7)

Sire 276 (83.7)  
Dam 253 (96.8)

Sire 253 (96.8)  
Dam 254 (96.4)

Sire 254 (96.4)  
Dam 257 (93.2)

Sire 257 (93.2)  
Dam 258 (88.2)

1 - Mean percentage kill for all eight strains of Salmonella pullorum, all others are for Strain No. 2. Duplicate tubes for each fowl were run simultaneously. All of the progeny were tested twice. Last numeral on the left in each number of the fowl specifies plan used in determining the bactericidal activity.

2 - Mean of three tests

Figure 9. Pedigrees and the percentage of organisms killed for the progeny of the high line - 1942 stock
Table 17. Summary of results for the high and low lines of the third generation - 1942 stock

<table>
<thead>
<tr>
<th></th>
<th>High Line</th>
<th></th>
<th></th>
<th>Low Line</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dam's</td>
<td>Per cent</td>
<td>Progeny</td>
<td>Dam's</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>kill</td>
<td>No.</td>
<td>kill</td>
<td>No.</td>
</tr>
<tr>
<td>249</td>
<td>91.9</td>
<td>3</td>
<td>45.5</td>
<td>243</td>
<td>53.8</td>
</tr>
<tr>
<td>253</td>
<td>96.8</td>
<td>9</td>
<td>71.0</td>
<td>239</td>
<td>45.6</td>
</tr>
<tr>
<td>257</td>
<td>93.2</td>
<td>4</td>
<td>34.3</td>
<td>233</td>
<td>44.0</td>
</tr>
<tr>
<td>256</td>
<td>68.2</td>
<td>3</td>
<td>55.5</td>
<td>244</td>
<td>58.2</td>
</tr>
<tr>
<td>254</td>
<td>96.4</td>
<td>5</td>
<td>45.1</td>
<td>236</td>
<td>37.7</td>
</tr>
<tr>
<td>Mean</td>
<td>53.2</td>
<td></td>
<td></td>
<td>Mean</td>
<td>27.3</td>
</tr>
</tbody>
</table>

1 - Percentages for females have been adjusted to equal those of the males. Each progeny was tested twice.
2 - Two sires used in each line.

Analysis of variance of angles corresponding to the percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>107</td>
<td>22717.8</td>
<td></td>
</tr>
<tr>
<td>Sires</td>
<td>3</td>
<td>6591.2</td>
<td>2190.4**</td>
</tr>
<tr>
<td>High Vs. Low</td>
<td>1</td>
<td>6259.1</td>
<td>6259.1**</td>
</tr>
<tr>
<td>Remainder</td>
<td>2</td>
<td>130.1</td>
<td>65.0</td>
</tr>
<tr>
<td>Dams for given sire</td>
<td>6</td>
<td>5755.6</td>
<td>959.3**</td>
</tr>
<tr>
<td>Progeny for given sire and</td>
<td>44</td>
<td>12531.7</td>
<td>284.8**</td>
</tr>
<tr>
<td>dam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between tests</td>
<td>54</td>
<td>4039.3</td>
<td>74.8</td>
</tr>
</tbody>
</table>

P values, Sires 7.48, Dams for a given sire 5.37, High Vs. Low 21.98
** Significant at 1 per cent level.
1 - Error term
organisms killed for the high line was 53.2 and that for the low line, 27.3. The two lines are significantly well separated, the mean difference in percentage of organisms killed being 25.9.

The dams showed significant differences, which suggests that selection could be carried further. Nearly all of the variation between the sires was included in that between the high and low lines. Sires within the high and low lines showed no significant differences.

Table 18 presents a comparison of the bactericidal activity of the plasma of the progeny of a high and a low dam mated to the same male. Sire 276 and Dam 253 are full sibs, selected from the high line. Dam 242 was from the low line. The mean difference between their progeny in organisms killed, 20.1 per cent, is significant.

The results from the use of a male of low bactericidal action are presented in Table 19. In this case, Sire 273 of the low line was mated to his sister, 243, and Dam 255 of the high line. Dam 255 is a full sister of Sire 276 and Dam 253 used in the other mating. The difference between the progeny of the high and the low dams, 24.7 per cent, is significant.

The results of another mating, son x dam, referred to as Mating 250, are presented in Table 20. Sire 231 (97.6 per cent kill) was mated to his Dam 250 (81.5 per cent kill), both of the high line. Dam 250 was probably highly
Table 16. Percentage of organisms killed for the progeny of a high and a low dam mated to a high male

<table>
<thead>
<tr>
<th>Sire 276¹ (83.7 per cent kill)</th>
<th>Dam 253¹ (96.6 per cent kill)</th>
<th>Dam 242 (39.0 per cent kill)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progeny No.</td>
<td>Per cent² kill</td>
<td>Progeny No.</td>
</tr>
<tr>
<td>26</td>
<td>93.3</td>
<td>23</td>
</tr>
<tr>
<td>215</td>
<td>84.3</td>
<td>213</td>
</tr>
<tr>
<td>212</td>
<td>72.5</td>
<td>214</td>
</tr>
<tr>
<td>211</td>
<td>70.4</td>
<td>22</td>
</tr>
<tr>
<td>21</td>
<td>68.8</td>
<td>211</td>
</tr>
<tr>
<td>22</td>
<td>68.1</td>
<td>27</td>
</tr>
<tr>
<td>210</td>
<td>68.0</td>
<td>225</td>
</tr>
<tr>
<td>216</td>
<td>48.3</td>
<td>Mean</td>
</tr>
<tr>
<td>217</td>
<td>Full sibs</td>
<td></td>
</tr>
<tr>
<td>217</td>
<td>Mean of two tests</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>29</td>
<td>6430.5</td>
<td></td>
</tr>
<tr>
<td>Dams</td>
<td>1</td>
<td>1022.4</td>
<td>1022.4¹</td>
</tr>
<tr>
<td>Progeny given dam</td>
<td>13</td>
<td>2827.7</td>
<td>217.5¹</td>
</tr>
<tr>
<td>Within progeny</td>
<td>15</td>
<td>2580.37</td>
<td>172.0</td>
</tr>
</tbody>
</table>

¹ - Error term

F value, Dams 4.70

* Significant at 5 per cent level.
Table 19. Percentage of organisms killed for progeny of a high and a low dam mated to a low male

<table>
<thead>
<tr>
<th>Sire 273(^1) (31.0 per cent kill)</th>
<th>Dam 255 (96.6 per cent kill)</th>
<th>Dam 243(^1) (53.3 per cent kill)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progeny No.</td>
<td>Per cent(^2) kill</td>
<td>Progeny No.</td>
</tr>
<tr>
<td>------------</td>
<td>----------------</td>
<td>------------</td>
</tr>
<tr>
<td>221</td>
<td>67.6</td>
<td>228</td>
</tr>
<tr>
<td>21</td>
<td>53.9</td>
<td>28</td>
</tr>
<tr>
<td>217</td>
<td>49.5</td>
<td>215</td>
</tr>
<tr>
<td>213</td>
<td>32.2</td>
<td>23</td>
</tr>
<tr>
<td>211</td>
<td>17.4</td>
<td>27</td>
</tr>
<tr>
<td>Mean</td>
<td>43.6</td>
<td>18.9</td>
</tr>
</tbody>
</table>

1 - Full sibs
2 - Mean of two tests

Analysis of variance of angles corresponding to percentage of organisms killed

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>21</td>
<td>3996.2</td>
<td></td>
</tr>
<tr>
<td>Dams</td>
<td>1</td>
<td>1314.9</td>
<td>1314.9*</td>
</tr>
<tr>
<td>Progeny given dam 9</td>
<td>9</td>
<td>2299.3</td>
<td>255.5(^1)</td>
</tr>
<tr>
<td>Within progeny</td>
<td>11</td>
<td>384.0</td>
<td>34.9</td>
</tr>
</tbody>
</table>

F value, Dams 5.15

* Significant at 5 per cent level.

\(^1\) - Error term
Table 20. A comparison of the mean percentage of organisms killed for Mating 250 with those for the high and the low lines

<table>
<thead>
<tr>
<th></th>
<th>No. of progeny</th>
<th>Mean per cent kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>High line</td>
<td>24</td>
<td>53.2</td>
</tr>
<tr>
<td>Mating 250</td>
<td>14</td>
<td>37.3</td>
</tr>
<tr>
<td>Low line</td>
<td>30</td>
<td>27.3</td>
</tr>
</tbody>
</table>

1 - Mean of two tests

Analysis of variance of angles corresponding to percentage of organisms killed - High Line and Mating 250

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean of squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>75</td>
<td>16325.1</td>
<td></td>
</tr>
<tr>
<td>Progeny given dam</td>
<td>32</td>
<td>7892.6</td>
<td>246.6</td>
</tr>
<tr>
<td>Dams</td>
<td>5</td>
<td>5694.2</td>
<td>1138.8</td>
</tr>
<tr>
<td>High Vs. 250</td>
<td>1</td>
<td>1513.0*</td>
<td>1513.0*</td>
</tr>
<tr>
<td>Means of dams</td>
<td>4</td>
<td>4181.2</td>
<td>1045.3</td>
</tr>
<tr>
<td>Within progeny</td>
<td>38</td>
<td>2738.3</td>
<td>72.1</td>
</tr>
</tbody>
</table>

1 - Error term
F value, High Vs. 250 6.13
*Significant at 5 per cent level.

Analysis of variance of angles corresponding to percentage of organisms killed - Low Line and Mating 250

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean of squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>87</td>
<td>20596.4</td>
<td></td>
</tr>
<tr>
<td>Progeny given dam</td>
<td>38</td>
<td>12661.9</td>
<td>333.2</td>
</tr>
<tr>
<td>Dams</td>
<td>5</td>
<td>2410.0</td>
<td>482.0</td>
</tr>
<tr>
<td>Low Vs. 250</td>
<td>1</td>
<td>703.6</td>
<td>703.6</td>
</tr>
<tr>
<td>Means of dams</td>
<td>4</td>
<td>1706.5</td>
<td>426.6</td>
</tr>
<tr>
<td>Within progeny</td>
<td>44</td>
<td>5324.5</td>
<td>121.0</td>
</tr>
</tbody>
</table>

1 - Error term
heterozygous since her progeny of the previous generation showed a wide range in bactericidal activity. When the results of this mating are compared with those for the high line, a significant difference of 15.9 per cent of organisms killed is found, Table 20. When compared with the low line, the difference of 10.0 per cent is not significant. The mean percentage of organisms killed by the progeny of this mating is between that of the high and low lines, but somewhat nearer the low line.

For the progeny of Mating 245, Sire 269 (98.7 per cent kill) of the high line mated to Dam 245 (76.2 per cent kill) of the low line, the mean percentage of organisms killed was 88.2. The progeny of Mating 245 gave less kill than that of the high line and greater kill than the low line, Table 21. The mean was slightly closer to the low line, but it did not differ significantly from that of either line.

From the results presented, it seems probable that the bactericidal action of the plasma is in part at least influenced by heritable factors. The high and low lines are significantly separated in the third generation. The means of the percentage of organisms killed for progeny from crosses of high and low fowls were between those of the high and low lines. The inheritance is probably very complex, involving many pairs of factors. Further selections should be made in an attempt to separate the lines more widely and to obtain more homozygous stock for additional matings.
Table 21. A comparison of the mean percentage of organisms killed for Mating 245 with that for the high and the low lines

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of progeny</th>
<th>Mean per cent kill&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Line</td>
<td>24</td>
<td>53.2</td>
</tr>
<tr>
<td>Mating 245</td>
<td>9</td>
<td>38.2</td>
</tr>
<tr>
<td>Low Line</td>
<td>30</td>
<td>27.3</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean of two tests

Analysis of variance of angles corresponding to percentage of organisms killed - High Line and Mating 245

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>65</td>
<td>6941.6</td>
<td>257.1&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Progeny given dam</td>
<td>27</td>
<td>5168.1</td>
<td>1033.6</td>
</tr>
<tr>
<td>Dams</td>
<td>5</td>
<td>986.9</td>
<td>197.3&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>High Vs. 245</td>
<td>1</td>
<td>4181.2</td>
<td>1045.3</td>
</tr>
<tr>
<td>Means of dams</td>
<td>4</td>
<td>1379.3</td>
<td>344.8</td>
</tr>
<tr>
<td>Within progeny</td>
<td>35</td>
<td>1379.3</td>
<td>344.8</td>
</tr>
</tbody>
</table>

<sup>1</sup> Error term

Analysis of variance of angles corresponding to percentage of organisms killed - Low Line and Mating 245

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>77</td>
<td>17933.0</td>
<td>234.9&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Progeny given dam</td>
<td>33</td>
<td>11710.9</td>
<td>354.9&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dams</td>
<td>5</td>
<td>2316.5</td>
<td>463.1</td>
</tr>
<tr>
<td>High Vs. 245</td>
<td>1</td>
<td>610.1</td>
<td>610.1</td>
</tr>
<tr>
<td>Means of dams</td>
<td>4</td>
<td>1706.5</td>
<td>426.6</td>
</tr>
<tr>
<td>Within progeny</td>
<td>39</td>
<td>3962.6</td>
<td>101.7</td>
</tr>
</tbody>
</table>

<sup>1</sup> Error term
DISCUSSION

The plasma of the domestic fowl showed bactericidal action with eight strains of Salmonella pullorum. Among the eight different strains of Salmonella pullorum used there was considerable variation in resistance to bactericidal action. The length of time since isolation seemed to be one cause for variation, those having been isolated the longest being less resistant. Even though there was a significant difference in resistance, when the groups were divided according to length of time since isolation, within each group there was considerable variation. The two strains isolated from poults were relatively susceptible to bactericidal action of the fowl. One of these had been isolated for slightly more than two years while the other had been isolated only a relatively short time. There was no difference between these two strains in their resistance to the bactericidal activity of the fowl.

One of the strains of Salmonella pullorum was found to be highly resistant to bactericidal action. The bactericidal activity of the plasma of White Leghorns was only slightly effective in destroying these cells, and only about half of the Rhode Island Reds showed any bactericidal action. This strain had been isolated from an abdominal exudate of a New
Hampshire cockerel. Other strains isolated from adult fowls did not show such resistance.

This study has revealed that strains of a given organism can vary widely in their resistance to fowl bactericidins.

The bactericidal activity varied widely between fowls of the same breed, and also between breeds. The plasma of White Leghorns showed greater bactericidal action than that of Rhode Island Reds against each of the eight strains of Salmonella pullorum employed. White Leghorns of another strain showed greater bactericidal activity than White Rocks against Salmonella pullorum Strain No. 2. The difference in bactericidal activity between White Leghorns and New Hampshires was not significant. Some of the variation observed could probably be attributed to strains. However, Stafaeth (1942) found White Leghorns to give 20 per cent greater kill than New Hampshires and 35 per cent greater kill than Barred Rocks.

White Leghorns usually show less infection to pullorum disease than Barred Rocks, White Rocks, Rhode Island Reds, or New Hampshires. The fact that White Leghorns have been found to show a higher bactericidal activity may at least partially account for the reduced infection among these fowls. Additional support is obtained from the fact that fowls reacting positively to the pullorum disease test show little or no bactericidal activity.
No significant regression could be found of the bactericidal activity of the plasma on body weight, body condition and sexual maturity of these fowls. There was no consistent change in the bactericidal activity of the plasma of fowls taken before, during and after a full body molt.

Changes in the organisms and in the fowl during an eight-week period were not important. Two tests taken during this period of time show insignificant differences. In the third generation the females of all matings showed greater bactericidal action than the males. This was not true for the other two generations. There is no apparent reason for this difference occurring in the third generation.

The bactericidal action appears to involve a natural bactericidin and complement. In most instances when the plasma was heated for 30 minutes at 54°C, the bactericidal action was completely destroyed. It was impossible to reactivate the plasma with fresh guinea pig plasma.

When the same fowl's fresh plasma was added to her heated plasma, there was an increase in bactericidal action for those fowls of the low line. Those of the high line showed an increase proportional to the amount of fresh plasma added. The reduction in bactericidal activity may be due to destruction of the bactericidins by heating. Pollard, Hall and Eichhorn (1943) found that the complement-fixing antibodies of the fowl were highly thermolabile.
When pooled fresh plasma of high kill fowls was added to heated plasma of low kill fowls, there was no increase in bactericidal action above that obtained when the same fowl's fresh plasma was added. If the killing action were due to complement alone, it seems that there should have been an increase in bactericidal action when adding the plasma of high kill fowls.

After absorption tests, using a heavy suspension of bacterial cells of *Salmonella pullorum* Strain No. 2, there was a reduction in bactericidal action, apparently due to the removal of the antibody. When the supernatant fluid of the absorbed plasma was added to heated plasma, there was a slight increase in bactericidal action. Complete reactivation may not have been possible because of the thermolability of the antibody.

After immunization, most of the fowls giving negative agglutination tests showed increased bactericidal action. The increase was probably due to the production of an immune antibody. Since complement is apparently unaffected by immunization, it seems that the increased bactericidal activity found in the low kill fowls provides further evidence that they were not deficient in complement. Morrison (1942) determined the hemolytic complement titre of 15 fowls used in this study. There was no relationship between the bactericidal activity of the plasma and the amount of
hemolytic complement. He found the variation in hemolytic complement between the fowls to be small.

Fowls of three breeds which reacted positively to the agglutination test for pullorum showed little bactericidal action. This suggests that there may be a relationship of the bactericidal action of the plasma of fowls with resistance to pullorum. Studies of differences between chicks in bactericidal activity during the time they are most susceptible would be helpful in answering this question.

After a series of inoculations with the same strain of *Salmonella pullorum* as used in running the bactericidal tests, five of the six fowls which reacted positively to the agglutination test for pullorum showed no increase in bactericidal action. They were apparently unable to produce specific immune bactericidins. Some of the negative reactors also failed to show increased bactericidal action. The failure to produce antibodies may be a characteristic of some fowls.

Wide variation was observed among the White Leghorns and the Rhode Island Reds in their bactericidal action against each strain of *Salmonella pullorum*. Such wide variability among stock raised in the same flock suggests inherent differences. An attempt was made to produce two lines of stock differing in bactericidal action. In the first two generations no important changes occurred. Wide variation between the progeny within the families was observed. To
produce the third generation, progeny giving low kill from the "Low" dams and those giving high kill from the "High" dams were selected. In this generation the lines were significantly separated. Progeny from high and low dams mated to the same male showed significant differences in bactericidal action. The differences observed indicate that the bactericidal action of the plasma may be in part influenced by heritable factors. The inheritance is probably very complex and further studies are needed before definite conclusions can be made.
SUMMARY AND CONCLUSIONS

The plasma of the domestic fowl apparently contains natural bactericidins capable of killing Salmonella pullorum organisms. Wide differences in resistance to the bactericidal action were found between the eight strains of Salmonella pullorum employed. Those strains that had been isolated most recently were more resistant to bactericidal action. However, there was considerable variation between the strains within this group.

The plasma of White Leghorns of two different strains showed higher bactericidal activity than that of Rhode Island Reds and White Rocks. The greater bactericidal action of the plasma of White Leghorns may partially account for less infection being observed in this breed.

The bactericidal activity of the plasma was not influenced by body weight, body condition, sexual maturity or molting, as these factors were measured in this study.

The variation between tests made during an eight-week period were small. Changes in the organisms and the fowls, as they were reflected in the bactericidal activity of the plasma, were not significant.

The bactericidal action of the plasma apparently involves a natural antibody and complement. The plasma of fowls
showing low bactericidal activity was apparently not deficient in complement.

Fowls which reacted positively to the agglutination test for pullorum disease showed little bactericidal action. These fowls after immunization with dead cells of *Salmonella pullorum* Strain No. 2 showed no increase in bactericidal activity. Some of the negative reactors also failed to respond to inoculations.

Wide differences in bactericidal activity have been found between fowls of a given strain. These wide differences observed among stock reared in the same flock indicate genetic differences. After three generations of selection two lines of Rhode Island Reds have been produced differing in bactericidal activity. The results obtained indicate that the bactericidal action is in part at least influenced by heritable factors. The inheritance is probably very complex, and additional data are needed before conclusions can be made.
LITERATURE CITED

Abdosh, Y. B.

Bahler, D. R.

Bordet, Jules

Buchner, Hans

Bull, Carroll G. and Tao, Shan Ming

Colebrook, L. and Storer, E. J.
On the reduction of the bactericidal power of blood which is effected by adding it to citrate of soda and other decalcifying agents; and on the question whether blood so treated should be employed for immune-transfusions. Brit. Jour. Exp. Path. 5:47-54. 1924.

Gordon, J. and Hoyle, L.

Gordon, J. and Hoyle, L.

Gordon, J. and Johnstone, K. I.
Hodes, S. S.

Hyde, R. R.

Irwin, W. R. and Hughes, T. P.
Inheritance as a factor in resistance to an infectious disease. VI. The correlation between resistance and the bactericidal power of the whole blood. Jour. Imm. 24:345-348. 1933.

Irwin, J. R. and Ferguson, L.G.

Mackie, T. J. and Finklestein, M. H.

Mackie, T. J., Finklestein, M. H., and Van Rooyen, C. E.

Malone, R., Avari, C. R., and Naidu, B. P. U.

Miller, W. R.

Milone, Nicholas A.

Morrison, S. M.
Factors influencing the quantitative determination of complement in chicken sera. Unpublished M.S. thesis. Library, Purdue University. 1942.
Noguchi, Hideyo and Bronfenbrenner, J.

Nuttal, G.

Pettersson, Alfred

Pettersson, Alfred

Pettersson, Alfred

Pfeiffer, R.

Pollard, M., Hall, W. J., and Eichhorn, A.

Roberts, E., Severna, J. M. and Card, L. E.

Schmetzer, E. E., and Hartsell, S. E.
Purdue University, West Lafayette, Indiana. Unpublished data. 1939.

Shrigley, E. W. and Irwin, M. R.

Silverthorne, Wells
The bactericidal power of blood and protection against meningococcal infection. Jour. Imm. 33:51-56. 1937.
Stafseth, H. J.
1943.

Torrey, John C.
Bacteriolysis of the gonococcus and of the meningococcus
with normal and specific immune rabbit serum. Jour.

Walsh, V. G., and Harmsworth, Daphne
The effect of clotting and of sodium citrate on the
7:129-132. 1926.

Walsh, V.G., and Harmsworth, Daphne
The influence of coagulation on the bactericidal power

Wulf, Fred
On thermostable bactericidal substance demonstrated in
human sera, particularly during fever. Jour. Imm.
27:451-468.
Department of Botany, Purdue University, West Lafayette,

and helpful suggestions offered by Dr. S. K. Harrell,
further the gratitude acknowledged the contributors
whose given in bundling the statistical data.

...the writer wishes to express his appreciation to Dr.

ACKNOWLEDGEMENTS