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VARIATION IN THE REACTIONS OBTAINED IN REPEATED AGGLUTINATION TESTS OF THE SAME FOWLS WITH *BACTERIUM PULLORUM* ANTIGEN

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INTRODUCTION

The studies by Rettger[1, 2, 4] and Rettger and Harvey,[3] reported in four papers published between 1900 and 1909, definitely established the disease of young chicks commonly known as "white diarrhea" to be a specific infectious disease, the causative organism of which was designated *Bacterium pullorum*. Further studies by Rettger and his associates were reported in 1909,[5] 1911[6] 1912,[7] and 1914.[8, 9] They determined that apparently healthy adult fowls may be carriers of *Bact. pullorum*. The infection in hens usually becomes localized in the ovaries and is eliminated in the eggs. When such eggs are used for hatching, the infection is transmitted to chicks. This is considered the usual source of *Bact. pullorum* infection in chicks. Jones[10, 11] in 1910 and 1911 and Gage[12] in 1911 published the results of investigations which confirmed the findings of Rettger and his associates.

The most important problem in the prevention of the disease in chicks, therefore, became the detection and elimination of infected breeding stock. In 1913 Jones[13] demonstrated that the agglutination test was of value for this purpose. His findings were confirmed by others and the testing of breeding flocks by this method has been practiced extensively for several years.

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All who have made careful study of the agglutination test for detecting carriers of *Bact. pullorum* recognize that repeated tests are necessary for the elimination of all infected fowls in a flock. The failure of a single test to detect all of the infected fowls is commonly considered to be due either to certain birds having acquired infection too recently for the production of sufficient agglutinins in their blood serum to cause an agglutination reaction or to certain birds becoming infected after the test, either from association with the infected birds or from contaminated litter and soil. It is commonly considered, however, that after agglutinins have become sufficiently abundant to cause a reaction, they will remain so as long as the fowl continues to harbor *Bact. pullorum.*

The workers in the laboratories of the Division of Veterinary Science, University of California had no reason to doubt that fowls with well-established infection with *Bact. pullorum* would uniformly react to the agglutination test until, in the routine testing that was being carried on, it became necessary to make tests of two lots of blood samples from the same birds at an interval of twenty-one days. On August 25, 1925, a test was made on a lot of 574 blood samples. Another lot of blood samples from this flock was received on September 15, 1925, and included duplicates of 388 samples that had been tested twenty-one days previously. The comparative results of the tests of the two samples of blood from 388 fowls were as follows:

Six fowls reacted to both tests.

Twelve fowls that gave a positive reaction to the first test failed to react to the second test.

Three fowls that were negative at the first test reacted to the second test.

Such discrepancies in the results of the two tests could not be ascribed to differences between the antigens or methods because these were the same for both tests. They could hardly be considered as due to certain fowls having become free from and others having acquired infection between the two tests because of the short interval between them. Therefore, it seemed probable that all of the fowls that had reacted to either one or both tests were infected at the time each test was made but certain of them had failed to react to one test. If this assumption is correct, it would indicate that fowls may not constantly give a positive reaction to the agglutination test while they are carriers of *Bact. pullorum.* It was to obtain information on this point that the studies herein reported were undertaken.

Other points regarding which it was thought information might be obtained were:

First, the correlation between intensity of egg production, age of the birds or season of the year and any variability in the reactions to repeated agglutination tests of the same individuals that is found to occur;

Second, the accuracy with which the results of an agglutination test may be interpreted to indicate the presence or absence of *Bact. pullorum* infection;

Third, the rapidity with which *Bact. pullorum* infection may spread among non-infected adult females from association with infected adult females.

Plan of the Experiment

In December, 1925, 200 White Leghorn pullets, from 8 to 9 months old, were obtained from a flock in which *Bact. pullorum* infection was known to exist. They were immediately leg-banded and subjected to an agglutination test. Seventy of the birds gave a positive reaction in at least one dilution and 130 failed to react. The seventy reactors and eighty of the non-reactors were placed together in one house and designated group 1 and group 2, respectively. The remaining fifty non-reactors were placed in a separate house and designated group 3. Outside runs were not provided. An agglutination test of the blood serum of all birds was made once each month. This procedure is to continue for at least two years, but this report is concerned only with the first twelve months.

A careful search for *Bact. pullorum* was made in all birds that died from any cause, except certain ones otherwise accounted for.

The antigen was prepared from a single strain of *Bact. pullorum* of known good agglutinability that had been isolated from a chick. Cultures were incubated on agar for 48 hours and the growth washed off with sterile, physiological salt solution containing 0.5 per cent phenol. Cultural and microscopic tests were made of each lot of antigen to insure freedom from contamination. The antigen in concentrated form was stored in an ice box. It was diluted with sufficient phenolized saline to give a reading of 3.5 cm. with a Gates' nephelometer at the time the tests were made.

In all of the tests, four dilutions of serum and antigen were used, i.e., 1–25, 1–50, 1–100 and 1–200. The tubes were incubated for 24 hours and kept at room temperature 24 hours longer. Readings were made after 24 and 48 hours.

Summary and Discussion of the Results of the Agglutination Tests of the Fowls in Group 1

This group consisted of seventy birds that gave a positive reaction to the first agglutination test. However, there was a marked variation in the number of these birds that reacted at each of the eleven subsequent monthly tests. A summary of the results of the tests and the average egg production during each month is given in table 1. The gradual decrease in the number of birds in the group is due to deaths that occurred.

TABLE 1

Summary of the Results of 12 Agglutination Tests and Egg Production of Group 1

Month	Number of birds in group	Number of reactors	Per cent reactors	Per cent egg production for the month
December	70	70	100.0	0.
January	70	38	54.2	1.3
February	68	39	57.3	2.9
March	60	44	73.3	27.2
April	59	29	49.1	39.5
May	58	26	44.8	45.7
June	56	23	41.0	41.4
July	54	31	57.3	28.2
August	53	26	49.0	21.7
September	51	25	49.0	21.9
October	51	22	43.1	12.2
November	50	19	38.0	3.6

In table 1, it is seen that at none of the tests after the first were reactions obtained from all of the birds that reacted to the first test. The nearest approach to this was in March, when 73.3 per cent of the birds reacted. In January, February and July, positive reactions were obtained from 54.2 per cent, 57.3 per cent and 57.3 per cent of the birds, respectively. Less than half of the birds gave a positive reaction at each of the seven other tests, the percentage ranging from 49 in April, August and September down to 38 in November. Table 1 also clearly shows that the variation in the number of birds that reacted at the different tests was not correlated to that of egg production.

The progressive decrease in the number of reactors to each of the tests after July suggests that the decrease may be in correlation with

the increasing age of the birds. However, a similar decline in number of reactors occurred between March and June, but was followed by an increase in the number of reactors to the test in July.

The difference in the number of the birds of group 1 that reacted to each of the twelve tests was not merely a progressive decrease due to certain of the birds ceasing to react, but was also due to a fluctuation between positive and negative of the reactions which some individual birds gave to the different agglutination tests. This is shown by the increase in the number of positive reactions obtained in March over that obtained in January or February and in the number in July over that obtained in April, May or June. It is more clearly brought out, however, by the following detailed summary of the reactions to the agglutination test of the fifty birds that lived during the entire year and were tested twelve times.

A general summary of the number of the fifty birds that reacted to each of the twelve tests is given in table 2.

TABLE 2

SUMMARY OF RESULTS OF AGGLUTINATION TESTS OF 50 BIRDS OF GROUP 1 THAT WERE TESTED TWELVE TIMES

Number of test	Month	Number of reactors	Per cent reactors
1	December	50	100.0
2	January	31	62.0
3	February	28	56.0
4	March	36	72.0
5	April	23	46.0
6	May	21	42.0
7	June	19	38.0
8	July	27	54.0
9	August	24	48.0
10	September	24	48.0
11	October	21	42.0
12	November	19	38.0

In table 2, it is seen that the variation in the percentage of the fifty birds that gave positive reactions to the different agglutination tests closely follows that shown in table 1 for the whole of group 1.

Of the fifty birds that were tested twelve times

10, or 20 per cent gave a positive reaction to all 12 tests.
4, or 8 per cent gave a positive reaction to 11 tests.
4, or 8 per cent gave a positive reaction to 10 tests.
2, or 4 per cent gave a positive reaction to 9 tests.

1, or 2 per cent gave a positive reaction to 8 tests.
3, or 6 per cent gave a positive reaction to 7 tests.
1, or 2 per cent gave a positive reaction to 6 tests.
4, or 8 per cent gave a positive reaction to 5 tests.
4, or 8 per cent gave a positive reaction to 4 tests.
5, or 10 per cent gave a positive rtaction to 3 tests.
4, or 8 per cent gave a positive reaction to 2 tests.
8, or 16 per cent did not react after the first test.

The distribution of the positive and negative reactions to the agglutination tests of the forty birds that did not give a positive reaction to all of the twelve tests is given in table 3.

A study of table 3 shows that the positive reactions of twenty-six of the thirty-two fowls that reacted to from two to eleven tests were interspersed with negative reactions to one or more consecutive tests. The most commonly occurring irregularity of this nature was one negative reaction between two positive reactions. This occurred in nineteen instances. Negative reactions to two consecutive tests between positive tests occurred in seven instances; to three consecutive tests in three instances; to four consecutive tests in three instances; to five consecutive tests in three instances; and to seven consecutive tests in one instance.

Table 3 also shows that certain of the birds, after giving positive reactions either consistently or irregularly to one or more tests, did not react to any subsequent test. The number of such birds and the last test to which a positive reaction was obtained is as follows:

8 birds did not react after the first test.
2 birds did not react after the third test.
3 birds did not react after the fourth test.
3 birds did not react after the fifth test.
3 birds did not react after the eighth test.
1 bird did not react after the ninth test.
5 birds did not react after the tenth test.
4 birds did not react after the eleventh test.

The disappearance of agglutinins from the blood serum of the sixteen birds that failed to react after the first, third, fourth or fifth test is possibly due to the birds having become free from *Bact. pullorum* infection. These birds cannot with certainty be regarded as free from infection, however, because, as will be shown later, *Bact. pullorum* was isolated from the ovaries of six birds of group 1 that died after having failed to react to from one to three agglutination tests next preceding their deaths.

TABLE 3

THE DISTRIBUTION OF THE POSITIVE AND NEGATIVE REACTIONS OF 40 BIRDS OF GROUP 1 THAT DID NOT REACT TO ALL OF THE TWELVE AGGLUTINATION TESTS

Number of positive reactions	Total number of birds	Number of birds that gave the same reaction to each test	Tests at which a positive reaction occurred	Tests at which a negative reaction occurred
11	4	1	First and second; fourth to twelfth	Third
		1	First to sixth; eighth to twelfth	Seventh
		2	First to eleventh	Twelfth
10	4	1	First to fifth; seventh to ninth; eleventh and twelfth	Sixth and tenth
		1	First to tenth	Eleventh and twelfth
		1	First to fourth; sixth; eighth to twelfth	Fifth and seventh
		1	First to third; fifth to eleventh	Fourth and twelfth
9	2	1	First to fourth; sixth to tenth	Fifth, eleventh and twelfth
		1	First and second; sixth to twelfth	Third, fourth and fifth
8	1	1	First; third to fifth; eighth and ninth; eleventh and twelfth	Second, sixth, seventh, tenth
7	3	1	First to fourth; ninth and tenth; twelfth	Fifth to eighth; eleventh
		1	First; seventh to twelfth	Second to sixth
		1	First to sixth; eleventh	Seventh to tenth; twelfth
6	1	1	First and second; fourth to sixth; eighth	Third; seventh; ninth to twelfth
5	4	1	First to fifth	Sixth to twelfth
		1	First and second; fourth and fifth; eighth	Third; sixth and seventh; ninth to twelfth
		1	First to fourth; twelfth	Fifth to eleventh
		1	First to third; eighth and ninth	Fourth to seventh; tenth to twelfth

TABLE 3—(*Continued*)

Number of positive reactions	Total number of birds	Number of birds that gave the same reaction to each test	Tests at which a positive reaction occurred	Tests at which a negative reaction occurred
4	4	1	First and second; fourth and fifth	Third; sixth to twelfth
		1	First; fourth; eighth; tenth	Second and third; fifth to seventh; ninth; eleventh and twelfth
		1	First to fourth	Fifth to twelfth
		1	First, third and fourth; tenth	Second; fifth to ninth; eleventh and twelfth
3	5	1	First and second; fourth	Third; fifth to twelfth
		1	First; fourth; tenth	Second and third; fifth to ninth; eleventh and twelfth
		1	First; fourth; eighth	Second and third; fifth to seventh; ninth to twelfth
		1	First; fourth and fifth	Second and third; sixth to twelfth
		1	First, second and third	Fourth to twelfth
2	4	3	First and fourth	Second and third; fifth to twelfth
		1	First and third	Second; fourth to twelfth
1	8	8	First	Second to twelfth

Any or all of the birds that gave positive reactions up to the eighth or subsequent tests can reasonably be expected to again react since the number of tests to which these birds have given a negative reaction is no greater than the number of consecutive negative reactions that occurred between the positive reactions of some of the birds that reacted irregularly to the tests.

The variation in the number of birds of group 1 that reacted to each of the twelve agglutination tests made at intervals of approximately one month is, therefore, manifested in two ways: first, by fluctuation between positive and negative of the reactions of some individuals to the different tests, and, second, by certain of the birds, after giving a positive reaction to one or more tests, ceasing to react to all subsequent tests.

Studies by Beach, Halpin and Lampman[14] that were carried on at the same time as those herein reported showed similar variation in the reactions to the agglutination test exhibited by a flock of hens that was tested twelve times in thirteen months.

Summary of Results of the Agglutination Tests and Postmortem Examination of the Fowls that Died in Group 1

The mortality in group 1 during the year was twenty fowls. Two were not examined. The remaining eighteen were carefully examined for the presence of gross ovarian or other lesions suggestive of *Bact. pullorum* infection. A bacteriologic examination, particularly for the purpose of determining the presence of *Bact. pullorum*, was made of the ovaries and yolks of these birds. The results are given in table 4:

TABLE 4

Results of Agglutination Tests and Postmortem Examination of Twenty Fowls that Died in Group 1

Number of agglutination tests	Tests giving positive reaction	Tests giving negative reaction	Condition of ovary	Ovarian lesions found	Results of bacteriologic examination of ovaries
2	First	Second	Active	None	Negative
3	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second and third	Dormant	Congestion. No abnormal yolks	*Bact. pullorum* isolated
4	First and second	Third and fourth	Dormant	Abnormal yolks	*Bact. pullorum* isolated
7	First, third and fourth	Second, fifth, sixth, seventh	Not examined		
8	All	None	Active	Abnormal yolks	*Bact. pullorum* isolated
10	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
11	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second and third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
2	First	Second	Dormant	None	*Bact. pullorum* isolated
3	First and third	Second	Dormant	Abnormal yolks	*Bact. pullorum* isolated
6	First to fifth	Sixth	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First and third	Second	Dormant	Abnormal yolks	*Bact. pullorum* isolated
5	First, third, fourth	Second, fifth	Active	Abnormal yolks	Negative
9	First, third, fourth, sixth, eighth	Second, fifth, seventh, ninth	Active	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second, third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
6	First	Second to sixth	Not examined.		
8	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second, third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
4	First	Second, third, fourth	Active	Abnormal yolks	*Bact. pullorum* isolated

A study of the summarized data concerning the twenty dead fowls from group 1, as given in table 4, shows the following:

No examination was made of two fowls.

Bact. pullorum was isolated from the ovaries of sixteen of the eighteen fowls examined. Gross ovarian lesions, in the form of abnormal yolks, were present in fourteen of these. Congestion of the ovary was the abnormality found in one. In the one remaining fowl from which *Bact. pullorum* was isolated, no ovarian abnormality nor other lesion suggestive of infection with *Bact. pullorum* was observed. Five of the sixteen fowls that yielded *Bact. pullorum* at the bacteriologic examination reacted to all agglutination tests before their death. Three failed to react to all tests but did to the one next preceding their death. Two did not react to the last agglutination test, five did not react to the last two tests, and one did not react to the last three tests, preceding their death. This definitely shows fowls with well-established ovarian infection with *Bact. pullorum* may not always have sufficient agglutinins in their blood serum to cause a reaction to the agglutination tests.

The ovary of one of the two reacting fowls from which *Bact. pullorum* was not isolated was normal in appearance. The ovary of the other bird contained abnormal yolks. The negative results of the bacteriologic examination of these two birds cannot be considered as positive evidence that they were not carriers of *Bact. pullorum.* The organism may have been present in them even though it was not recovered in cultures.

The comparative results of the agglutination tests and bacteriologic examination of the eighteen dead fowls indicate that a high percentage of fowls that give a positive reaction to an agglutination test for the detection of *Bact. pullorum* infection are carriers of that organism. This is true even though such fowls fail to react to agglutination tests made one to three months later. As previously stated, the term "positive reaction" in this paper is applied to partial or complete clearing of any one or all of the four serum-antigen dilutions, 1–25, 1–50, 1–100 and 1–200.

Summary of Results of the Agglutination Tests of the Fowls in Group 2

Group 2 consisted of eighty of the fowls that did not react to the first agglutination test and that were confined in the same pen with group 1, the fowls that reacted to the first test.

Twelve of these fowls reacted to some of the subsequent agglutination tests. The tests to which these birds gave positive reactions were as follows:

One fowl gave a positive reaction to the second, third, fourth and fifth tests, and a negative reaction to the sixth test. This bird afterwards disappeared from the pen and no further data concerning it was obtained.

One fowl gave a positive reaction to the fourth, fifth and eighth to twelfth tests.

One fowl gave a positive reaction to the fourth, fifth, eighth and ninth tests.

One fowl gave a positive reaction to the fourth and eighth tests.

Five fowls gave a positive reaction to the fourth test only. The reactions of two of these consisted only of a partial clearing of the lowest of the four serum-antigen dilutions. Since these two birds gave only a slight reaction to the one test and no reaction to the other tests, it is perhaps incorrect to classify them as reactors.

One fowl gave a positive reaction to the eighth and ninth tests.

One fowl gave a positive reaction to the tenth test.

One fowl gave a positive reaction to the eleventh and twelfth tests.

With the exception of the one that disappeared from the pen, all of the fowls that became reactors to the agglutination test are still living. No opportunity for postmortem and bacteriologic examination of any of them has, therefore, been afforded.

Nine of the fowls were negative to from one to three agglutination tests before they gave a positive reaction. This number of negative reactions is no greater than the number of consecutive negative reactions that occurred between the positive reactions of some of the birds of group 1. Therefore, it seems just as probable that the positive reactions of these birds resulted from *Bact. pullorum* infection which they were harboring when the experiment started as from infection which they acquired from association with the infected birds.

The other three birds that became reactors did not react until the eighth, tenth and eleventh tests, respectively. It does not seem improbable, therefore, that the positive reaction to the agglutination test of these birds was due to infection with *Bact. pullorum* acquired after the experiment started. This indicates that transmission of *Bact. pullorum* infection among adult fowls by association of infected and non-infected may be an important factor in increasing the extent of the infection in breeding flocks.

Results of Postmortem Examinations of the Fowls in Group 2 that Died

Twenty-four hens died during the year. None of these had given a positive reaction to an agglutination test. Three of the dead were not examined. Twenty-one were given a careful postmortem examination for gross lesions, particularly of the ovary, that might be suggestive of *Bact. pullorum* infection. Cultures were made from the ovaries of these birds.

The bacteriologic examination of eighteen birds gave negative results. The ovaries of eleven of these birds were normal in appearance; a small cyst was attached to the ovaries of two; the ovaries of two birds were congested; and a few small abnormal yolks were present in three birds.

Bact. pullorum was isolated from the ovaries of the remaining three of the twenty-one birds examined. Abnormal yolks were present in all three birds. Two of these had been negative to three agglutination tests and one to five tests. This is additional evidence that fowls with infection of the ovaries with *Bact. pullorum* may fail to react to the agglutination test.

Summary of the Results Obtained with Group 3

This group consisted of fifty fowls that did not react to the first test and were kept separated from all other fowls during the year.

Forty-nine of the birds did not react to any of the agglutination tests during the year. Twelve of these birds died during the year. The deaths occurred after they had been tested from two to ten times. Examination was not made in the case of six of these. Postmortem examination of the remaining six showed the ovaries to be normal in appearance with the exception of a pea-sized tumor-like mass of tissue attached to the ovary of one bird. The bacteriologic examination of these six fowls gave negative results.

One fowl reacted to the fifth and sixth tests. This fowl died soon after the sixth test. The ovary contained several large abnormal yolks from which *Bact. pullorum* was isolated. It seems unlikely that this bird became infected from association with the other birds of group 3 since the experiment started as the results of the agglutination tests and the postmortem examination of those that died indicate that none of the other birds were infected. Furthermore, the lesions, found in

this fowl seemed too extensive to have resulted from recently acquired infection. It appears probable, therefore, that although this fowl did not give a positive reaction until the fifth month of the experiment, it was harboring infection when the experiment started.

Variation in the Degree of the Positive Reactions to the Agglutination Tests

In preceding pages, it has been pointed out that reactions to the agglutination tests of many of the birds fluctuated between positive and negative. A reaction was considered to be positive when there was entire or partial clearing of any one of the four serum-antigen dilutions. It was found that a great variation existed between the degree of the positive reactions of the birds that reacted more than once. In fact, the blood serum of no bird that reacted to more than one test caused the same degree of agglutination in all of the tests in which a positive reaction was secured. The total number of positive reactions obtained was 418. The variation in the degree of reactions is summarized in table 5.

TABLE 5

Variation in the Degree of the 418 Positive Reactions to the Agglutination Test

Dilutions in which agglutination occurred*	Number of reactions	Per cent of total reactions
Partial in 1–25 dilution. No agglutination in others	26	6.1
Complete in 1–25 dilution. No agglutination in others	72	17.2
Partial or complete in 1–25 and 1–50 dilutions. No agglutination in others	133	29.4
Partial or complete in 1–25, 1–50 and 1–100 dilutions. No agglutination in others	71	16.9
Partial or complete in 1–25, 1–50, 1–100 and 1–200 dilutions	116	25.3

* In every instance, there was agglutination in all dilutions below the highest dilution in which agglutination occurred.

It is shown by table 5 that fewer positive reactions would have been obtained if the lowest serum-antigen dilution had been higher than 1–25. If the lowest dilution had been 1–50 there would have been 98 or 23.4 per cent less positive reactions, if the lowest dilution had been 1–100, there would have been 231 or 55.2 per cent less positive reactions.

Such variation in the results of the agglutination tests gives rise to the question of whether an agglutination in low dilution only can be interpreted as indicating infection with *Bact. pullorum*. Some information on this point is furnished by the positive reactions to the agglutination test of seventeen reacting fowls which died and from which *Bact. pullorum* was isolated. Fifty-nine positive reactions were obtained from these seventeen birds. Table 6 gives a summary of the variation in the degree of the reactions.

TABLE 6

VARIATION IN THE DEGREE OF 59 POSITIVE REACTIONS TO THE AGGLUTINATION TESTS OF 17 FOWLS THAT DIED AND FROM WHICH *Bact. pullorum* WAS ISOLATED

Dilutions in which agglutination occurred	Number of reactions	Per cent of total reactions
Partial in 1–25 dilution. No agglutination in others	3	5.0
Complete in 1–25 dilution. No agglutination in others	13	22.0
Partial or complete in 1–25 and 1–50 dilutions. No agglutination in others	20	33.8
Partial or complete in 1–25, 1–50 and 1–100 dilutions. No agglutination in others	11	18.6
Partial or complete in 1–25, 1–50, 1–100 and 1–200 dilutions	12	20.3

By comparing table 6 with table 5, it is seen that the variation in the degree of the positive reaction to the agglutination tests of the seventeen fowls that were known to harbor *Bact. pullorum* closely follows the variation in the degree of the positive reactions of all of the birds. The fact that in more than half of the positive reactions of these seventeen known infected fowls agglutination was obtained only in one or both of the 1–25 and 1–50 dilutions indicates that any fowl that gives a positive agglutination reaction in these dilutions but not in higher dilutions may be a carrier of *Bact. pullorum*. It would, therefore, be expected that any agglutination test procedure for the detection of carriers of *Bact. pullorum* should include a serum-antigen dilution as low as 1–25.

Conclusions

This paper presents the results of the first twelve of a series of at least twenty-four monthly agglutination tests of the same fowls for the detection of *Bact. pullorum* infection, together with the results of the bacteriologic examinations of the fowls that have died during the twelve month period. Complete interpretation of the results of these tests cannot be made until the experiment is terminated and a postmortem and bacteriologic examination is made of all of the fowls. The information obtained from the results of the first year of the experiment, however, would seem to warrant the following conclusions:

Adult fowls with well-established ovarian infection with *Bact. pullorum* may not always react to an agglutination test. This factor seriously affects the dependability of the agglutination test as a means of detecting *Bact. pullorum* carriers and therefore detracts from the practical value of the tests as a means for the complete eradication of the infection from a breeding flock.

A fowl that has reacted to an agglutination test may not react to subsequent tests even though it is still infected with *Bact. pullorum.* Therefore, a fowl that has once reacted to a test cannot be considered as free from the infection if it fails to react to tests that are made subsequently.

A positive reaction to the agglutination test may be considered as a highly accurate indication of *Bact. pullorum* infection. A negative reaction to a test, however, appears to less accurately indicate freedom from *Bact. pullorum* infection, either recently acquired or of long standing.

In an agglutination test procedure with an antigen of equal or greater density than that used in these studies, a serum-antigen dilution at least as low as 1–25 should be included. Clearing of the 1–25 dilution alone or accompanied by clearing of one or more higher dilutions of the same serum can be interpreted as a positive reaction.

No information regarding the interpretation of proagglutination or paradoxical reactions was obtained in these studies since this phenomenon was not encountered.

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1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
3. The Formation of Sodium Carbonate in Soils, by Arthur B. Cummins and Walter P. Kelley. March, 1923.
4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
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