A BACTERIOLOGICAL STUDY, WITH SPECIAL REFERENCE TO BACTERIUM PULLORUM, OF CHICKS DEAD IN SHELL AND CHICKS DYING WITHIN THREE WEEKS AFTER HATCHING

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INTRODUCTION

The poultryman has many obstacles with which to contend. In addition to the problems of feeding, breeding and marketing of the products, many others present themselves. The most important ones are those bearing on the health of the birds. The question of preventing this or that disease from entering the flock and how to control and eradicate these diseases when once they have occurred among the poultry are questions of economic importance.

Among the several diseases of chicks bacillary white diarrhea probably causes greater losses than any other infection.

The term "white diarrhea" has unfortunately been used to designate almost any type of intestinal trouble in which the fecal discharges are of a more or less whitish nature. As a matter of fact anything that interfers with the normal metabolism of the chick may cause the symptom of "white diarrhea". However bacillary white diarrhea infection is caused by a known specific agent, Bacterium pullorum. This microorganism is responsible for the death of thousands of baby chicks each year. It has also been shown to be a cause of

infertile eggs, chicks dying in the shell and of chicks maturing in a stunted condition. Some infected chicks grow rapidly, reach maturity and are apparently free from the disease, but nevertheless harbor the microorganism within their bodies.

The economic importance of this disease is very great and needs further investigation. This thesis, which is entitled, "A Bacteriological Study, With Special Reference to Bacterium Pullorum, of Chicks Dead in Shell and Chicks Dying Within Three Weeks After Hatching", is a part of a larger problem in which all the effects of bacillary white diarrhea are being studied.

REVIEW OF LITERATURE

Rettger (1) (2) (1900 and 1901) was the first to make extensive studies of bacillary white diarrhea. He isolated a bacillus from the livers and spleen of affected baby chicks, injected living cultures of it into other chicks and recovered the microorganism from them.

The bacillus No. 9 isolated in 1908 by Pernot (3) from unabsorbed yolk and heart blood of chickens that did not hatch or that died a few days after hatching was probably Bacterium pullorum.

In 1909, Rettger and Stoneburn (4) reported the finding of the organism in (1) the ova in the ovaries of hens, (2) yolk of fresh eggs, (3) eggs incubated for varying lengths of time and (4) yolk sacs of fully developed chicks still within the shell, which they considered as evidence that the hen was the original source of infection to the chick. They pointed out that the disease may be transmitted through an infected food supply. An experiment was run on 50 chicks whereby they received a few drops of a watery suspension of Bacterium pullorum when taken from the incubator. The microorganisms were also added to their food and drinking water. As a result of this 34 of the 50 died. Of 180 chicks treated in a similar way at 2 to 3 days of age, 13 per cent succumbed. Of 396 chicks infected in a similar way when 24 hours old about 24 per cent died as compared to 17 per cent in the control lot. Eleven strong chicks were injected subcutaneously with a pure culture of the microorganism and all died and Bacterium pullorum was recovered from the internal organs of each dead chick.

In their second report Rettger and Stoneburn (5) (1911) deal much with the source of infection and recorded finding Bacterium pullorum in the ovary of a number of hens. The disease was produced at will by 3 strains of the microorganism isolated from infected ovaries, eggs and chicks respectively. The organism was isolated from

the liver and unabsorbed yolks of the chicks. They state that infection from chick to chick cannot apparently take place after they are 3 or 4 days old. The female chicks which survive often harbor the infection and may become bacillus carriers.

Rettger, Kirkpatrick, and Stoneburn (6) (1912) in their third report state that the greatest mortality occurred among the chicks that were infected within 3 days after hatching. The most critical period of infection being the first 48 hours after hatching. They further state that with few exceptions, the deaths from typical bacillary white diarrhea occur while the chicks are under one month of age. The most satisfactory method of diagnosing bacillary white diarrhea of chicks is by a bacteriological examination.

In 1913 Jones (7) reports experiments which show that infection takes place in 3 ways, (1) through egg, (2) direct contact in the incubator from those acquiring infection through the egg and (3) by day old chicks placed in contaminated surroundings. Bacillary white diarrhea usually develops in chicks from the third to fifth day and lasts about 1 month. Four days after hatching the chicks are apparently immune to the infection.

Hadley et al (8) (1918) report that Bacterium pullorum is dextrin, dulcite and maltose negative. Bacterium pullorum A causing bacterial white diarrhea in chicks is aerogenic and produces acid and gas on dextrose, while Bacterium pullorum B causes infections in adult

fowls and is amaerogenic and only produces acid in dextrose.

Ward and Gallagher (9) (1920) contribute the following information in regard to bacillary white diarrhea. It is an acute, highly fatal septicaemia of young chicks caused by Bacterium pullorum. The infection may occur in several ways. The most common source being localized usually in the ovary of the hen. Should one or more infected eggs hatch then others in the brood are exposed and nearly all become infected. Chicks are most susceptible during the first 48 hours of life, but are practically insusceptible after the third day. The mortality rate among broods affected runs from 40 to 90 per cent. The incubation period of the disease in chicks is from 4 to 10 days. Deaths begin to occur soon after hatching and continue until the third or fourth week. They also state that artificial cultural methods are the more precise for a correct diagnosis. The causative agent may be readily isolated from the heart blood, liver, spleen, kidneys or unabsorbed yolk. It may be differentiated from other microorganisms by its cultural and biochemic characteristics and by its agglutinating properties against a homologous serum.

Canfield (10) (1925) states that the greatest losses from this disease occur among young chicks from 4 to 16 days old. Some infected chicks survive and become carriers of the disease for life. The infection also spreads through contaminated food and drinking water.

Experiments upon the livability of chicks from infected and non-infected stock showed only 22.4 per cent from reacting hens while the non-reactors showed 94.02 per cent living chicks. (He did not state whether the dead chicks were examined bacteriologically.)

Gwatkin (11) (1926) pointed out that 53 cultures obtained from outbreaks of bacillary white diarrhea were shown to be Salmonella pullora. Four strains were anaerogenic. Three out of four cultures from infections in adult fowls were anaerogenic.

Three cases of lung infection were observed in 65 chicks and not in the heart and liver. Three cases were observed in the heart and liver, when not in the lungs. He concludes that this necessitates the culturing of all three tissues.

Brunett and Bradley (1926) reports that the infected hen transmitts the microorganism from her ovary to the egg and thence to the chick. The mortality is high. The microorganism may be found in the blood, liver, lungs and the persistent yolk sac.

Many other pieces of literature were reviewed, however, most of them confirm the results obtained by the early investigators.

METHODS OF INVESTIGATION

The Experimental Fowls

Breeds.

Sixty-six fowls of the Barred Rock, Rhode Island Red and White Leghorn breeds were chosen for this experiment. Four of the birds died during the course of the project leaving 62 to complete the problem.

They were classed as reactors and non-reactors to the macroscropic agglutination test for bacillary white diarrhea. Agglutination in the 1-50 serum-antigen dilution was used as a basis for this classification. They were tested at monthly intervals.

The numbers of the reactors are as follows:

White Leghorn hens.

11	1556
753	1731
135	4284
232	4296
966	4282
1508	4287

Rhode Island Red hens.

15	17741
73	17753
95	17769
K262	17772

Barred Rock hens.

17701	17717
17707	17718
17708	17723
17711	17728
17712	17733
17714	

Three of the reactors died during the experiment, two Barred Rock hens numbered 17708 and 17717 and 1 White Leghorn numbered 1556. The ovary of 17708 macroscopically appeared normal and an attempt to isolate the Bacterium pullorum was fruitless. The ovary of hen 17717 appeared pathological and upon culturing for Bacterium pullorum, the microorganism was isolated. They both died February 23, 1927. Fowl 1556 died April 4, 1927. Her ovary presented marked alterations and Bacterium pullorum was isolated from it.

The numbers of the non-reactors are as follows:

White Leghorn hens.

18	352
26	357
116	380
142	385
185	724
293	727
347	729
348	766
	785

Rhode Island Red hens.

12	92
13	152
66	262
76	278
86	299
88	

Fowl numbered 116 died March 29. Her ovaries appeared normal and when cultured, Bacterium pullorum was not isolated.

Four non-reacting White Leghorn roosters were kept with the White Leghorn hens. There numbers are as follows:

283	0
369	774

The numbers of the non-reacting Rhode Island Red roosters are as follows:

286	96
161	

The fowls that ceased to be non-reactors are as follows: numbers 352, 76, 161 and 96. The serum of the White Leghorn hen numbered 352 agglutinated pullorum antigen at a 1-50 dilution during the March and April tests. At the time of the March and April tests the serum of a Rhode Island Red hen numbered 76 was positive to pullorum antigen in a 1-50 dilution. The serum of a Rhode Island Red rooster numbered 161 contained agglutinins for Bacterium pullorum antigen sufficient to cause a positive reaction in a 1-50 dilution at the time of the March and April tests. The serum of a Rhode Island rooster numbered 96 was positive for pullorum antigen in a 1-50 dilution at the February and April tests.

The Barred Rock hens, Rhode Island Red reacting hens, Rhode Island Red non-reacting hens and the 3 Rhode Island Red non-reacting roosters were kept together. The White Leghorn reacting hens and non-reactors and 4 non-reacting roosters were kept together, but separate from the other breeds. Each of the two groups of fowls had a poultry house of suitable construction for its quarters.

Feeding.

The birds were fed a laying mash mixed with water about ten o'clock in the morning. The evening feeding consisted of two-thirds cracked yellow corn and one-third whole wheat. Dry laying mash was before the fowls in self-feeders at all times. They also had grit and fresh water.

Handling of the Eggs.

All of the laying birds were trapnested. When a fowl was removed from the nest her number and the date of laying were recorded on the egg. The eggs were stored in an egg crate, placed in a basement room where the temperature and humidity were uniform. The egg crate was placed in a slanting position which was changed 2 or 3 times daily. No eggs for hatching were kept longer than 10 days. Double yolked or very small eggs were not set, otherwise no eggs were discarded.

When the eggs were sorted for incubation an equal number of eggs laid by non-reacting White Leghorn hens were set with those laid by reacting White Leghorn hens. For the heavy breeds an equal number of eggs from Rhode Island Red hens were set with those of the reacting Rhode Island Red and reacting Barred Rock hens combined.

Incubation.

Two incubators of a standard make were used and the directions were followed as accurately as possible. The chicks from 4 hatches are

included in this experiment. Settings were made February 16, February 24, March 13 and March 20.

The eggs from each hen were placed in wire baskets or gauze sacks on the eighteenth day of incubation. The eggs from the reactors were kept in baskets or sacks on one side of the incubator and from the non-reactors on the other side.

Handling of Chicks.

The chicks were removed from the incubator 24 hours after the date due to hatch. The identity of the non-reactors was maintained with numbered leg bands. After banding they were placed in a box by themselves. The reactors were then taken care of in the same way. Both groups of chicks were kept under the same conditions at all times, but these conditions changed as the broods were moved during the three weeks after hatching.

The first brood was kept in a coop in the veterinary hospital for one week. This coop had a kerosene burning brooder stove in the center of it and was divided into 2 equal parts by a tight partition. They were then removed to 2 brooder houses for the other two weeks. The reactors were put in one brooder house and non-reactors in the other. A coal burning brooder stove was used in each house.

The second brood was housed the entire 3 weeks in the coop in the veterinary hospital.

The third brood was housed the entire time in the 2 brooder houses.

The fourth brood was shipped to Richmond, Virginia on the afternoon of April 11. They were received there the morning of April 12 and reshipped to Blacksburg, Virginia that afternoon. They were received here the morning of April 13 and no mortality had resulted. They were brooded in the coop in the veterinary hospital for 3 weeks.

The coop in the veterinary hospital and the brooder houses were cleaned thoroughly and disinfected each time before placing a new brood in them. The brooders were kept at a temperature varying from 95°F to 100°F during the 3 weeks after each hatch.

Feeding.

The chicks were fed four times per day with a standard brand of chick mash. As a safeguard against so-called "leg weakness" about 1 per cent of cod liver oil was added to the mash. Fresh water and grit were kept before the chicks all the time.

Technic of Chick Examination.

The yolk of each chick that had "pipped" the shell was cultured for Bacterium pullorum. The egg was well flamed and the shell picked off the chick with flamed forceps. The area of the abdomen over the yolk sac was well flamed and then seared with a red hot knife. The abdomen was picked up with a flamed forceps and a red hot knife

Omission

When the fourth hatch was shipped to Richmond, Virginia the chicks from non-reacting and reacting hens were shipped in separate shipping boxes.

The following chicks were too weak to ship, so they were killed and autopsied:

White Leghorn non-reactors

694	696
695	697

Rhode Island Red non-reactors

698	693

White Leghorn reactors

720	722
721	718
	719

used to open the yolk sac with one quick insert. The contents of the yolk sac were aspirated into a sterile pipette and put into a test tube containing nutrient broth. The tube was labeled with the hen number and date laid, and allowed to incubate from 36 to 48 hours at 37°C. All nutrient broth and agar used in this problem was adjusted to a pH of 7.4 to 7.6 by the Medalia Method (13).

The dead chicks were examined by the following method: The leg band was removed and number recorded on a petri dish cover along with the date. The petri dish cover and bottom were marked off in 3 parts and labeled liver, heart and yolk, respectively. The chick was then flamed until the feathers were burned off. It was then placed, ventral side uppermost, on a dissecting board which had been previously washed with a 2 per cent trikresol solution. The skin was picked up with a flamed forceps and a median incision was made from above the anus through the breast bone with a pair of flamed scissors. The thoracic and abdominal organs were then exposed. A lobe of the liver was then secured with a flamed forceps and cut off with a pair of flamed scissors. The tissue was then flamed and a small piece was cut off, leaving fresh tissue exposed. The exposed end was gently rubbed over the surface of agar in the petri dish. The same procedure was followed for the heart. The technic used with the yolk sac varied with the consistency of its contents. If the yolk was unabsorbed, the sac was seared with a red hot knife, picked up with a pair of flamed forceps,

punctured and about 1 cc. of the contents aspirated into a sterile pipette. It was then transferred to nutrient broth. If the yolk was inspissated it was removed from the chick, flamed, severed in two and streaked on agar. The bone marrow was cultured by cutting the femur from the body with hot scissors, flaming the end of the bone, and making a transfer with a sterile straight platinum needle from the marrow to nutrient broth.

The agar plate and tubes of nutrient broth were placed in the incubator at a temperature of 37°C.

The inoculated agar plates and tubes of nutrient broth were allowed to incubate for 36 to 48 hours. The inoculated agar plates were then examined and any colonies resembling Bacterium pullorum were fished and transferred to agar slants for further study. The tubes of nutrient broth which had been inoculated from bone marrow were examined for cloudiness of the medium. Any that remained clear after 48 hours incubation were considered to be free of Bacterium pullorum. Those that were cloudy or the least suspicious were plated on agar by the loop dilution method using two plates for each broth culture. Tubes of nutrient broth containing yolk were plated out in a way similar to that employed with the tubes inoculated from the bone marrow. These plates were allowed to incubate for 48 hours at a temperature of 37°C. The plates were then removed from the incubator and examined macroscopically and microscopically for colonies of

Bacterium pullorum. Any suspicious colonies were marked. The marked colonies were then transferred to agar slants, incubated for 72 hours at a temperature of 37°C, and then placed in a dark chamber at room temperature where all cultures were held until ready for a differential diagnosis by fermentation and agglutination tests.

IDENTIFICATION OF CULTURES

Fermentation Tests

The cultures were transferred from the agar slants to nutrient broth and incubated for 24 hours at a temperature of 37°C.

The cultures were then transferred to the following carbohydrate media: dextrose, dextrine, dulcite and maltose. They were then incubated for 72 hours at 37°C after which readings were taken and recorded. The carbohydrate media were prepared as follows: nutrient broth was made which consisted of the following ingredients;- .3 per cent Meat Extract "Difco" standardized, .5 per cent sodium chloride (C. P), 1 per cent peptone "Difco" standardized, and sufficient distilled water to make the desired amount required. This was autoclaved for 2 hours at 15 pounds pressure. The medium was then removed and titrated to a pH of 7.2 by the "Medalia Method" (13). The carbohydrates were added in an amount to make a .5 per cent solution. Andrade's indicator was added in an amount to make a 1 per cent solution. Each carbohydrate medium was put into sterile test tubes which contained Dunham fermentation tubes inverted. The media were then autoclaved for 30 minutes at a pressure of 5 pounds. When the autoclaving process was about over the pressure was momentarily increased to 10 pounds and then released quickly. This procedure as a rule was successful in filling the fermentation tubes.

Regardless of the fermentative characteristics of the various cultures, agglutination tests were run on each.

Agglutination tests.

Agglutination tests were run on every culture isolated during this experiment. The culture was washed from the agar slant with sterile .2 per cent phenolated physiological saline solution. The bacterial suspension was then passed through a sterile glass-wool filter into a sterile test tube. The antigen was then standardized to a turbidity corresponding with McFarland's nephelometer tube .75. The antigen was then put into Wassermann vials as follows: In one row consisting of five vials, 1 cc. of antigen was put into the first vial and .5 cc. into each of the other four. The last vial was used as a control. In another row of four vials, 1 cc. of antigen was put into the first vial and .5 cc. into the other three. This row of four vials was used to test the antigen with known negative serum.

Into the first vial of the row of five tubes was put 4 cc. of known positive serum making a dilution of 1-25. The contents were then mixed with a sterile pipette and .5 cc. removed and put into tube number 2, making a serum-antigen dilution of 1-50. This same procedure was repeated in the other two tubes, which made serum-antigen dilutions of 1-100 and 1-200 respectively. The same technic was repeated with the row of four vials using negative serum. The

serum-antigen dilutions and control were then incubated at 37°C for 24 hours, removed and placed at room temperature for 24 hours then readings were taken and recorded.

RESULTS OF INVESTIGATION

The results of this problem are such that they can be presented very well by tables. The tabulated results are given by breeds, with the breeds divided into groups according as to whether the serum agglutination reaction of the birds in each group was positive or negative. This data includes the number of settings, number of eggs set, number eggs pipped, number of pipped eggs from which Bacterium pullorum was isolated, total number hatched, total number of chicks dying within 3 weeks after hatching, percentage of the same, number of dead chicks from which Bacterium pullorum was isolated and percentage of the same.

Tables number 1 through 5 show the results as just described.

Table I. Tabulated data of the 4 settings of White Leghorn non-reactors.

Setting	No. eggs set	No. Pipped	Pipped B.P.*	Hatched	Died 3wks	Per Cent died	Died of B.P.*	Per cent dead B.P.* Isolated
1	28	2	-	17	1	5.8	-	-
2	48	1	1	35	17	48.5	15	42.8
3	50	-	-	38	10	26.3	3	7.8
4	44	-	-	32	9	28.1	3	9.3
Total	170	3	1	122	37	**30.3	21	***17.2

*B. P. Bacterium pullorum
**Average percentage of total dead in 3 weeks
***Average percentage of dead in which Bacterium pullorum was isolated

Table II. Tabulated data of the 4 settings of White Leghorn reactors.

Setting	No.eggs set	No.Pipped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
1	28	1	-	11	3	27.2	3	27.2
2	48	1	1	23	10	43.4	7	30.4
3	50	1	-	35	12	34.2	9	25.7
4	44	2	1	26	11	42.3	5	19.2
Total	170	5	2	95	36	**37.8	24	***24.2

Table III. Tabulated data of the 4 settings of Rhode Island Red non-reactors.

Setting	No.eggs set	No.Pipped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
1	51	1	-	27	3	11.1	3	11.1
2	39	-	-	18	17	94.4	17	94.4
3	47	2	-	17	8	47.0	7	41.1
4	53	2	1	27	20	74.0	14	51.8
Total	190	5	1	89	48	**53.9	41	*** 46.0

Table IV. Tabulated data of the 4 settings of Rhode Island Red reactors.

Setting	No.eggs set	No.Pipped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
1	19	1	-	9	7	77.7	7	77.7
2	14	1	1	9	8	88.8	8	88.8
3	19	-	-	10	10	100.0	5	50.0
4	20	1	1	15	15	100.0	13	86.6
Total	72	3	2	43	40	** 93.0	33	***76.7

Table V. Tabulated data of the 4 settings of Barred Rock reactors.

Setting	No.eggs set	No.Pipped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
1	32	1	-	12	8	66.6	8	66.6
2	25	-	-	3	2	66.6	2	66.6
3	28	-	-	9	6	66.6	5	55.5
4	33	4	1	7	7	100.0	6	85.7
Total	118	5	1	31	23	** 74.1	21	***67.7

*B. P. Bacterium pullorum
**Average percentage of total dead in 3 weeks
***Average percentage of dead in which Bacterium pullorum was isolated

The results of the fermentative and agglutinative studies of microorganisms isolated from tissues of baby chicks are tabulated in the following tables, 6 through 23:

Legend for tables 6 through 23

NR following "chick" signifies that the egg from which the chick was hatched was laid by a non-reacting hen. R by a reacting hen. The number after either of the above is the leg band number which identifies the chick. The letters BR, RIR or WL after the word "hen" designate the breeds, Barred Rock, Rhode Island Red or White Leghorn respectively. The number after the above mentioned letters indicates the hen that laid the egg from which the chick was hatched.

Tissue exam	Tissues examined
L	Liver
H	Heart
Y	Yolk
BM	Bone marrow
D'ose	Dextrose
D'ine	Dextrine
Dulc	Dulcite
Malt	Maltose
Agg. Reaction	Agglutination Reaction
25	serum-antigen dilution 1-25
50	serum-antigen dilution 1-50
100	serum-antigen dilution 1-100
200	serum-antigen dilution 1-200
Positive serum	Identifying number of reacting fowl whose serum was used to test the agglutination affinity of the culture.

A blank space after any one of the letters L. H. Y. or BM indicates that Bacterium pullorum was not isolated.

N. S.	Indicates that chicks were not shipped to Richmond, Virginia.
D. B.	Indicates chick died in a basket in the incubator.

- -	Carbohydrates not fermented
+ -	Acid production
+ +	Acid and gas production

Table VI. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

First Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 541	L									
Hen WL 348	H									
Date died	Y									
3/14/27	BM									
Chick R 407	L									
Hen WL 753	H									
Date died	Y									
3/19/27	BM	+ -	- -	- -	- -	4	4	4	-	4284
Chick R 414	L	+ -	- -	- -	- -	4	4	4	-	232
Hen WL 966	H									
Date died	Y									
3/28/27	BM									
Chick R 431	L	+ +	- -	- -	- -	4	4	4	4	232
Hen WL 1508	H									
Date died	Y	C								
3/17/27	BM	+ +	- -	- -	- -	4	4	4	4	17753
Chick NR 532	L	+ -	- -	- -	- -	4	4	4	-	232
Hen RIR 12	H									
Date died	Y									
3/30/27	BM	+ -	- -	- -	- -	4	4	-	-	15
Chick NR 538	L	+ +	- -	- -	- -	4	4	4	4	966
Hen RIR 13	H	+ +	- -	- -	- -	4	4	4	4	966
Date died	Y									
3/23/27	BM	+ -	- -	- -	- -	4	4	4	4	17707
Chick NR 540	L	+ +	- -	- -	- -	4	4	4	4	232
Hen RIR 13	H	+ -	- -	- -	- -	4	4	4	4	17701
Date died	Y									
3/30/27	BM	+ +	- -	- -	- -	4	4	4	4	17707
Chick R 418	L	+ +	- -	- -	- -	4	4	4	4	1756
Hen RIR 73	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	K262
3/16/27	BM	+ +	- -	- -	- -	4	4	4	4	K262
Chick R 402	L	+ +	- -	- -	- -	4	4	4	4	17741
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	73
3/17/27	BM	+ +	- -	- -	- -	4	4	4	4	17741
Chick R 420	L									
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	4	17772
Date died	Y	+ +	- -	- -	- -	4	4	4	-	15
3/16/27	BM	+ +	- -	- -	- -	4	4	4	4	966
Chick R 421	L									
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17723
3/14/27	BM	+ +	- -	- -	- -	4	4	4	4	753

C* Contaminated

Table VII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

First Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ose	D'ine	Dulc	Malt	25	50	100	200	
Chick R 422	L									
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17723
3/15/27	BM									
Chick R 423	L									
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	-	73
3/16/27	BM									
Chick R 424	L									
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
3/20/27	BM									
Chick R401	L	+ +	- -	- -	- -	4	4	4	4	17728
Hen BR 17708	H	+ +	- -	- -	- -	4	4	4	4	17714
Date died	Y	+ +	- -	- -	- -	4	4	4	4	4296
3/24/27	BM									
Chick R411	L	+ -	- -	- -	- -	4	4	4	4	95
Hen BR 17708	H									
Date died	Y	C*								
3/17/27	BM	+ -	- -	- -	- -	4	4	4	4	73
Chick R 412	L	+ +	- -	- -	- -	4	4	4	4	95
Hen BR 17708	H									
Date died	Y	+ -	- -	- -	- -	4	4	4	-	15
3/16/27	BM	+ -	- -	- -	- -	4	4	4	4	15
Chick R 413	L	+ -	- -	- -	- -	4	4	4	4	73
Hen BR 17708	H	+ -	- -	- -	- -	4	4	4	-	4284
Date died	Y	+ +	- -	- -	- -	4	4	4	-	4282
3/19/27	BM	+ -	- -	- -	- -	4	4	4	4	4287
Chick R 3	L									
Hen BR 17712	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	753
3/14/27	BM									
Chick R 408	L									
Hen BR 17712	H									
Date died	Y									
3/25/27	BM	+ +	- -	- -	- -	4	4	4	4	17723
Chick R 404	L									
Hen BR 17723	H									
Date died	Y	+ -	- -	- -	- -	4	4	4	4	1556
3/16/27	BM	+ -	- -	- -	- -	4	4	4	4	K262
Chick R 426	L	+ +	- -	- -	- -	4	4	4	4	15
Hen BR 17733	H	+ +	- -	- -	- -	4	4	4	4	K262
Date died	Y									
3/17/27	BM	+ +	- -	- -	- -	4	4	4	4	17769

*C Contaminated

Table VIII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Second Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 557	L	+ +	- -	- -	- -	4	4	4	4	17728
Hen WL 18	H	+ +	- -	- -	- -	4	4	4	-	232
Date died	Y	+ -	- -	- -	- -	4	4	4	-	17712
3/27/27	BM									
Chick NR 560	L	+ +	- -	- -	- -	4	4	4	4	966
Hen WL 26	H	+ +	- -	- -	- -	4	4	4	4	966
Date died	Y	+ +	- -	- -	- -	4	4	4	4	4282
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	17707
Chick NR 561	L	+ +	- -	- -	- -	4	4	4	-	1556
Hen WL 26	H	+ +	- -	- -	- -	4	4	4	-	1556
Date died	Y									
3/23/27	BM	+ +	- -	- -	- -	4	-	-	-	17712
Chick NR 563	L									
Hen WL 26	H									
Date died	Y									
3/24/27	BM	+ -	- -	- -	- -	4	4	4	4	17712
Chick NR 564	L	+ +	- -	- -	- -	4	4	4	4	4296
Hen WL 26	H									
Date died	Y									
3/28/27	BM	+ +	- -	- -	- -	4	4	4	4	17712
Chick NR 590	L	+ +	- -	- -	- -	4	4	4	4	4296
Hen WL 185	H	+ +	- -	- -	- -	4	4	4	4	4296
Date died	Y									
3/28/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick NR 592	L	-								
Hen WL 185	H									
Date died	Y									
3/24/27	BM									
Chick NR 593	L									
Hen WL 185	H									
Date died	Y	+ +	- -	- +	- -	4	4	4	4	17728
3/28/27	BM	- -	- -	- -	- -					
Chick NR 594	L	+ -	- -	- -	- -	4	4	4	4	17731
Hen WL 185	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17707
3/28/27	BM									
Chick NR 581	L									
Hen WL 293	H	+ +	- -	- -	- -	4	4	4	4	753
Date died	Y	+ +	- -	- -	- -	4	4	-	-	4282
3/22/27	BM	+ +	- -	- -	- -	4	4	4	4	4282
Chick NR 582	L	+ +	- -	- -	- -	4	4	4	4	17714
Hen WL 293	H									
Date died	Y	+ +	- -	- -	- -	4	-	-	-	4282
3/24/27	BM	+ +	- -	- -	- -	4	-	-	-	17733

Table IX. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Second Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ose	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 583	L	+ +	- -	- -	- -	4	-	-	-	2328
Hen WL 293	H									
Date died	Y	C								
3/28/27	BM									
Chick NR 566	L	+ +	- -	- -	- -	4	4	4	4	753
Hen WL 727	H	+ +	- -	- -	+ -	4	4	4	-	753
Date died	Y									
3/22/27	BM	+ -	- -	- -	- -	4	4	4	-	1756
Chick NR 568	L									
Hen WL 727	H									
Date died	Y									
3/28/27	BM									
Chick NR 595	L	+ +	- -	- -	- -	4	4	4	4	17707
Hen WL 729	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17707
3/26/27	BM	+ +	- -	- -	- -	4	4	4	-	17712
Chick NR 578	L	+ +	- -	- -	- -	4	4	4	4	17701
Hen WL 766	H									
Date died	Y									
4/2/27	BM									
Chick NR 579	L									
Hen WL 766	H	+ -	- -	- -	- -	4	4	4	44	4287
Date died	Y	+ -	- -	- -	- -	4	4	4	4	17701
4/3/27	BM	-								
Chick R 441	L									
Hen WL 232	H									
Date died	Y	+ -	- -	- -	- -	4	4	4	-	17701
3/25/27	BM	+ -	- -	- -	- -	4	4	4	4	17728
Chick R 442	L									
Hen WL 232	H	+ +	- -	- -	- -	4	4	-	-	17731
Date died	Y									
3/24/27	BM									
Chick R 443	L	+ +	- -	- -	- -	4	4	4	-	753
Hen WL 232	H	+ +	- -	- -	- -	4	4	4	4	17723
Date died	Y									
3/22/27	BM	+ +	- -	- -	- -	4	4	4	4	753
Chick R 444	L									
Hen WL 232	H	+ -	- -	- -	- -	4	4	4	4	17731
Date died	Y									
3/26/27	BM									
Chick R 445	L	+ +	- -	- -	- -	4	4	4	-	17728
Hen WL 232	H	+ +	- -	- -	- -	4	4	4	-	17728
Date died	Y	+ +	- -	- -	- -	4	4	4	-	17723
3/24/27	BM									

C* Contaminated

Table X. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Second Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ose	D'ine	Dulc	Malt	25	50	100	200	
Chick R 453	L	+ -	- -	- -	- -	4	4	4	4	17731
Hen WL 4284	H	+ +	- -	- -	- -	4	4	4	4	17714
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17718
3/28/27	BM	+ -	- -	- -	- -	4	4	4	4	232
Chick R 455	L									
Hen WL 4284	H									
Date died	Y									
3/28/27	BM									
Chick R 452	L	+ +	- -	- -	- -	4	4	4	4	4296
Hen WL 4296	H									
Date died	Y									
3/25/27	BM									
Chick R 465	L									
Hen WL 4282	H									
Date died	Y									
3/23/27	BM									
Chick R 466	L									
Hen WL 4282	H									
Date died	Y									
3/24/27	BM									
Chick NR 596	L									
Hen RIR 12	H									
Date died	Y	+ +	- -	- -	- -	4	-	-	-	4282
3/22/27	BM									
Chick NR 597	L									
Hen RIR 12	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	-	17712
3/23/27	BM									
Chick NR 548	L	+ +	- -	- -	- -	4	4	-	-	17728
Hen RIR 13	H									
Date died	Y									
3/25/27	BM	+ -	- -	- -	- -	4	4	4	4	4287
Chick NR 549	L	+ +	- -	- -	- -	4	4	4	4	17714
Hen RIR 13	H	+ -	- -	- -	- -	4	4	4	-	4282
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17707
3/27/27	BM	+ +	- -	- -	- -	4	4	4	4	4287
Chick NR 550	L	+ +	- -	- -	- -	4	4	4	4	17728
Hen RIR 13	H	+ +	- -	- -	- -	4	4	4	4	17714
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17731
3/27/27	BM	+ +	- -	- -	- -	4	4	4	4	17714
Chick NR 551	L									
Hen RIR 13	H									
Date died	Y	+ -	- -	- -	+ -	4	4	4	4	4296
3/27/27	BM									

Table XI. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Second Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 570	L									
Hen RIR 76	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17714
3/24/27	BM									
Chick NR 571	L	- -	-							
Hen RIR 76	H									
Date died	Y	+ -	- -	- -	+ -	4	4	4	4	4282
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	4296
Chick NR 572	L	+ -	- -	- -	+ -	4	4	4	-	1556
Hen RIR 76	H	+ +	- -	- -	- -	4	4	4	4	1556
Date died	Y									
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	4282
Chick NR 573	L	+ +	- -	- -	- -	4	4	4	4	966
Hen RIR 76	H	+ +	- -	- -	- -	4	4	4	4	966
Date died	Y									
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	K262
Chick NR 547	L	+ +	- -	- -	- -	4	4	4	4	4296
Hen RIR 88	H	+ +	- -	- -	- -	4	4	4	4	4296
Date died	Y									
3/28/27	BM									
Chick NR 598	L	+ +	- -	- -	- -	4	4	4	4	17707
Hen RIR 88	H	+ +	- -	- -	- -	4	4	4	4	17707
Date died	Y									
3/26/27	BM	+ +	- -	- -	- -	4	4	4	4	17712
Chick NR 574	L									
Hen RIR 152	H	+ +	- -	- -	- -	4	4	4	4	966
Date died	Y	+ -	- -	- -	- -	4	4	4	4	17712
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	4287
Chick NR 575	L	+ +	- -	- -	- -	4	4	4	-	17707
Hen RIR 152	H	+ +	- -	- -	- -	4	4	4	4	17731
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17707
3/26/27	BM	+ +	- -	- -	- -	4	4	-	-	17701
Chick NR 577	L	+ +	- -	- -	- -	4	4	4	4	17731
Hen RIR 152	H	+ +	- -	- -	- -	4	4	4	4	4282
Date died	Y									
3/31/27	BM									
Chick NR 552	L									
Hen RIR 262	H	+ +	- -	- -	- -	4	4	4	4	4287
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
3/27/27	BM									
Chick NR 553	L									
Hen RIR 262	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17731
3/26/27	BM	+ +	- -	- -	- -	4	4	4	4	17731

Table XII. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Second Hatch	Tissue exam	Carbohydrate Reaction D'ese	D'ine	Dulc	Malt	Agg. Reaction 25	50	100	200	Positive serum
Chick R 437	L	C								
Hen RIR 73	H	C								
Date died	Y	+ +	- -	- -	- -	4	-	-	-	4282
3/25/27	BM	+ -	- -	- -	- -	4	4	-	-	15
Chick R 438	L	+ +	- -	- -	- -	4	4	4	4	966
Hen RIR 73	H	+ +	- -	- -	- -	4	4	4	-	1556
Date died	Y									
3/23/27	BM	+ +	- -	- -	- -	4	4	4	4	17712
Chick R 440	L	+ +	- -	- -	- -	4	4	4	4	17714
Hen RIR 73	H	-								
Date died	Y	+ +	- -	- -	- -	4	4	4	4	4284
3/24/27	BM	+ +	- -	- -	+ -	4	4	4	4	17718
Chick R 446	L	+ +	- -	- -	- -	4	4	4	4	17714
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	4	17714
Date died	Y	+ +	- -	- -	- -	4	-	-	-	4296
3/24/27	BM	+ +	- -	- -	- -	4	4	4	4	17712
Chick R 447	L									
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	4	4282
Date died	Y	+ +	- -	- -	- -	4	4	4	4	966
3/22/27	BM	+ +	- -	- -	- -	4	4	4	4	4282
Chick R 448	L	+ +	- -	- -	- -	4	4	4	4	753
Hen RIR 95	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	753
3/22/27	BM	+ +	- -	- -	- -	4	4	4	4	966
Chick R 433	L	+ +	- -	- -	- -	4	4	4	4	17728
Hen RIR 17772	H	+ -	- -	- -	- -	4	4	4	4	332
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17728
3/28/27	BM	+ -	- -	- -	- -	4	4	4	4	232
Chick R D.B.	L									
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	-	4284
Date died	Y	+ +	- -	- -	- -	4	4	4	4	4282
3/18/27	BM									
Chick R 432	L									
Hen BR 17708	H									
Date died	Y									
3/30/27	BM	+ +	- -	- -	- -	4	4	4	4	4287
Chick R 435	L									
Hen BR 17728	H	+ +	- -	- -	- -	4	4	4	-	17733
Date died	Y	+ +	- -	- -	- -	4	4	4	-	17712
3/31/27	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									

C* Contaminated

Table XIII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Third Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 599	L									
Hen WL 26	H									
Date died	Y									
4/16/27	BM									
Chick NR 631	L	+ +	- -	- -	- -	4	4	4	4	15
Hen WL 142	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	15
4/21/27	BM									
ChickNR 614	L									
Hen WL 185	H									
Date died	Y									
4/15/27	BM									
Chick NR 635	L									
Hen WL 293	H									
Date died	Y	C								
4/10/27	BM									
Chick NR 623	L									
Hen WL 347	H									
Date died	Y									
4/9/27	BM									
Chick NR 619	L	+ +	- -	- -	- -	4	4	4	4	17733
Hen WL 348	H	+ +	- -	- -	- -	4	4	4	-	17733
Date died	Y									
4/13/27	BM									
Chick NR 628	L									
Hen WL 352	H									
Date died	Y									
4/9/27	BM									
Chick NR 605	L									
Hen WL 385	H									
Date died	Y									
4/23/27	BM									
Chick NR 601	L									
Hen WL 766	H									
Date died	Y									
4/23/27	BM									
Chick NR 602	L									
Hen WL 766	H									
Date died	Y									
4/9/27	BM	+ +	- -	- -	- -	4	4	4	4	135
Chick R 499	L	+ -	- -	- -	- -	4*	4	4	4	17723
Hen WL 966	H									
Date died	Y									
4/24/27	BM									

*Cloudy
C Contaminated

Table XIV.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Third Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick R 500	L									
Hen WL 966	H									
Date died 4/13/27	Y									
	BM									
Chick R 473	L									
Hen WL 1731	H									
Date died 4/10/27	Y									
	BM									
Chick R 713	L									
Hen WL 4284	H									
Date died 4/17/27	Y									
	BM									
Chick R 701	L	+ +	- -	- -	- -	4	4	4	4	135
Hen WL 4296	H	+ +	- -	- -	- -	4	4	4	4	11
Date died 4/19/27	Y	+ +	- -	- -	- -	4	4	4	4	135
	BM									
Chick R 702	L	+ +	- -	- -	+ -	4	4	4	4	17733
Hen WL 4296	H	+ +	- -	- -	- -	4	4	4	4	4284
Date died 4/12/27	Y	+ +	- -	- -	- -	4	4	4	4	4284
	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 703	L	+ +	- -	- -	- -	4	4	4	4	11
Hen WL 4296	H	+ +	- -	--	- -	4	4	4	4	135
Date died 4/20/27	Y	+ +	- -	- -	- -	4	4	4	4	17733
	BM									
Chick R 704	L	+ +	- -	- -	- -	4	4	4	4	17711
Hen WL 4296	H									
Date died 4/15/27	Y									
	BM									
Chick R D.B.	L									
Hen Wl 4296	H									
Date died 4/4/27	Y									
	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 705	L									
Hen Wl 4287	H									
Date died 4/12/27	Y	+ +	- -	- -	- -	4	4	-	-	4284
	BM									
Chick R 708	L	+ +	- -	- -	- -	4	4	4	4	17701
Hen WL 4287	H	+ +	- -	- -	- -	4	4	4	4	17701
Date died 4/9/27	Y	+ +	- -	- -	- -	4	4	4	4	17733
	BM									
Chick R 709	L									
Hen WL 4287	H									
Date died 4/17/27	Y	+ -	- -	- -	- -	4	4	4	4	17701
	BM									

Table XV. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Third Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 647	L	+ -	- -	- -	+ -	4	4	-	-	17733
Hen RIR 13	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	-	4287
4/12/27	BM									
Chick NR 636	L									
Hen RIR 66	H									
Date died	Y									
4/10/27	BM									
Chick NR 640	L	+ +	- -	- -	- -	4	4	4	-	17728
Hen RIR 88	H									
Date died	Y									
4/11/27	BM									
Chick NR 642	L									
Hen RIR 92	H	+ -	- -	- -	- -	4	4	4	4	17728
Date died	Y									
4/11/27	BM	+ +	- -	- -	- -	4	4	4	4	4284
Chick NR 643	L	+ +	- -	- -	- -	4	4	4	4	17701
Hen RIR 92	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17733
4/10/27	BM	+ +	- -	- -	- -	4	4	4	4	17718
Chick NR 652	L									
Hen RIR 262	H	+ -	- -	- -	+ -	4	4*	4*	4*	K262
Date died	Y									
4/15/27	BM									
Chick NR 653	L	+ +	- -	- -	- -	4	4	4	4	4284
Hen RIR 262	H									
Date died	Y									
4/16/27	BM	+ +	- -	- -	- -	4	4	4	4	17753
Chick NR 641	L	+ +	- -	- -	- -	4	4	-	-	17733
Hen RIR 299	H									
Date died	Y									
4/11/27	BM									
Chick R 481	L	+ +	- -	- -	- -	4	4	4	4	135
Hen RIR 15	H									
Date died	Y									
4/14/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 482	L									
Hen RIR 15	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	135
4/9/27	BM									
Chick R D.B.	L									
Hen RIR 15	H									
Date died	Y									
4/4/27	BM									

Table XVI.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Third Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick R 483	L									
Hen RIR 15	H									
Date died	Y	N-C								
4/12/27	BM									
Chick R 477	L	+ +	- -	- -	- -	4	4	4	-	4284
Hen RIR 73	H									
Date died	Y									
4/13/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 478	L									
Hen RIR 73	H									
Date died	Y									
4/18/27	BM									
Chick R 479	L									
Hen RIR 73	H									
Date died	Y									
4/13/27	BM									
Chick R 480	L									
Hen RIR 73	H									
Date died	Y									
4/16/27	BM	+ -	- -	- -	- -	4	4	4	4	17723
Chick R 467	L	+ +	- -	- -	- -	4	4	4	4	17718
Hen RIR 95	H									
Date died	Y									
4/11/27	BM									
Chick R 468	L									
Hen RIR 95	H									
Date died	Y									
4/11/27	BM									
Chick R 475	L	+ +	- -	- -	- -	4	4	4	4	135
Hen BR 17723	H	+ +	- -	- -	- -	4	4	4	-	17701
Date died	Y									
4/15/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 476	L									
Hen BR 17723	H									
Date died	Y									
4/9/27	BM									
Chick R 474	L	+ -	- -	- -	- -	4	4	4	4	17701
Hen BR 17728	H	+ +	- -	- -	- -	4	4	4	4	17701
Date died	Y	+ +	- -	- -	- -	4	4	4	-	17718
4/10/27	BM									
Chick R 717	L									
Hen BR 17728	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
4/5/27	BM									

N-C* Not cultured

Table XVII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Third Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick R 472	L	+ -	- -	- -	- -	4	4	4	4	17701
Hen BR 17733	H	+ -	- -	- -	- -	4	4	4	4	17701
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17733
4/11/27	BM	+ +	- -	- -	- -	4	4	4	4	17716
Chick R D.B.	L									
Hen BR 17733	H									
Date died	Y									
4/4/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									

Table XVIII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 681	L	+ -	- -	- -	- -	4	4	4	4	17741
Hen WL 26	H									
Date died	Y									
4/20/27	BM									
Chick NR 682	L	+ +	- -	- -	- -	4	4	4	4	17723
Hen WL 26	H									
Date died	Y									
4/20/27	BM									
Chick NR 684	L									
Hen WL 26	H									
Date died	Y									
4/23/27	BM									
Chick NR 691	L									
Hen WL 116	H									
Date died	Y									
4/20/27	BM									
Chick NR 685	L	+ +	- -	- -	- -	4	4	4	4	17723
Hen WL 352	H									
Date died	Y									
5/1/27	BM									
Chick NR 694	L									
Hen WL 385	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick NR 695	L									
Hen WL 385	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick NR 696	L									
Hen WL 385	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick NR 697	L									
Hen WL 729	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick R 726	L	+ +	- -	- -	- -	4	4	4	4	17701
Hen WL 753	H									
Date died	Y									
4/20/27	BM									
Chick R 720	L									
Hen WL 1508	H									
Date died	Y									
4/12/27 N.S.	BM									

Table XIX.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction D'ese	D'ine	Dulc	Malt	Agg. Reaction 25	50	100	200	Positive serum
Chick R 721	L									
Hen WL 1508	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick R 722	L									
Hen WL 1508	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick R 734	L	+ +	- -	- -	- -	4	4	4	4	135
Hen WL 4284	H	+ +	- -	- -	- -	4	4	4	4	135
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
4/19/27	BM	+ +	- -	- -	- -	4	4	4	4	753
Chick R 718	L									
Hen WL 4296	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick R 719	L									
Hen WL 4296	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick R D.B.	L									
Hen WL 4296	H									
Date died	Y	N-C								
4/11/27	BM									
Chick R 729	L	+ +	- -	- -	- -	4	4	4	4	11
Hen WL 4282	H									
Date died	Y									
4/19/27	BM									
Chick R 730	L	+ +	- -	- -	- -	4	4	-	-	4284
Hen WL 4282	H									
Date died	Y									
4/16/27	BM	+ +	- -	- -	- -	4*	4	-	-	1508
Chick R 761	L	+ -	- -	- -	- -	4	4	4	-	15
Hen WL 4287	H									
Date died	Y									
4/23/27	BM									
Chick NR 672	L	+ +	- -	- -	- -	4	4	4	4	15
Hen RIR 12	H	+ +	- -	- -	- -	4	4	4	4	11
Date died	Y	+ -	- -	- -	- -	4	-	-	-	966
4/20/27	BM	+ +	- -	- -	- -	4	4	4	-	17712
Chick NR 673	L	+ +	- -	- -	- -	4	4	4	4	135
Hen RIR 12	H	+ +	- -	- -	- -	4	4	4	4	11
Date died	Y									
4/19/27	BM	+ +	- -	- -	- -	4	4	4	4	17731

N-C* Not cultured

Table XX. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ese	D'ine	Dulc	Malt	25	50	100	200	
Chick NR 674	L									
Hen RIR 12	H									
Date died	Y									
4/16/27	BM									
Chick NR 675	L	+ +	- -	- -	- -	4	4	-	-	17701
Hen RIR 12	H	+ +	- -	- -	- -	4	4	4	4	135
Date died	Y									
4/19/27	BM									
Chick NR 676	L									
Hen RIR 12	H	+ -	- -	- -	- -	4	4	4	-	753
Date died	Y									
4/18/27	BM	+ +	- -	- -	- -	4	4	4	4	17723
Chick NR 656	L	+ -	- -	- -	- -	4	4	4	4	17723
Hen RIR 13	H	+ +	- -	- -	--	4	4	4	4	4296
Date died	Y	+ -	- -	- -	- -	4	4	4	4	15
4/20/27	BM	+ +	- -	- -	- -	4	4	4	4	17718
Chick NR 657	L									
Hen RIR 13	H									
Date died	Y									
4/20/27	BM									
Chick NR 658	L	+ -	- -	- -	- -	4	4	4	4	17714
Hen RIR 13	H	+ +	- -	- -	- -	4	4	4	4	15
Date died	Y	+ +	- -	- -	- -	4	4	4	4	15
4/20/27	BM									
Chick NR 698	L									
Hen RIR 66	H									
Date died	Y									
4/12/27 N.S.	BM									
Chick NR 700	L									
Hen RIR 276	H	+ +	- -	- -	- -	4	4	4	4	753
Date died	Y									
4/17/27	BM									
Chick NR 667	L	+ +	- -	- -	- -	4	4	-	-	11
Hen RIR 88	H	+ +	- -	- -	- -	4	-	-	-	135
Date died	Y	+ -	- -	- -	- -	4	4	4	-	11
4/18/27	BM	+ +	- -	- -	- -	4	-	-	-	17718
Chick NR 669	L	+ -	- -	- -	- -	4	4	4	4	11
Hen RIR 88	H	+ -	- -	- -	- -	4	4	4	4	11
Date died	Y									
4/19/27	BM	+ -	- -	- -	- -	4	4	4	4	15
Chick NR 670	L									
Hen RIR 88	H	+ +	- -	- -	- -	4	4	4	4	135
Date died	Y									
4/19/27	BM	+ +	- -	- -	- -	4	4	4	4	11

Table XXI. A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction D'ese	D'ine	Dulc	Malt	Agg. Reaction 25	50	100	200	Positive serum
ChickNR 671	L									
Hen RIR 88	H									
Date died	Y									
4/19/27	BM									
ChickNR 804	L									
Hen RIR 92	H	+ +	- -	- -	- -	4	4	4	4	15
Date died	Y	+ +	- -	- -	- -	4	4	4	4	753
4/20/27	BM	+ +	- -	- -	- -	4	4	4	4	17711
ChickNR 692	L									
Hen RIR 152	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	-	17701
4/16/27	BM									
ChickNR 693	L									
Hen RIR 152	H									
Date died	Y									
4/12/27 N.S.	BM									
ChickNR 664	L	+ +	- -	- -	- -	4	4	4	4	11
Hen RIR 262	H	+ +	- -	- -	- -	4	4	42	4	11
Date died	Y	+ -	- -	- -	- -	4	4	4	4	135
4/20/27	BM									
ChickNR 666	L									
Hen RIR 262	H									
Date died	Y									
4/20/27	BM									
Chick NR 654	L									
Hen RIR 278	H									
Date died	Y	C								
4/19/27	BM	+ -	- -	- -	- -	4	4	4	4	753
Chick R 744	L	+ +	- -	- -	- -	4	4	4	4	753
Hen RIR 15	H	+ -	- -	- -	- -	4	-	-	-	17701
Date died	Y									
4/19/27	BM	+ +	- -	- -	- -	4	4	4	-	15
Chick R 745	L	C								
Hen RIR 15	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17728
4/16/27	BM									
Chick R 746	L									
Hen RIR 15	H									
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
4/17/27	BM									
Chick R 747	L	+ +	- -	- -	- -	4	4	4	4	11
Hen RIR 15	H	+ -	- -	- -	+ -	4	4	4	4	135
Date died	Y	+ -	- -	- -	- -	4	4	4	4	135
4/19/27	BM	+ +	- -	- -	- -	4	4	4	4	15

C* Contaminated

Table XXII.A Fermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction D'ose	D'ine	Dulc	Malt	Agg.Reaction 25	50	100	200	Positive serum
Chick R 736	L									
Hen RIR 73	H									
Date died	Y									
4/17/27	BM									
Chick R 737	L	+ +	- -	- -	- -	4	4	4	4	11
Hen RIR 73	H	+ +	- -	- -	- -	4	4	4	4	11
Date died	Y									
4/18/27	BM	+ +	- -	- -	- -	4	4	4	4	17707
Chick R 751	L									
Hen RIR 73	H									
Date died	Y									
4/17/27	BM									
Chick R 752	L									
Hen RIR 73	H	+ +	- -	- -	- -	4	-	-	-	17731
Date died	Y									
4/20/27	BM	+ -	- -	- -	+ -	4	4	4	4	17707
Chick R 750	L	+ -	- -	- -	- -	4	4	4	4	135
Hen RIR 95	H	+ -	- -	- -	+ -	4	4	4	4	11
Date died	Y									
4/18/27	BM	+ +	- -	- -	- -	4	4	4	4	17769
Chick R 757	L	+ +	- -	- -	- -	4	4	4	4	11
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	4	11
Date died	Y									
4/20/27	BM	+ +	- -	- -	- -	4	4	4	4	17701
Chick R 760	L	+ +	- -	- -	- -	4	4	4	4	17701
Hen RIR 95	H	+ +	- -	- -	- -	4	4	4	-	4284
Date died	Y									
4/17/27	BM									
Chick R 755	L									
Hen RIR 17753	H	+ +	- -	- -	- -	4	4	4	4	17701
Date died	Y									
4/16/27	BM									
Chick R 756	L	+ +	- -	- -	- -	4	-	-	-	17753
Hen RIR 17753	H	+ -	- -	- -	- -	4	4	4	-	17701
Date died	Y	+ -	- -	- -	- -	4	4	-	-	135
4/17/27	BM									
Chick R 758	L	+ +	- -	- -	- -	4*	4	4	4	17723
Hen RIR 17753	H									
Date died	Y									
4/20/27	BM									
Chick R 759	L	+ +	- -	- -	- -	4	4	4	-	17733
Hen RIR 17753	H	+ +	- -	- -	+ -	4	4	-	-	11
Date died	Y	+ +	- -	- -	- -	4	4	-	-	753
4/18/27	BM	+ +	- -	- -	- -	4	4	4	-	17723

4* Cloudy

Table XXIII.AFermentative and Agglutinative Study of Microorganisms Isolated from Tissues of Baby Chicks.

Fourth Hatch	Tissue exam	Carbohydrate Reaction				Agg. Reaction				Positive serum
		D'ose	D'ine	Dulc	Malt	25	50	100	200	
Chick R 748	L									
HenBR 17701	H									
Date died	Y									
4/20/27	BM									
Chick R 749	L									
HenBR 17701	H	+ -	- -	- -	- -	4	4	4	4	966
Date died	Y									
4/21/27	BM	+ +	- -	- -	- -	4	4	4	4	K262
Chick R 724	L	+ -	- -	- -	- -	4	4	4	4	11
HenBR 17712	H	+ -	- -	- -	- -	4	4	4	4	753
Date died	Y	+ -	- -	- -	- -	4	4	4	4	11
4/18/27	BM	+ -	- -	- -	- -	4	4	4	4	95
Chick R 725	L	+ -	- -	- -	- -	4	4	4	4	135
HenBR 17712	H	+ -	- -	- -	- -	4	4	4	4	135
Date died	Y	+ +	- -	- -	- -	4	4	4	4	17701
9/19/27	BM	+ +	- -	- -	- -	4	4	4	4	4296
Chick R 753	L									
HenBR 17718	H	+ +	- -	- -	- -	4	4	4	-	238
Date died	Y									
4/20/27	BM									
Chick R 754	L									
HenBR 17723	H									
Date died	Y									
4/21/27	BM	+ +	- -	- -	- -	4	4	4	4	73
Chick R 723	L									
Hen BR 17733	H	+ -	- -	- -	- -	4	4	4	4	135
Date died	Y	+ +	- -	- -	- -	4	4	4	-	753
4/17/27	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									
Chick	L									
Hen	H									
Date died	Y									
	BM									

Tables 24 through 28 show by breeds the settings, the number of pipped eggs from which Bacterium pullorum was isolated, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Table XXIV. Showing the number of eggs pipped, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Setting	No. eggs set	No. pipped	No. pipped Bacterium pullorum	Hen	White Leghorn non-reactors								
					Carbohydrate Reaction				Agg. Affinities				Positive
					D'ose	D'ine	Dulc	Malt	25	50	100	200	serum
1	28	2	-										
2	48	1	1	724	+ -	- -	- -	- -	4	4	4	4	4282
3	50	-	-										
4	44	-	-										
To.	170	3	1										

Table XXV. Showing the number of eggs pipped, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Setting	No. eggs set	No. pipped	No. pipped Bacterium pullorum	Hen	White Leghorn reactors								
					Carbohydrate Reaction				Agg. Affinities				Positive
					D'ose	D'ine	Dulc	Malt	25	50	100	200	serum
1	28	1	-										
2	48	1	1	4282	+ -	- -	- -	- -	4	4	4	-	4282
3	50	1	-										
4	44	2	1	4296	+ +	- -	- -	- -	4	4	4	4	17728
To.	170	5	2										

See legend on page 20.

Table XXVI. Showing the number of eggs pipped, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Setting	No. eggs set	No. pipped	No. pipped Bacterium pullorum	Rhode Island Red non-reactors									
					Carbohydrate Reaction				Agg. Affinities				Positive
				Hen	D'ose	D'ine	Dulc	Malt	25	50	100	200	serum
1	51	1	-										
2	39	-	-										
3	47	2	-										
4	53	2	1	299	+ +	- -	- -	- -	4	4	4	4	17728
To.	190	5	1										

Table XXVII. Showing the number of eggs pipped, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Setting	No. eggs set	No. pipped	No. pipped Bacterium pullorum	Rhode Island Red reactors									
					Carbohydrate Reaction				Agg. Affinities				Positive
				Hen	D'ose	D'ine	Dulc	Malt	25	50	100	200	serum
1	19	1	-										
2	14	1	1	73	+ -	- -	- -	- -	4	4	4	4	4282
3	19	-	-										
4	20	1	1	95	+ +	- -	- -	- -	4	4	4	4	4284
To.	72	3	2										

See legend on page 20.

Table XXVIII. Showing the number of eggs pipped, hens which laid them and fermentative and agglutinative studies of the microorganisms isolated.

Setting	No. eggs set	No. pipped	No. pipped Bacterium pullorum	Barred Rock reactors									
					Carbohydrate Reaction				Agg. Affinities				Positive
				Hen	D'ose	D'ine	Dulc	Malt	25	50	100	200	serum
1	32	1	-										
2	25	-	-										
3	28	-	-										
4	33	*4	1	11771	8 + +	- -	- -	- -	4	4	4	-	17701
To.	118	5	1										

See legend on page 20.

*One not cultured.

DISCUSSION AND SUMMARY

Tables 29 and 30 summarize the data of the problem from the standpoint of the non-reacting and reacting groups irrespective of breeds.

Table 29 shows that of the 211 chickens hatched from 360 eggs laid by non-reacting hens, 40.2 per cent died during the 3 weeks following hatching. From 29.3 per cent Bacterium pullorum was isolated.

Table 30 presents data to the effect that of 169 chicks hatched from 360 eggs laid by reacting hens, 58.5 per cent died within 3 weeks after hatching. The per cent of the total dead, found to be infected with Bacterium pullorum was 46.1.

Table XXIX. A summary of tabulated data for the non-reacting group.

Set-ting	No.eggs set	No.Pi-pped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
	170	3	1	122	37	30.3	21	17.2
	190	5	1	89	48	53.9	41	46.0
Total	360	8	2	211	85	**40.2	62	***29.3

Table XXX. A summary of the data for the reacting group of fowls.

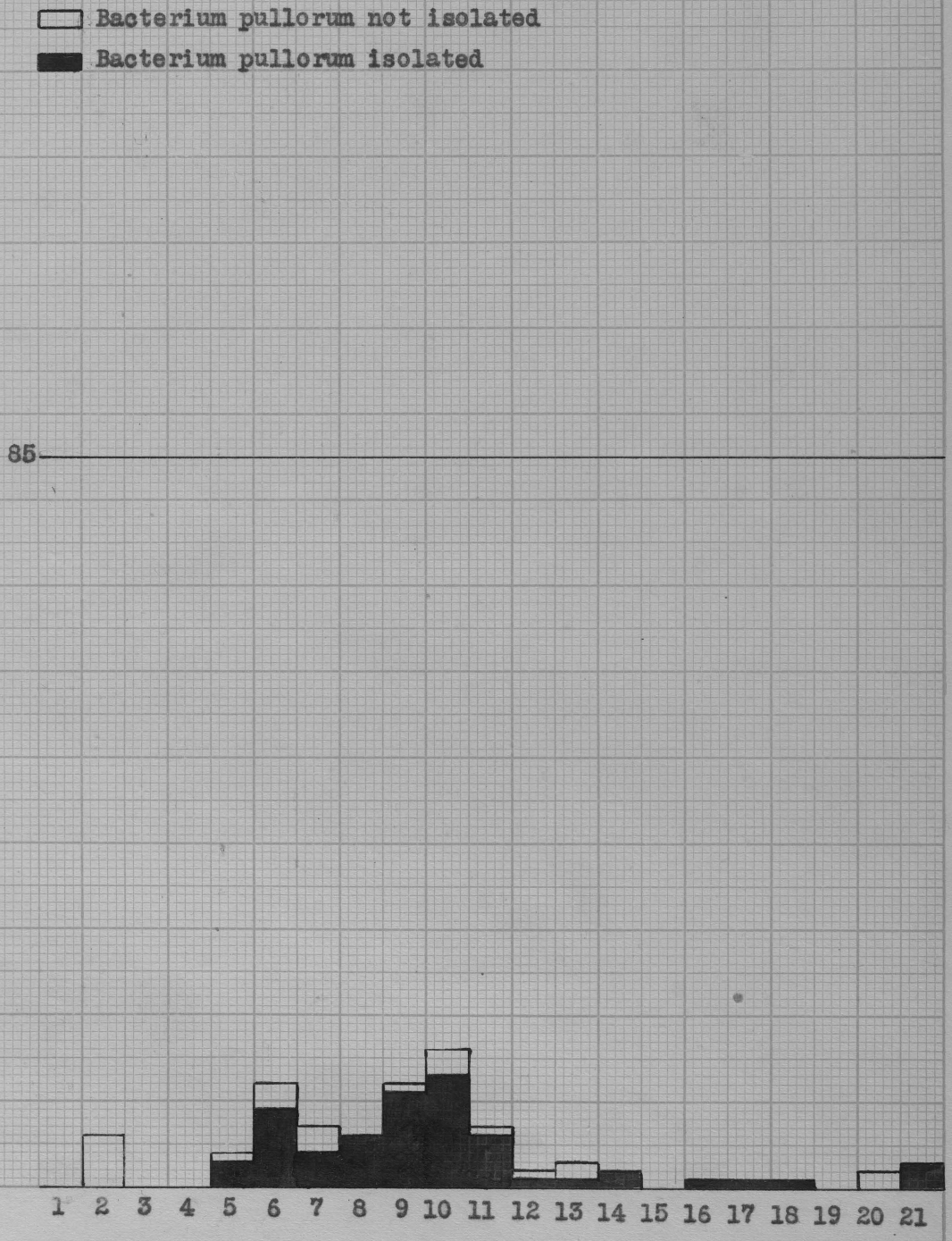
Set-ting	No.eggs set	No.Pi-pped	Pipped B.P.*	Hatched	Died 3wks	Per cent died	Died of B.P.*	Per cent dead B.P.*Isolated
	120	5	2	95	36	37.8	24	24.2
	72	3	2	43	40	93.0	33	76.7
	118	5	1	31	23	74.1	21	67.7
Total	360	13	5	169	99	**58.5	78	***46.1

*B.P. Bacterium pullorum
**Average percentage of total dead in 3 weeks
***Average percentage of dead in which Bacterium pullorum was isolated.

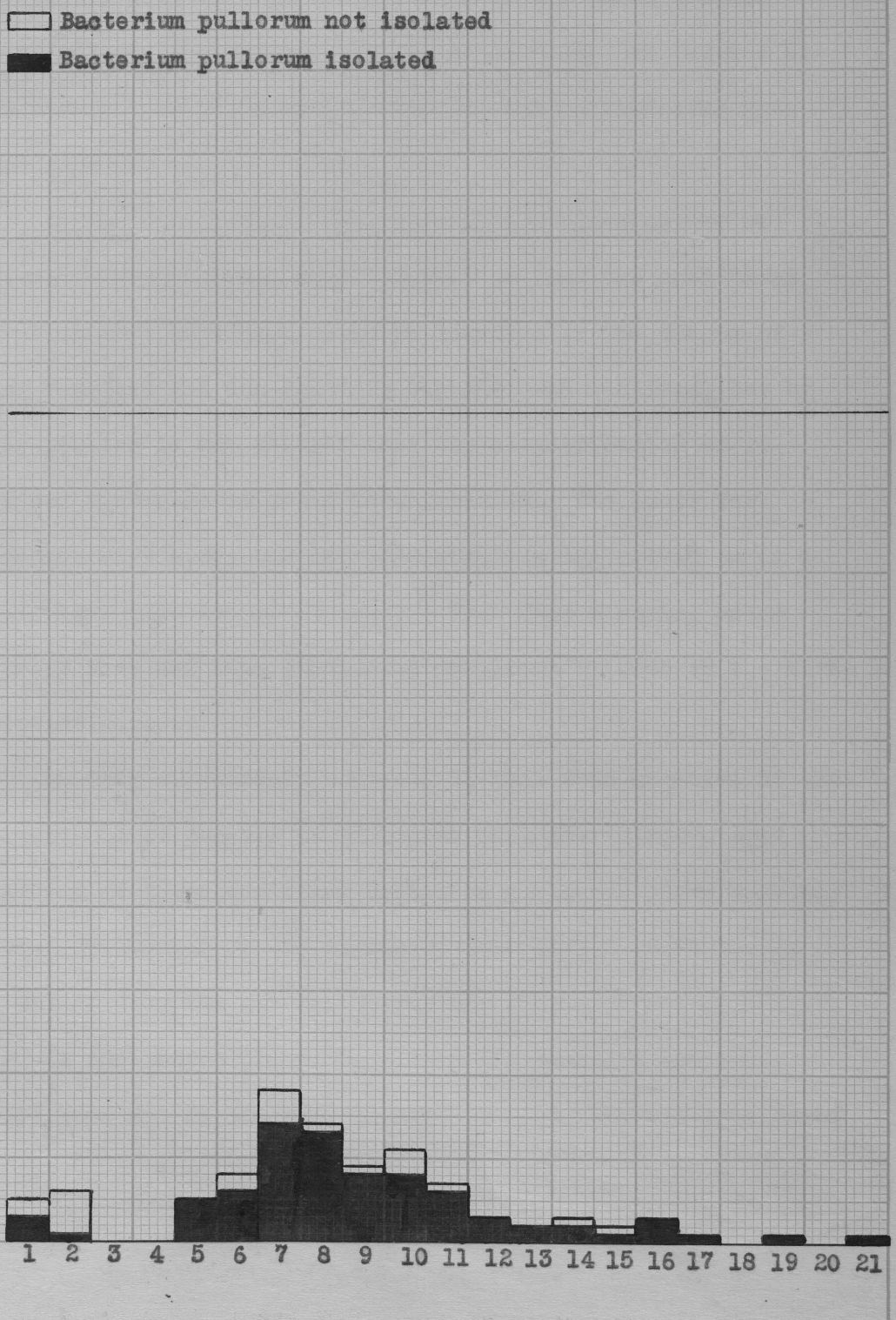
Graph I summarizes the mortality of chicks from non-reactors and the number dead from which Bacterium pullorum was isolated.

Graph II summarizes the mortality of chicks that were hatched from reactors and the number of dead from which Bacterium pullorum was isolated.

Graph I. Shows the mortality by days of chicks from non-reactors and the number dead from which Bacterium pullorum was isolated.



Graph II. Shows the mortality by days of chicks from reactors and the number dead from which Bacterium pullorum was isolated.



Some of the results of this experiment may seem erratic to the casual observer, but a knowledge of two factors which entered into the problem may help to explain why the results obtained with the chicks from the reacting and non-reacting hens are not more marked.

In the first place, the reactors and non-reactors of each breed were purposely kept together so that the conditions under which they were kept would be identical. The danger of keeping reactors and non-reactors together was realized, but by monthly testing it was thought that the classification of reactors and non-reactors could be maintained. The basis for declaring a bird a reactor has been complete agglutination in a serum-antigen dilution of at least 1-50. Hens reacting below this point have not been considered dangerous by some of the foremost investigators.

In this experiment, however, 11 that were negative at the first test, later reacted at dilutions of 1-25. Since pipped eggs from 2 of these contained Bacterium pullorum, these hens may have been the source of the infection that caused heavy losses among chicks from the non-reactors.

In the second place, eggs from the reactors and non-reactors were set in the same incubator. This was not considered

a dangerous procedure. There is experimental evidence to show that there is danger of transmission of bacillary white diarrhea infection in forced draft incubators, but none which shows a similar danger in the type employed in this experiment. Although the eggs from the reactors and non-reactors were separated by being placed in wire baskets or gauze sacks prior to hatching there is a possibility that infection of chicks from reactors may have taken place in the incubator.

In this problem 21 eggs had been pipped but had not hatched. The yolk sacs of 20 of the unhatched chicks were cultured and 7 were found to contain Bacterium pullorm. As stated previously, from 2 eggs laid by non-reacting hens, number 724 and number 299, Bacterium pullorum was isolated.

Of the 184 chicks examined bacteriologically 309 cultures were isolated. The tissues from which they were isolated varied as follows: 87 were from the liver, 72 from the heart, 71 from the yolk and 79 from the bone marrow.

Seventy-three of the 309 cultures were anaerogenic, that is they produced no gas in dextrose broth.

Thirteen of the 309 cultures produced acid in maltose broth.

The study of the antigenic affinities revealed that 235 cultures were agglutinated by serum from reacting hens at dilutions

of 1-200. Forty-five of the cultures were only agglutinated in dilutions of 1-100. The serum from reacting hens agglutinated 16 in dilutions of 1-50 while 13 were only antigenic enough to be agglutinated in dilutions of 1-25.

In the first hatch a very small percentage (6.8) of the chicks from the non-reactors died of Bacterium pullorum infection while 56.2 percentage died of bacillary white diarrhea in the reacting group. The percentage of mortality from this cause in the non-reactors increased markedly in the next three hatches which is as follows: percentage of the groups of non-reacting chicks from which Bacterium pullorum was isolated, second hatch 60.3, third hatch 18.1, and the fourth hatch 28.8. The percentage of the broods of reacting chicks from which Bacterium pullorum was isolated, second hatch 48.5, third hatch 35.1, fourth hatch 50.0. This may indicate that the non-reacting hens previously referred to were contracting the infection from contact with the reactors and were transmitting it to the chicks.

CONCLUSIONS

1. Bacillary white diarrhea is an acute infection disease of baby chicks which in this experiment caused the greatest number of deaths between the fourth and twelfth days after hatching.

2. The mortality rate was exceedingly high in the chicks both from the reacting and non-reacting hens. Ordinarily it would be expected that no deaths due to Bacterium pullorum should occur among the chicks from the non-reacting hens provided they are kept under conditions that prevent infection. In this problem it may be concluded that the chicks may have become infected from the egg yolks of the reacting or infected mother hens and also from the yolks of low reacting hens in the so-called non-reacting group and from infection transmitted in the incubator. Since the mortality rate among the chicks from the reacting groups of hens was 16.8 per cent more than among the chicks from the non-reacting hens it may be concluded that the infected mother hen is the greatest source of danger.

3. Bacterium pullorum was isolated from one-third of the eggs that were pipped but which did not hatch. It appears that the infection may be responsible for the inability of many chicks to emerge from the shell.

4. Both aerogenic and anaerogenic strains of Bacterium pullorum were responsible for chick mortality.

5. A small per cent of cultures of Bacterium pullorum isolated from baby chicks are likely to be more or less non-antigenic.

6. All four tissues, liver, heart, yolk and bone marrow should be cultured in order to make certain that Bacterium pullorum is detected.

7. Bacterium pullorum is dextrose positive and dextrine, and dulcite/ maltose negative, however, thirteen strains formed acid in maltose.

8. In view of the fact that the rate of mortality from Bacterium pullorum infection was very low among the non-reacting chicks of the first hatch as compared to chicks of the same group in the three subsequent hatches and also in view of the fact that the serum-agglutination reaction changed from negative to a weak positive reaction in some of the non-reacting birds it may indicate that these hens were gradually contracting the infection from contact with the reactors and were transmitting it to the chicks.

An interesting fact noted in connection with this experiment was that the percentage of eggs hatching from the non-reacting group was 58.6 while from the reacting flock it was 46.9, in spite of the fact that some of the eggs laid by the non-reacting group were infected.

From data presented in this investigation it may be further concluded that hens negative to the agglutination test cannot safely be kept with hens positive to the test without danger of transmission of Bacterium pullorum from the infected birds to the others, and until an investigation of the possibility of transmission of Bacterium pullorum in the non-forced draft type of incubator is conducted, one should be rather cautious about setting eggs from reacting and non-reacting hens together in this type of machine.

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